

Increased percentage of mast cells within sinonasal mucosa of chronic rhinosinusitis with nasal polyp patients independent of atopy

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Background: Initial attention on the pathophysiology of chronic rhinosinusitis (CRS) has focused on eosinophils. Other immune cells such as mast cells (MCs) have been identified and appear to be elevated in CRS with nasal polyp (NP) patients. MCs are commonly linked to immunoglobulin E (IgE)-mediated inflammatory changes characterized by elevated T helper 2 cytokines. Although atopy is a common comorbid condition with CRS, the objective of this study was to determine if elevated MCs are linked primarily to atopic status in CRS patients and to understand the significance of MCs in the pathophysiology of CRS.

Methods: Ethmoid sinonasal mucosa from patients undergoing endoscopic sinus surgery was harvested from 3 groups: healthy control (HC), CRS without NP (CRSsNP), and CRS with NP (CRSwNP) and analyzed by flow cytometry to quantify CD117⁺/CD203c⁺ MCs and CRTH2⁺ CD4⁺ T cells. Relative expression of prostaglandin D₂ synthase in ethmoid mucosa was determined by quantitative real-time polymerase chain reaction (PCR).

Results: MCs were significantly elevated in CRSwNP patients as compared to CRSsNP patients and HCs. This elevation was not solely dependent on the presence of IgE-mediated hypersensitivity. Relative expression of prostaglandin D₂ synthase was also increased in CRSwNP patients along with an associated presence of a CRTH2⁺ memory CD4⁺ T cell population.

Conclusion: Elevated percentages of MCs are found in the sinonasal mucosa of CRSwNP patients, regardless of atopic status. Secreted by MCs, elevated prostaglandin D₂ may play a role in the recruitment of CRTH2⁺ cells to the inflamed mucosa of CRSwNP patients © 2012 ARS-AAOA, LLC.

Key Words:

mast cells; Chronic rhinosinusitis; nasal polyps; prostaglandin D₂ synthase; CRTH2; atopy

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Chronic rhinosinusitis (CRS) describes a heterogeneous condition of chronic inflammation of the sinonasal mucosa that presents with a similar constellation of symptoms lasting for at least 3 months. Clinically, it is categorized

by the absence or presence of nasal polyps (NPs). This classification is supported molecularly by the differential expression of T helper cytokines between these 2 clinical CRS subsets.¹ Despite this understanding, the etiology and immunopathology initiating and sustaining the chronic inflammation present in CRS and its subtypes remain unclear.

Initial studies on CRS focused on eosinophils. Their prevalence within the diseased mucosa, especially in CRS with NP (CRSwNP) patients suggested that eosinophils play a central role in the chronic inflammatory response. Within these diseased states, eosinophils appear to have a number of effects including the recruitment of other inflammatory cells, damage to the epithelial cell barrier, and alteration of mucus production.² However, the clinical efficacy of anti-eosinophil therapies such as mepolizumab (an anti-interleukin (IL)-5 antibody) have had mixed success in treatment of chronic respiratory diseases, suggesting that other immune cells and pathways must be important.³

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Mast cells (MCs) are most well known for their association with allergy and immunoglobulin E (IgE)-mediated responses. Often located within the epithelial cell layer as well as near blood vessels, MCs can impact the barrier function and modulate the immune response through recruitment of other immune cells.⁴ In addition, MCs have been implicated in a number of chronic inflammatory diseases, including psoriasis, rheumatoid arthritis, and asthma,⁵ and have also been linked to tumors.⁶ Finally, MCs when activated, can release a number of proinflammatory and T helper type 2 (Th2) cytokines, including IL-4 and IL-5.

Prostaglandin D₂ synthase (PGDS) is the terminal synthase in the production of prostaglandin D₂ (PGD₂) from arachidonic acid. Two subtypes of PGDS exist, the lipocalin type and the hematopoietic type. Only the hematopoietic type has been identified in the sinonasal mucosa. PGDS levels closely correlate to PGD₂ levels. MCs are the main source of PGD₂, which is important in a number of chronic Th2 inflammatory diseases. Two receptors for PGD₂ have been characterized, including prostaglandin D₂ receptor 1 (DP1) and chemoattractant receptor homologous molecule expressed on T helper type 2 cells (CRTH2). CRTH2, also known as DP2, is a PGD₂ receptor that is found on hematopoietic cells such as eosinophils, basophils, and some T lymphocytes.

With the goal of obtaining a better understanding of CRS, we characterized the presence of MCs within the diseased sinonasal mucosa and assessed possible correlations among CRS subtypes and/or atopic status. In addition, we assessed the relative expression of hematopoietic type PGDS (h-PGDS) within the inflamed sinonasal mucosa of CRS patients and the percentage of CRTH2⁺ CD4⁺ cells to investigate the potential role of MCs in the recruitment of and activation of other effector cells.

