Role of tissue eosinophils in chronic rhinosinusitis–associated olfactory loss

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Background: **Olfactory dysfunction is 1 of the hallmark symptoms of chronic rhinosinusitis (CRS). Eosinophilic inflammation has been implicated as a potential causative factor. However, prior studies have been limited by retrospective study designs, concomitant use of systemic corticosteroids, and other confounding factors.**

Methods: **CRS and healthy non-CRS control subjects undergoing endoscopic sinus or skull-base surgery were prospectively enrolled and completed olfactory testing utilizing the 40-item Smell Identification Test (SIT) immediately prior to surgery. Histopathological evaluation of tissue excised from the ethmoid bulla was performed by a pathologist in a blinded fashion. Disease severity and patient-reported outcomes were measured via the Lund-Mackay computed tomography (CT) grading system and 22-item Sino-Nasal Outcome Test (SNOT-22), respectively. The associations between olfactory function, tissue eosinophilia, and disease severity were analyzed using Spearman rank order correlation and multiple linear regression.**

Results: **Twenty-seven (27) subjects with CRS without nasal polyps (CRSsNP), 32 subjects with CRS with nasal polyps (CRSwNP), and 10 healthy non-CRS controls were enrolled. CRSwNP was associated with higher mean tissue eosinophil counts (71.6 vs 28.1 eosinophils/high-power field [HPF], $p < 0.05$) and lower age/sex-adjusted SIT scores (-17.4 vs -6.2, $p < 0.001$) when compared to CRSsNP. SIT scores were strongly negatively correlated with tissue eosinophil counts in CRSwNP ($r = -0.60$, $p = 0.0003$), but not CRSsNP ($r = 0.16$, $p = 0.42$). The correlation between olfactory function and tissue eosinophilia in CRSwNP persisted after adjusting for disease severity.**

Conclusion: **Tissue eosinophilia is associated with olfactory loss in CRSwNP, independent of disease severity. These results suggest a possible role for eosinophils or eosinophil-associated cytokines in CRS-associated olfactory loss.** © 2017 ARS-AAOA, LLC.

Key Words:
olfaction; smell; rhinosinusitis; sinusitis; eosinophil; polyps

***How to Cite this Article*:**
Hauser LJ, Chandra RK, Li P, Turner JH. Role of tissue eosinophils in chronic rhinosinusitis–associated olfactory loss. *Int Forum Allergy Rhinol*. 2017;7:957–962.

Olfactory dysfunction is 1 of the 4 cardinal symptoms of chronic rhinosinusitis (CRS), affecting up to 84% of patients.[1,2] Loss of smell puts individuals at increased risk due to an inability to sense noxious odors such as smoke, gas, and spoiled foods, and these patients typically score lower on quality of life (QoL) surveys and have worse baseline disease severity than their normosmic counterparts.[3] However, the etiology of olfactory loss in CRS remains largely unknown. Previous studies have suggested that eosinophilic inflammation may have adverse effects on olfactory function. For example, Yee et al.[4] evaluated olfactory mucosal biopsies from CRS patients and noted higher numbers of infiltrating eosinophils in the olfactory epithelium compared to tissue from healthy controls. Likewise, a separate study found that allergic sensitization of mice with *Aspergillus fumigatus* results in elevated eosinophilic infiltration and olfactory sensory neuron apoptosis.[5]

Eosinophilic CRS (eCRS) is a particularly recalcitrant form of CRS with poor QoL outcomes and high rates of disease recurrence.[6–9] The etiology of eCRS remains controversial. Some investigators have proposed that superantigens derived from *Staphylococcus aureus* can result in polyclonal production of immunoglobulin E and subsequent tissue infiltration by eosinophils.[10,11] Others

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Funding sources for the study: NIH (National Institute of Deafness and Communication Disorders [NIDCD] RO3 DC014809 to J.H.T.; National Institute of Allergy and Infectious Diseases [NIAID] L30 AI113795 to J.H.T.; National Center for Advancing Translational Sciences [NCATS] UL1 TR000445).
Potential conflict of interest: None provided.
Presented at the ARS Meeting at the annual Combined Otolaryngology Spring Meetings (COSM) on April 26–30, 2017, San Diego, CA

Received: 2 March 2017; Revised: 5 June 2017; Accepted: 28 June 2017
DOI: 10.1002/alr.21994
View this article online at wileyonlinelibrary.com.

have proposed that local release of interleukin 5 (IL-5) and IL-13 due to fungal organisms inhabiting the sinonasal mucosa of a susceptible host can lead to unrelenting eosinophilic inflammation.[12–14] Interestingly, while eCRS is commonly associated with presence of nasal polyps, up to 40% of CRSsNP patients also have elevated tissue eosinophils.[6,7,15,16]

Tissue eosinophilia has been associated with olfactory dysfunction in a small number of clinical studies.[7,17,18] However, these studies have been largely retrospective and have often failed to account for confounding factors such as disease severity or phenotype. Soler et al.[7] found that eCRS patients (defined as >5 eosinophils/high-power field [HPF]) had lower olfaction scores than those with non-eCRS; however, smell function did not directly correlate with eosinophil counts in these patients. Of note, subjects in this study received preoperative systemic corticosteroids, which are known to promote eosinophil apoptosis,[19,20] a confounding factor that could have potentially masked the true effect of eosinophilia on olfactory function. Additional prospective studies that account for disease severity and other confounding factors are needed to help clarify the role of tissue eosinophilia on olfactory function. The objective of this prospective study was to evaluate the putative association between olfaction, tissue eosinophils, and disease severity in patients with CRS without polyps (CRSsNP) and CRS with nasal polyps (CRSwNP).

Patients and methods

Study design and population

This study was approved by the Vanderbilt University Institutional Review Board. Patients presented to the Vanderbilt Asthma, Sinus, and Allergy Program (ASAP) and Otolaryngology clinic at the Vanderbilt Bill Wilkerson Center. CRS was diagnosed according to the European Position Paper on Rhinosinusitis and Nasal Polyps and the International Consensus Statement on Allergy and Rhinology: Rhinosinusitis, and therefore patients were initially managed medically.[21,22] Patients with continued symptoms who elected to undergo endoscopic sinus surgery were prospectively enrolled. Control cases included patients undergoing pituitary or skull-base surgery without evidence of CRS. Patients were excluded if they had received systemic steroids within 4 weeks of surgery. Patients with cystic fibrosis, autoimmune, or granulomatous diseases were excluded. The presence of concomitant allergic rhinitis and asthma was recorded. Allergic rhinitis was defined as positivity on skin-prick testing or clinical history suggestive of seasonal variation in atopic symptoms with improvement with topical nasal steroids or oral antihistamine. Asthma was diagnosed according to a positive methacholine challenge test or consistent pulmonary function tests. Patient reported symptom severity was measured utilizing the 22-item Sino-Nasal Outcome Test (SNOT-22).[23]

Computed tomography

All patients underwent a high-resolution computed tomography (CT) scan of the paranasal sinuses. Each scan was evaluated by 2 authors (L.H. and J.T.) who were blinded to subject identifiers and diagnosis. A standard Lund-Mackay scoring system was used to assess overall extent of CRS. The degree of opacification in the olfactory cleft (OC) was determined according to previously published protocols[24,25] using a semiquantitative Likert scale ranging from 0 to 3 (0 = 0–24%; 1% = 25–49%; 2 = 50–74%; 3 = 75–100%). As described by other investigators,[25] the OC is the area between the middle turbinates extending from the cribriform plate to the sphenoid sinus face and spanning 1 cm below the skull base. OC opacification was assessed in 2 locations. The anterior OC was defined as the coronal plane from the anterior limit of the OC to the posterior limit of the globe and the posterior OC was defined as the coronal plane from the posterior limit of the globe to the face of the sphenoid sinus.

Olfactory testing

Subjects enrolled in the study completed the 40-item Smell Identification Test (SIT) immediately prior to surgery. The SIT has excellent sensitivity, correlates closely with scores attained via formal threshold testing, and has the advantage of being easily and quickly administered to subjects on the day of surgical intervention.[26] Raw scores were adjusted for patient age and gender by subtracting the mean normative age- and sex-appropriate SIT score from the total SIT score for each subject. Thus a negative adjusted SIT score represents reduced sense of smell compared to the mean for that subjects age and gender. Normative SIT scores were extracted from the Smell Identification Test Administration Manual (Sensonics International, Haddon Heights, NJ).

Histopathologic evaluation

Sinonasal tissue was collected from the ethmoid bulla in all patients undergoing endoscopic sinus surgery for CRS. Tissue from healthy controls was collected from either the ethmoid sinus or sphenoid face. Histopathological evaluation of excised tissue from both locations was performed by a pathologist in a blinded fashion and the mean number of eosinophils counted over 5 randomly selected HPFs was recorded. eCRS was defined by a count of more than 10 eosinophils per HPF, similar to published studies.[6,8,27,28]

Statistical analysis

The association between mean eosinophil counts and age- and sex-adjusted SIT scores was analyzed using Spearman rank order correlation because the data for each variable did not demonstrate parametric distribution and was not suggestive of a linear relationship. Bivariate linear regression was used to assess interactions between additional variables. All tests of the null hypothesis were evaluated at $\alpha = 0.05$. Analyses were performed using GraphPad Prism version 6 (GraphPad Software, La Jolla, CA).