Patients and methods

Human subjects

This study's research protocol was approved by the Institutional Review Board of The University of Texas Health Science Center at Houston. Ethmoid sinus mucosa removed during the course of a medically-indicated endoscopic sinus surgery was collected from those patients providing informed consent. Normal control tissue was taken from patients undergoing sinus surgery for benign pituitary tumor resection or undergoing cerebrospinal fluid leak repair. Diseased tissue was taken from CRS patients undergoing endoscopic sinus surgery—at least involving total ethmoidectomy—after failing medical therapy. Specimens were deidentified prior to processing in the laboratory.

Healthy controls (HCs) are defined as patients with no chronic sinonasal symptoms or objective findings on nasal endoscopy or imaging consistent with chronic rhinosinusitis or rhinitis. Definitions of CRSwNP and CRSsNP were based on the criteria outlined by The Rhinosinusitis Initiative.⁷ All CRS patients had 2 or more sinonasal symp-

toms for longer than 12 weeks with nasal endoscopic exam and computed tomography (CT) imaging consistent with paranasal sinus inflammation or obstruction. CRSwNP patients had evidence of nasal polyps either on nasal endoscopy or CT, whereas CRSsNP patients did not. Allergic fungal rhinosinusitis (AFRS), a subtype of CRSwNPs, was defined as meeting criteria for CRSwNP with the addition of eosinophilic mucin positive for the presence of fungi, documented fungal hypersensitivity, and CT imaging showing heterogeneous signal within diseased sinuses.⁷ Allergic rhinitis (AR) was defined as history consistent of inhalant allergies such as pruritus, clear rhinorrhea, sneezing, or nasal congestion with either positive skin test(s) or elevated aeroantigen-specific serum IgE levels for 20 common aeroallergens found within the Southwest United States, such as *Alternaria tenuis*, *Dermatophagoides farina*, Johnson grass, and marsh elder weed. Patients taking topical or systemic corticosteroids within 1 week of tissue harvesting were excluded from this study.

Histology

A sample of ethmoid sinus mucosa obtained at the time of surgery was placed immediately in 10% formalin and analyzed by histology. Paraffin sections of the tissue were made. Hematoxylin and eosin (H&E) stains were performed to characterize the overall inflammatory infiltrate. MCs were detected by staining with mouse anti-human mast cell tryptase monoclonal antibody clone AA1 (Dako North America, Carpinteria, CA).

Transmission electron microscopy analysis

Inflamed sinonasal mucosa was obtained from 1 CRSwNP patient, during functional endoscopic sinus surgery. The tissue was kept in chilled normal saline for a few hours and then fixed with a solution containing 3% glutaraldehyde plus 2% paraformaldehyde in 0.1 mol/L cacodylate buffer (pH 7.3) for 1 hour. After fixation, the samples are washed and treated with 0.1% Millipore-filtered cacodylate-buffered tannic acid, postfixed with 1% buffered osmium tetroxide for 30 minutes, and stained en bloc with 1% Millipore-filtered uranyl acetate. The samples were dehydrated in increasing concentrations of ethanol, infiltrated, and embedded in Spurr's low-viscosity medium. The samples were polymerized in a 70°C oven for 2 days. Ultrathin sections were cut in a Leica Ultracut microtome (Leica Microsystems Inc., Buffalo Grove, IL), stained with uranyl acetate and lead citrate in a Leica EM Stainer, and examined in a JEM 1010 transmission electron microscope (JEOL USA, Inc., Peabody, MA) at an accelerating voltage of 80 kV. Digital images were obtained using an AMT Imaging System (Advanced Microscopy Techniques Corp., Woburn, MA).

RNA extraction/reverse transcription

Harvested inflamed sinonasal mucosa tissue was quick frozen in liquid nitrogen immediately after removal in the

operating room. The tissue was stored at -80°C until RNA was extracted. Total RNA was isolated with RNeasy Mini kit (Qiagen, Valencia, CA) using the manufacturer's protocol and complementary DNA (cDNA) was synthesized using the iScript cDNA synthesis Kit (BioRad, Hercules, CA) per the manufacturer's protocol.

Real-time polymerase chain reaction

Real-time polymerase chain reaction (PCR) was performed with an ABI Prism 7500 Sequence Detection system (Applied Biosystems, Foster City, CA) using iTaq SYBR Green Supermix (BioRad) and primers at a concentration of 250 nM. Primer sequences for hemopoietic-type PGDS were forward 5'-GAGAGCAGAAATTATTCGTTAC-3' and reverse 5'-CCAGAGTGTCCACAATAGCA-3' as previously described.⁸ Reactions were incubated for 2 minutes at 50°C , denatured for 3 minutes at 95°C , and subjected to 40 two-step amplification cycles with denaturation at 95°C for 15 seconds followed by annealing-extension at 60°C for 45 seconds. A dissociation curve was run to ensure that the correct amplicon was detected. The threshold cycle (Ct) was determined for the gene of interest and the housekeeping gene β -actin and these values were used to calculate relative Ct using the following formula, relative Ct = $(1.8^{(Ct(\beta\text{-ACTIN}) - Ct(\text{UNKNOWN}))})1000000$. Relative Ct values were plotted and used to compare the relative expression level of the genes of interest.

Flow cytometry

Harvested sinonasal tissue initially placed in saline was rinsed thoroughly in Roswell Park Memorial Institute (RPMI) culture medium and incubated with Collagenase D (2 mg/mL; Roche Diagnostics, Indianapolis, IN) and DNase I (50 U/mL; Roche Diagnostics) for 60 minutes at 37°C in sterile phosphate-buffered saline (PBS) containing 0.5% bovine serum albumin (BSA), 10 mM 4-(2-hydroxyethyl)-1-piperazine ethanesulfonic acid (HEPES), 5 mM KCl, 1 mM MgCl₂, and 1.8 mM CaCl₂. Digestion was stopped by adding ethylenediamine tetraacetic acid (EDTA) at a final concentration of 2 mM. The resulting cell suspension was rinsed twice in PBS containing 1% BSA and 2 mM EDTA and filtered through a 40- μm cell strainer. Cells were resuspended in PBS containing 1% BSA and 2 mM EDTA and stained for 20 minutes on ice with allophycocyanin (APC)-labeled anti-human CD117 (clone 104D2; e-Bioscience, San Diego CA) together with either phycoerythrin (PE)-labeled anti-human CD203c (clone 97A6; Beckman Coulter Inc, Indianapolis, IN) or PE-labeled mouse IgG₁ isotype control (BD Biosciences, San Jose, CA) to analyze MCs. To analyze CRTH2⁺ T cells, cells were stained with PE-Cy7-labeled anti-CD4 (clone RPA-T4; e-Bioscience) together with APC-labeled anti-human CD45RO (clone UCHL1; BD Biosciences) and either PE-labeled anti-human CRTH2 (clone BM16; Miltenyi Biotec, Auburn, CA) or PE-labeled rat IgG_{2a} isotype control (BD Bioscience). Cells were ana-

lyzed using a FACSCalibur (BD Biosciences) and dead cells were excluded on the basis of morphology. Data were analyzed using FlowJo software (Tree Star Inc, Ashland, OR).

Statistical analysis

Statistical analysis was performed with GraphPad Prism software version 5.0 (La Jolla, CA). Data on MC and CD4⁺ T cell percentages are displayed as column graphs with error bars representing the standard error of the mean. Real-time PCR data are represented on scatter vertical plots with a horizontal line representing the median. One-way analysis of variance (ANOVA) with Tukey post test was used to compare MC and CD4⁺ T cell percentages and relative gene expression levels of PGDS among the groups. A 2-tailed *t* test was used to compare MC and CD4⁺ T cell percentages and relative gene levels of PGDS between the nonallergic and allergic groups. The significance level was set at $\alpha = 0.05$.

Results

Increased presence of MCs within inflamed sinus mucosa was most notable in CRSwNP patients, even in absence of elevated IgE-mediated hypersensitivity

To determine the presence of MCs within inflamed sinus mucosa from CRS patients, paraffin-embedded sinus mucosa tissue from HC and CRSwNP patients with no evidence of IgE-mediated hypersensitivity either on skin test or modified radioallergosorbent test (mRAST) ($n = 3$ in each group) were stained for MC tryptase. Figure 1A shows representative immunohistochemistry (IHC) slides from each group. This panel illustrates the visibly greater presence of MCs seen in CRSwNP patients, even in absence of IgE-mediated hypersensitivity.

For 1 of the CRSwNP patients examined by IHC, tissue was also treated for analysis by transmission electron microscopy. The images confirmed a significant presence of MCs along with eosinophils within the inflamed sinus mucosa (Fig. 1B). A number of MCs were identified, based on presence of round, monolobed nucleolus within cell-containing granules of various sizes and densities,⁹ in proximity to blood microvessels and the epithelium.

CRSwNP patients have elevated MCs within inflamed sinus mucosal tissue regardless of atopic status

The presence of activated MCs within diseased sinonasal mucosa from CRSwNP and CRSsNP patients was evaluated by flow cytometry. Representative data for a CRSsNP and CRSwNP patient with and without AR is shown in Figure 2A. A population of activated MCs, defined as CD117/CD203c double-positive cells, was observed in all patient tissue, even in the CRSwNP patients without AR. This population of activated MCs was small in CRSsNP