TABLE 1. Subject demographics

	Control (n = 10)	CRSsNP (n = 27)	CRSwNP (n = 32)
Age (years), mean	48.2	46.56	46.59
Sex (male), n (%)	4 (40)	11 (40.7)	20 (62.5)
Asthma, n (%)	0 (0)	9 (33.3)	19 (59.4)
Allergic rhinitis, n (%)	2 (20)	15 (55.6)	25 (78.1)
	Non-eCRS (n = 23)	eCRS (n = 36)	
Age (years), mean	48.39	45.42	
Sex (male), n (%)	13 (56.5)	18 (50)	
CRSwNP (n, %)	8 (34.4)	24 (66.7)	
Asthma, n (%)	11 (47.8)	17 (47.2)	
Allergic rhinitis, n (%)	14 (60.9)	26 (72.2)	

CRS = chronic rhinosinusitis; CRSsNP = CRS without nasal polyps; CRSwNP = CRS with nasal polyps; eCRS = eosinophilic CRS.

Results

Subject demographics

A total of 69 subjects met inclusion criteria and were enrolled in the study, including 27 with CRSsNP, 32 with CRSwNP, and 10 healthy non-CRS controls. Demographic data is presented in Table 1. There were no differences in age or gender among the 3 groups. Control subjects had a significantly lower incidence of asthma (0.0%) and a history of positive allergy testing (20%) compared to both CRSsNP (33.3%, $p = 0.001$; and 55.6%, $p = 0.044$; respectively) and CRSwNP (59.4%, $p < 0.001$; and 78.1%, $p = 0.002$; respectively). Subjects with nasal polyps had a higher incidence of asthma than those without polyps ($p = 0.046$).

Tissue eosinophil counts and SIT scores in CRS subtypes

A total of 36 subjects (61.0%) had eCRS, defined as greater than 10 eosinophils/HPF (Table 1). Patients with eCRS were significantly more likely to have nasal polyps than non-eCRS patients (66.6% vs 54.5%, $p = 0.017$). CRSwNP was associated with higher mean tissue eosinophil counts (71.6 vs 28.1 eosinophils/HPF, $p < 0.05$) and lower age/sex-adjusted SIT scores (−17.4 vs −6.2, $p < 0.001$) when compared to CRSsNP. Controls had a mean tissue eosinophil count of 0.5 eosinophils/HPF and a mean age/sex-adjusted SIT score of −3.9, which were significantly different than CRSwNP ($p < 0.001$ and < 0.001, respectively). CRSsNP had higher eosinophils/HPF than controls ($p = 0.001$), but the difference in SIT scores between these 2 groups did not reach statistical significance ($p = 0.27$). eCRS was associated with significantly lower SIT scores compared to non-eCRS (−15.11 vs −7.80, $p = 0.011$) (Fig. 1).