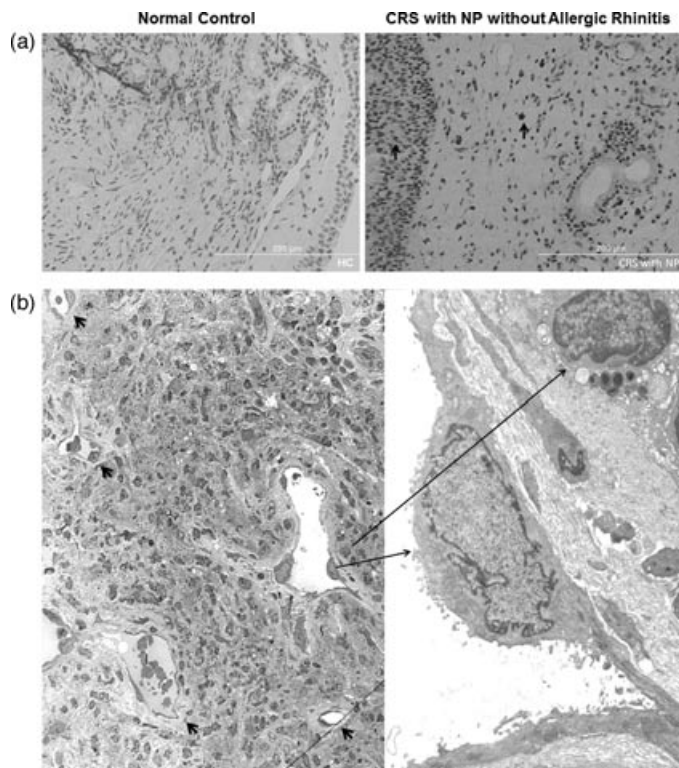


FIGURE 1. Imaging of ethmoid mucosa from HC and diseased ethmoid sinus mucosa suggest increased mast cell presence in CRSwNP patients. (A) Representative IHC stained with anti-mast cell tryptase comparing mast cell presence within ethmoid mucosa from HC and CRSwNP patient without AR at $\times 400$ magnification. Arrows indicate mast cells staining positive. (B) Transmission electron microscopy image from diseased ethmoid mucosa of a CRSwNP patient. The right image is a $\times 5000$ magnification of an overlapping area depicted on the left image at $\times 500$ magnification. The short arrowhead from the left to the right panel highlights an endothelial cell and the long arrowhead shows a representative MC, which were commonly found in close proximity to blood microvessels. AR = allergic rhinitis; CRSwNP = chronic rhinosinusitis with nasal polyps; HC = healthy controls; IHC = immunohistochemistry; MC = mast cell.

in the absence of AR as compared to CRSwNP, with or without AR.

Figure 2B graphically represents the percentage of MCs compiled from all patient tissue analyzed. Even in the presence of AR, only a small percentage of MCs (range, 0.19% to 0.76%) was observed in CRSsNP patients, which is not significantly different than the percentage of MCs observed in healthy individuals ($p > 0.05$). On the other hand, the percentage of MCs in CRSwNP patients without AR was typically 2- to 6-fold higher than in CRSsNP patients even with AR (range, 1.02% to 2.87%), and the percentage of MCs in CRSwNP patients with AR (range, 1.51% to 4%; $p < 0.05$) was significantly increased compared to HCs. Of the 7 CRSwNP with AR analyzed, 5 met criteria for AFRS. The MC percentages for the 5 AFRS patients were 1.51%, 2.5%, 2.66%, 1.86%, and 1.77%, whereas the MC percentages for the 2 CRSwNP and AR patients were 4% and 2.35%. MC percentages did not seem higher in AFRS patients relative to CRSwNP and AR. Interestingly, the percentage of MCs in CRSwNP patients with AR is significantly higher than in CRSsNP patients also with AR

($p < 0.05$). In the absence of AR, there was a trend toward higher percentages of MCs in CRSwNP patients as compared to CRSsNP.

Figure 2C graphically represents the percentage of MCs from the same CRS patients separated by status of AR, as confirmed by either skin or serum IgE level testing. There was a significantly higher percentage of MCs in the AR group as compared to the non-AR group ($p < 0.05$). However, as was observed in Figure 2B, despite the presence of AR, CRSwNP patients have a significantly higher percentage of MCs than CRSsNP patients.

PGDS messenger RNA expression upregulated in CRSwNP

To examine one downstream effect of the elevated percentage of activated MCs in CRSwNP, we examined the relative expression of h-PGDS in inflamed ethmoid sinus mucosa of CRS patients. PGDS levels closely correlate with PGD₂ levels and MCs are the predominant source of h-PGDS within inflamed sinonasal mucosa.¹⁰ Although expression of h-PGDS has been reported in other cells including dendritic cells, we found that these cells are observed at a much lower percentage ($<0.1\%$) than MCs in inflamed sinonasal mucosa (data not shown). Figure 3 illustrates the relative gene expression levels of h-PGDS within ethmoid sinus mucosa from HC, CRSsNP with and without AR, and CRSwNP with and without AR. As shown in Figure 3A, patients with CRSwNP and AR had significantly higher levels of h-PGDS expression as compared to CRSsNP with AR and HC ($p < 0.05$ and $p < 0.001$, respectively). There was also a significantly higher level of h-PGDS expression in CRSwNP without AR as compared to HC ($p < 0.05$). There is an increasing trend in the median levels of h-PGDS expression going from CRSsNP to CRSsNP and AR to CRSwNP to CRSwNP and AR. Figure 3B shows that in CRS patients the absence or presence of AR has no effect on the relative expression levels of h-PGDS.

CRSwNP patients have an elevated percentage of CD4⁺ cells, including a population of CRTH2⁺ lymphocytes

The elevated h-PGDS levels in CRSwNP patients prompted evaluation of CRTH2-positive cells. As shown in Figure 4A, analysis of T cell populations within the sinonasal mucosa by flow cytometry revealed an increased percentage of CD4⁺ T cells in CRSwNP patients (median% CD4⁺ cells = 21.65%; range, 13.5% to 28.0%) compared to CRSsNP patients (median% CD4⁺ cells = 11.3%; range, 0.51% to 21.8%) and HCs ($p < 0.01$ and $p < 0.01$, respectively). The number of CD4⁺ T cells did not correlate with atopic status, as illustrated in Figure 4B ($p = 0.86$). Subsequent analysis of the surface phenotype of the CD4⁺ T cells within diseased sinonasal mucosa of CRS patients showed that the majority of the cells are CD45RO⁺ memory cells including a population of CRTH2⁺ cells. Representative data is shown in Figure 4C.

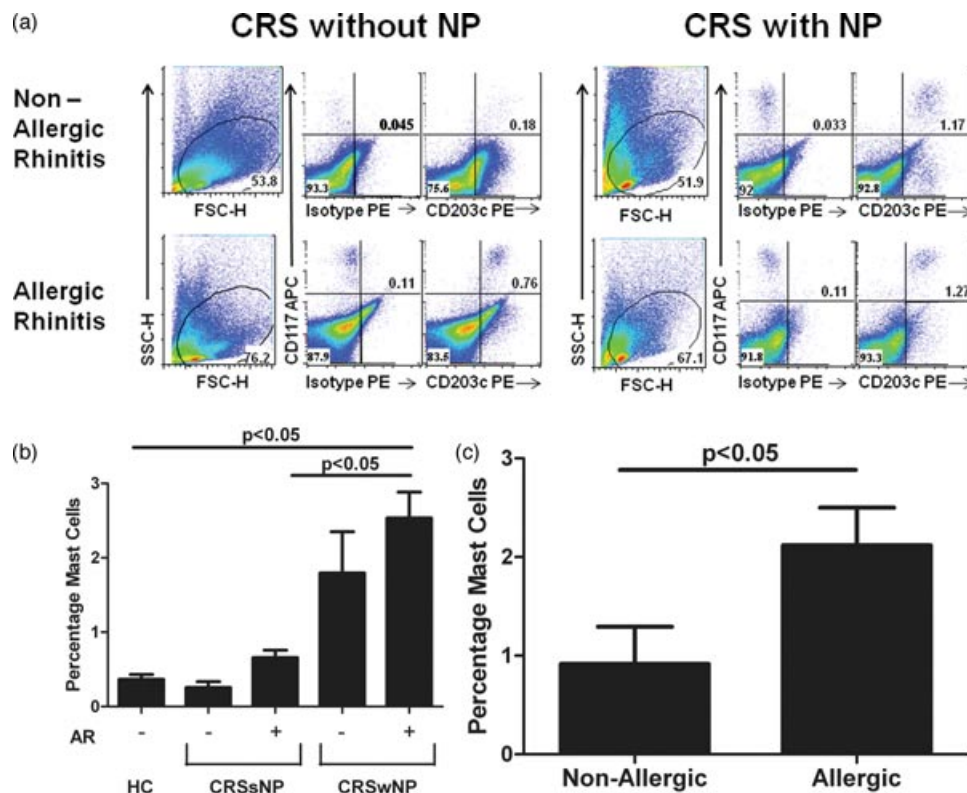


FIGURE 2. Mast cells present within ethmoid sinus mucosa of CRSwNP patients, independent of atopic status. (A) Representative FACS plots showing a population of CD117/CD203c double-positive activated mast cells in inflamed sinonasal mucosa from CRSsNP and CRSwNP patients with and without AR. (B) Percentage of mast cells within ethmoid sinus mucosa expressed as the arithmetic mean $\pm$ standard error from HC ($n = 2$), CRSsNP ($n = 4$), CRSsNP with AR ($n = 2$), CRSwNP ($n = 3$), and CRSwNP with AR ($n = 6$). (C) Percentage of mast cells within ethmoid sinus mucosa from atopic ($n = 9$) and nonatopic ($n = 7$) CRS patients. Data are expressed as the arithmetic mean $\pm$ standard error, $p < 0.05$ is considered significant. AR = allergic rhinitis; CRS = chronic rhinosinusitis; CRSsNP = CRS without nasal polyps; CRSwNP = CRS with nasal polyps; FACS = fluorescence-activated cell sorting; HC = healthy control.

Although a population of CRTH2⁺ CD4⁺ cells is observed in both CRSsNP patients and CRSwNP patients regardless of atopic status, the total percentage of CD4⁺ cells is significantly increased in CRSwNP compared to CRSsNP, indicating an increase in absolute numbers of CRTH2⁺ CD4⁺ cells in CRSwNP compared to CRSsNP.