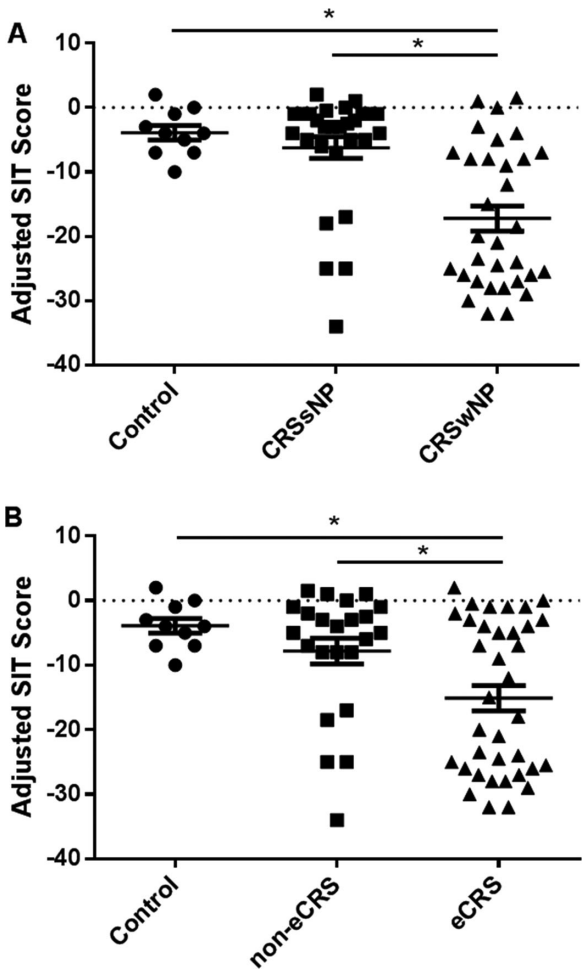

FIGURE 1. SIT scores for all study subjects. Scatter plots of age- and sex-adjusted SIT scores for (A) control, CRSsNP, and CRSwNP subjects, and (B) control, non-eCRS, and eCRS subjects. CRSwNP had significantly lower SIT scores compared to CRSsNP ($p < 0.001$) and controls ($p < 0.001$). eCRS had significantly lower SIT scores compared to non-eCRS ($p = 0.011$) and controls ($p < 0.001$). *$p < 0.05$. CRS = chronic rhinosinusitis; CRSsNP = CRS without nasal polyps; CRSwNP = CRS with nasal polyps; eCRS = eosinophilic CRS; SIT = 40-item Smell Identification Test.

Relationship between tissue eosinophilia, olfaction, and disease severity

Spearman rank correlation confirmed that there was a strong negative correlation between tissue eosinophil counts and SIT scores in CRSwNP ($r = -0.60$, $p = 0.0003$), but not CRSsNP ($r = 0.16$, $p = 0.42$) (Fig. 2). On bivariate regression, the correlation between olfactory function and tissue eosinophilia in CRSwNP persisted after adjusting for CT score ($p = 0.004$), anterior olfactory cleft score ($p = 0.002$), posterior olfactory cleft score ($p = 0.003$), and SNOT-22 score ($p = 0.009$).

Discussion

Anosmia or hyposmia is 1 of the 4 cardinal symptoms of CRS[22] and affects up to 84% of patients with CRS[1,2]; however, the etiology of this olfactory loss remains poorly defined. This study evaluated the association between tissue

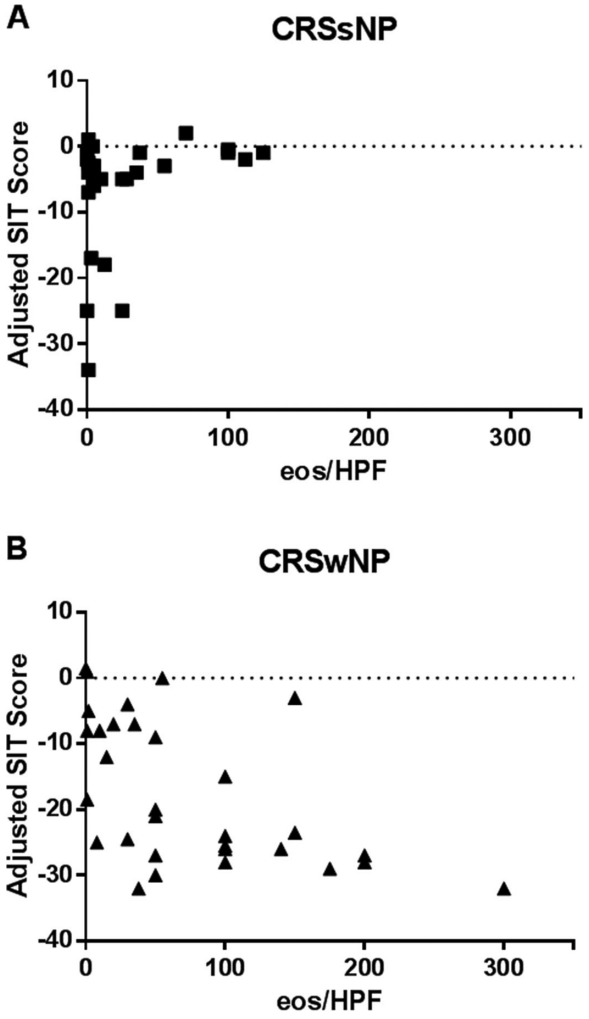

FIGURE 2. Correlation of olfaction function and tissue eosinophilia. Scatter plot of age- and sex-adjusted SIT scores compared to mean tissue eosinophil counts for (A) CRSsNP and (B) CRSwNP. There was a strong negative correlation ($R = -0.60$, $p = 0.0003$) between SIT scores and tissue eosinophil counts for CRSwNP subjects, but not for CRSsNP subjects ($R = 0.16$, $p = 0.42$). CRS = chronic rhinosinusitis; CRSsNP = CRS without nasal polyps; CRSwNP = CRS with nasal polyps; HPF = high-power field; SIT = 40-item Smell Identification Test.

eosinophilia, disease severity, and olfactory loss. We found that tissue eosinophilia was directly correlated with olfactory loss in CRSwNP, but not in CRSsNP.