Discussion

This study highlights that CRSwNP patients have elevated numbers of MCs within their diseased sinus mucosa, and that this is not solely dependent on the patients' atopic status. MCs are the major source of PGD₂ and this study shows a significant increase in PGDS, the enzyme responsible for its synthesis, in CRSwNP. There are 2 main receptors for PGD₂, DP1 and CRTH2. CRSwNP patients with and without AR have a distinct population of CRTH2⁺ memory CD4⁺ T cells within the diseased sinus mucosa.

MCs are most commonly associated with allergies and parasitic infections. Given the high prevalence of AR in CRS patients, the presence of MCs within sinonasal mucosa has often been thought to reflect the patient's atopic status. However, recent focus on MCs and their role in angiogenesis in tumors has lead to closer evaluation of the role of MCs in the inflammatory response.¹¹ Resid-

ing primarily in tissue that borders the environment, such as skin and mucosa, MCs play important roles in the innate immune response to many pathogens and in the adaptive immune response through their interactions with dendritic and T cells.¹² In the respiratory system, non-IgE-mediated activation of MCs is essential for bronchoconstriction and tracheal vascular hyperpermeability in the murine model of nonatopic asthma.¹³ In CRS, MCs have primarily been studied for their roles in IgE-mediated pathways. One study focusing on MCs in CRSwNP highlighted the colocalization of MCs and myofibroblasts within NPs and suggested that MCs were important in NP formation.¹⁴ Of the 12 CRSwNP patients included in the study, 7 had neither AR nor asthma.

In this study, we found that in the presence of AR where one would expect high levels of MCs, MCs were significantly more abundant within diseased ethmoid mucosa of CRSwNP patients as compared to CRSsNP patients. Although not significant, in the absence of AR, the percentage of MCs trended higher in CRSwNP vs CRSsNP patients. The signal responsible for non-IgE-mediated MC proliferation in CRSwNP is currently unknown. Stem-cell factor and IL-3 are the main growth factors identified in rodent models.¹⁵ MCs are the only terminally differentiated hematopoietic cell that expresses the receptor for

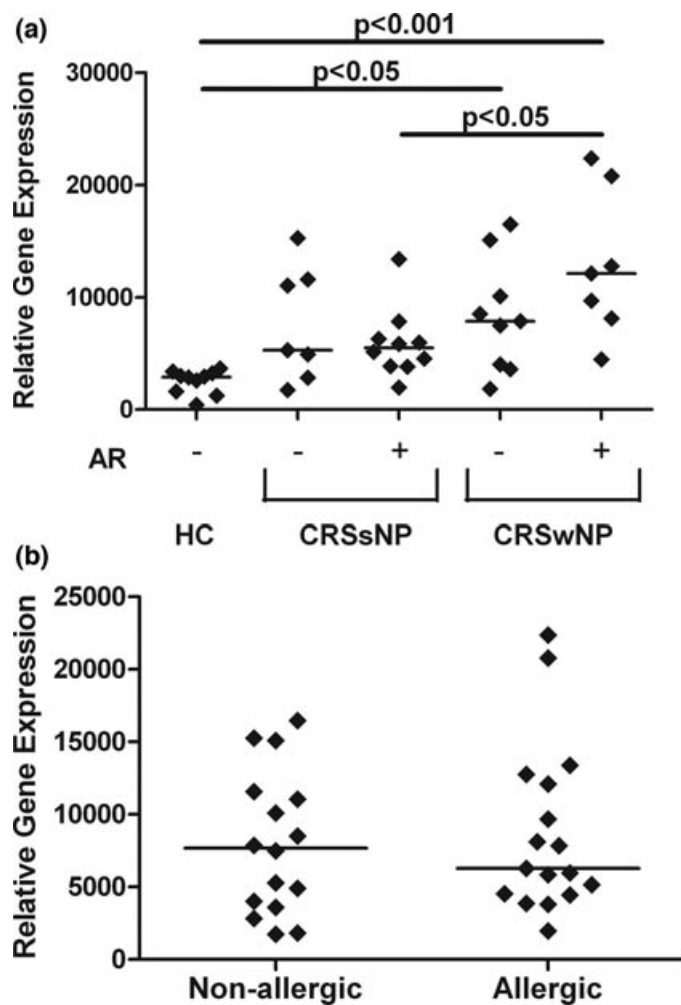


FIGURE 3. Prostaglandin D₂ synthase relative expression levels are elevated in CRSwNP patients. (A) Real-time PCR was performed with primers for h-PGDS on cDNA generated from RNA isolated from ethmoid sinus mucosa from HC (n = 10), CRSsNP (n = 7), CRSsNP with AR (n = 10), CRSwNP (n = 9), and CRSwNP with AR (n = 7). Each point represents the average of duplicate results for an individual sample and the horizontal line represents the median. Data were analyzed by 1-way ANOVA with Tukey post test, $p < .05$ was considered significant. (B) Relative expression level of PGDS comparing atopic (n = 17) and nonatopic (n = 16) CRS patients. ANOVA = analysis of variance; AR = allergic rhinitis; CRS = chronic rhinosinusitis; CRSsNP = CRS without nasal polyps; CRSwNP = CRS with nasal polyps; h-PGDS = hematopoietic type PGDS; PGDS = prostaglandin D₂ synthase.

stem cell factor, also known as c-kit. Stromal cells are the main source of c-kit, although expression of c-kit has also been observed in epithelial cells and fibroblasts within NP tissue.¹⁶ MCs express a number of surface receptors, including toll-like receptors, complement receptors, protease-activated receptors, cytokine receptors, chemokine receptors, and Fc receptors, that can respond to a variety of viral, bacterial, and fungal pathogens.¹⁵ Residing within mucosal tissue at the environmental border, MCs are poised to recognize a number of potential pathogens directly and these responses can be modulated based on the environmental cytokine and chemokine milieu.¹⁵

As in other tissues, this study showed that MCs within inflamed sinus mucosa from CRSwNP patients are associated with blood vessels and epithelial cells border (Fig. 1B). Localization near blood vessels enables MCs to effectively recruit other effector cells and alter vascular permeability to facilitate both innate and adaptive immune responses to pathogens.¹⁷ In this study, we demonstrate an increased presence of MCs in CRSwNP patients, even in those with no evidence of IgE-mediated hypersensitivity suggesting that MC host defense may play an important role in the Th2 cytokine inflammatory response characterizing CRSwNP in both IgE- and non-IgE-mediated pathways. A number of published studies have demonstrated the presence of MCs within NPs using IHC, however these studies provide conflicting results regarding the relative numbers of MCs in NPs from atopic and nonatopic individuals, with some indicating a significant difference^{10,18} and others not.^{8,19–21} Here, we have examined the percentage of MCs in inflamed ethmoid sinus mucosa using flow cytometry, a technique not employed in the previous studies, and demonstrated a significant elevation of MCs in inflamed mucosa of atopic CRSwNP patients compared to atopic CRSsNP patients supporting published data.¹⁸

To examine the downstream effects of the increased percentage of activated MCs in CRSwNP, we focused on PGD₂, the major prostaglandin produced by MCs, which functions to recruit Th2 lymphocytes, eosinophils, and basophils. MCs are the main cellular source for PGD₂ with a minor contribution from dendritic cells and T lymphocytes.¹⁸ Published data indicates a direct correlation between PGDS levels and PGD₂ levels, and MCs are the predominant cell type expressing h-PGDS.⁸ In patients with IgE-mediated hypersensitivity, significantly higher levels of h-PGDS were noted in CRSwNP vs CRSsNP. There was also a trend in patients without AR to have higher h-PGDS levels in CRSwNP vs CRSsNP. Figure 3B illustrates that h-PGDS levels are independent of IgE-mediated hypersensitivity. PGD₂ is a lipid mediator and is recognized by 2 possible receptors: D prostanoid receptor (DP) and CRTH2. CRTH2 is the most recently identified PGD₂ receptor and is specifically found on hematopoietic cells including eosinophils, basophils, and T lymphocytes.¹⁸ PGD₂ functions to recruit and activate CRTH2⁺ cells in response to MC activation, ultimately supporting the Th2 cytokine immune response characteristic of inflammatory diseases such as asthma.^{21,22}

We observed a significantly elevated percentage of CD4⁺ T cells in diseased sinonasal mucosa from CRSwNP patients compared to CRSsNP patients and HCs. Additional characterization of the CD4⁺ T cells within CRSwNP mucosa showed that the majority of these CD4⁺ cells were CD45RO⁺ memory cells, including a distinct population of CRTH2⁺ CD4⁺ T lymphocytes. The presence of this population of CRTH2⁺ CD4⁺ T cells may reflect a response to elevated PGD₂ produced by PGDS, which is expressed at elevated levels in diseased sinonasal mucosa of CRSwNP patients.

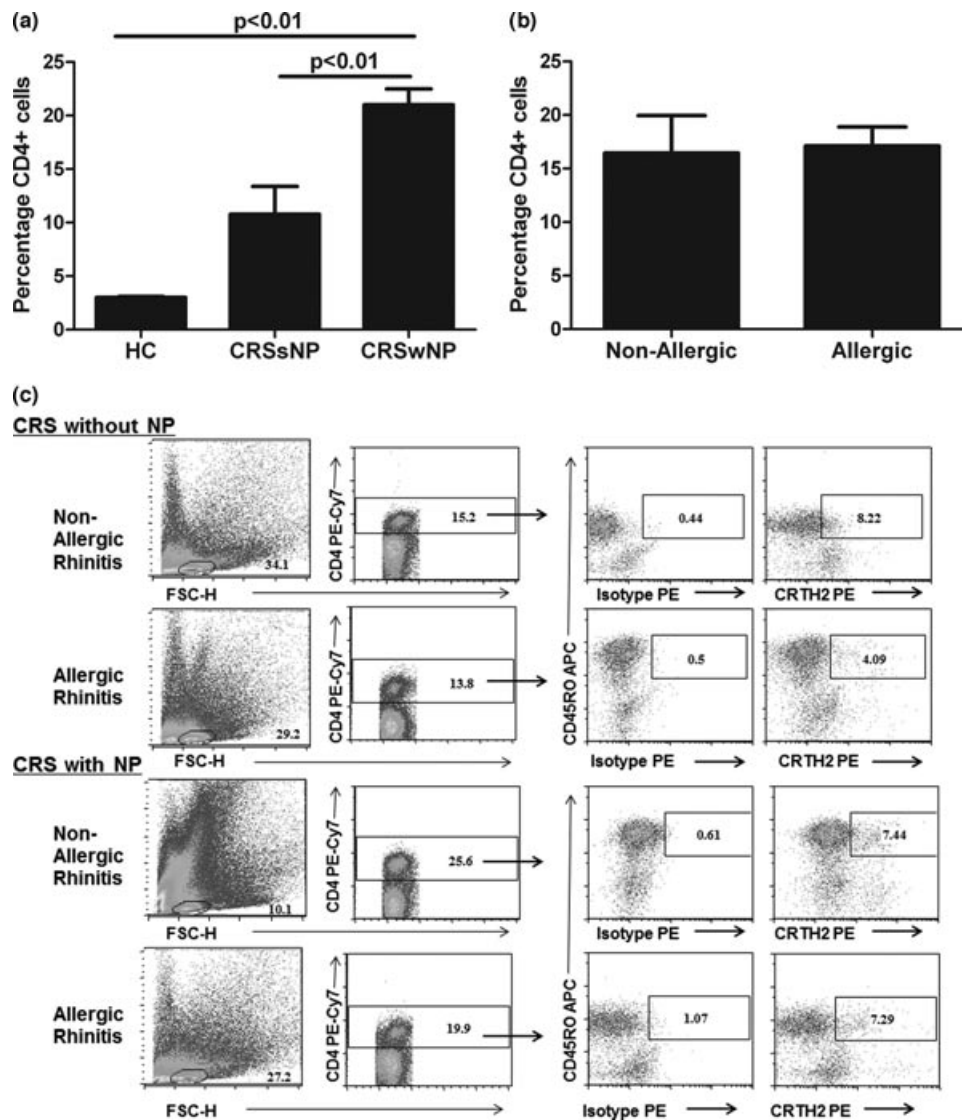


FIGURE 4. Elevated CRTH2⁺ memory CD4⁺ T cells in CRSwNP patients, even in absence of AR. (A) Percentage of CD4⁺ cells in ethmoid mucosa from HC (n = 2), CRSsNP (n = 7), and CRSwNP (n = 11). (B) Percentage of CD4⁺ cells in ethmoid mucosa from atopic (n = 10) and nonatopic (n = 8) CRS patients. Data represent arithmetic mean $\pm$ standard error, $p < 0.05$ was considered significant. (C) Representative flow cytometry plots from CRSsNP with (n = 3) and without (n = 4) AR and CRSwNP with (n = 4) and without (n = 6) AR showing a population of CRTH2⁺ CD45RO⁺ CD4⁺ T cells in CRSsNP and CRSwNP patients. AR = allergic rhinitis; CRS = chronic rhinosinusitis; CRSsNP = CRS without nasal polyps; CRSwNP = CRS with nasal polyps; HC = healthy control.

Asthma is diagnosed in up to 50% of CRS patients, primarily in patients with NPs.²³ Given the presence of CRTH2 on numerous Th2 effector cells and studies supporting a central role for CRTH2 in leukocyte recruitment, mucus production, IgE-mediated activation and production of Th2 cytokines in asthma, blocking CRTH2 represents a new potential means of treating asthma. A recent randomized, double-blinded, placebo controlled study evaluated the use of an oral CRTH2 antagonist in the treatment of moderate persistent asthma in 132 patients. In the 107 patients that were compliant with the protocol, there were significant improvements in pulmonary function, quality of life measures, and night-time symptom scores.²² This is the first clinical trial on one of several emerging synthetic CRTH2 antagonist targeted as potential treatment options for asthma, AR, chronic obstructive

pulmonary disease, atopic dermatitis, and now possibly CRS.

Future studies on MCs and CRTH2⁺ cells, including eosinophils, basophils, and T lymphocytes in the pathophysiology of CRS are clearly warranted. Similar to asthma, CRTH2 antagonist may represent a possible new therapeutic option to explore for the management of CRSwNP.

Conclusion

MCs have been recognized as important mediators of allergic inflammation. This study suggests that MCs may play an important role in the inflammation of CRSwNP even in the absence of IgE-mediated hypersensitivity. The elevated expression of PGDS in CRSwNP mucosa suggests

that activated MCs present within the diseased mucosa may produce PGD₂, resulting in recruitment and activation of CRTH2⁺ T cells. In light of recent evidence demonstrating the central role CRTH2 plays in the chronic inflammation

of asthma, atopic dermatitis, and AR, and positive data from the first clinical trial with a CRTH2 antagonist in the management of asthma, CRTH2 antagonist may represent a novel therapeutic option for patients with CRSwNP. ④

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