In general, olfactory loss in CRS has 2 potential broad etiologies: conductive loss due to obstruction of airflow to the olfactory cleft and sensorineural loss due to cell damage at the neuronal level likely from inflammatory infiltrate. CRSwNP patients are susceptible to both etiologies due to the presence of obstructive polyps and higher levels of T-helper 2 (Th2) and other pro-inflammatory cytokines. Similar to prior studies, SIT scores were significantly lower for subjects with CRSwNP compared to those with CRSsNP in our cohort. A prior study of 445 subjects found that patients with CRSwNP had significantly worse scores on the brief smell identification test compared to those with CRSsNP or recurrent acute rhinosinusitis.[29] Olfactory function was closely associated with disease severity among patients with nasal polyps, weakly associated among those without polyps, and not associated at all in patients with recurrent acute rhinosinusitis. However, previous studies have shown that patients with more severe disease are significantly less likely to gain improvement in their sense of smell postoperatively, suggesting that removal of polyps and restoration of olfactory cleft airflow alone may not sufficiently address olfactory loss in these patients.[2,30] Conversely, continued olfactory dysfunction may persist despite polyp removal due to persistent inflammatory changes to the sinonasal cavity and olfactory cleft. Additional studies have confirmed that disease severity alone cannot consistently account for decreases in olfactory function, further arguing against a purely conductive etiology of olfactory loss in CRS patients.[8,29] Collectively, though olfactory dysfunction may be multifactorial, these findings point to a role for inflammation as a major causative factor in CRS-associated olfactory dysfunction, with tissue eosinophilia having been previously implicated.

eCRS has long been recognized as a recalcitrant subtype of CRS with increased disease severity and worse clinical outcomes. High levels of eosinophils and eosinophil-associated cytokines are more common in patients with nasal polyps, and are generally correlated with a Th2-dominant inflammatory pathway.[31–35] We found that patients with eCRS (>10 eosinophils per HPF) scored significantly worse on the SIT compared to non-eCRS and healthy control subjects. To reduce confounding factors we assured that all patients enrolled in the study had been off systemic steroids for at least 30 days and completed their SIT on the day of tissue collection. We also adjusted raw SIT scores for patient age and sex prior to data analysis. While few studies have clearly associated tissue eosinophilia with olfactory function, several have associated eCRS with indicators of disease severity, including CT and endoscopy scores.[6–8] eCRS has also been associated with high rates of polyp regrowth[9] and significantly poorer QoL outcomes compared to patients with non-eCRS.[8,28]

Our study showed a direct and strong correlation between tissue eosinophil counts and SIT scores among CRSwNP patients regardless of disease severity, suggesting that eosinophilic infiltration may adversely affect olfactory function in this patient population. We find this logical given that there is a strong body of evidence linking eosinophils and eosinophil-associated cytokines with CRSwNP.[31–35] Surprisingly, however, a correlation between olfactory function and tissue eosinophilia was not identified in subjects without nasal polyps despite a fairly high (>40%) incidence of eosinophilic disease in this patient population. A previous study by Soler et al.[7] found a weak correlation between eosinophil counts and SIT scores in CRS patients as a whole, but did not determine correlations separately for CRSsNP and CRSwNP subgroups. In addition, all patients received perioperative systemic corticosteroids that may have altered tissue eosinophil

counts and olfactory testing results. Our results confirm that eosinophilia adversely affects olfactory function in CRSwNP patients, and that smell loss is dependent on the degree of eosinophilia. The lack of a similar association in CRSsNP patients suggests that this effect may be uniquely linked to the CRSwNP inflammatory environment, and may not be due to the presence of eosinophils alone.

The mechanisms through which eosinophils affect olfaction remain unclear. Eosinophil granule proteins are known to be neurotropic and even neurotoxic, suggesting that local release of these eosinophil secretory products could affect olfactory neuron survival or regeneration.[32,36,37] However, the fact that eosinophilia does not alter olfactory function in CRSsNP patients suggests that this mechanism may be less likely, as local effects of eosinophil granule proteins would be expected to have similar effects on olfactory neurons, regardless of CRS subtype. Alternatively, the association between eosinophilia and smell loss may be secondary to the effects of eosinophil-associated cytokines. Certain cytokines have the potential to negatively modulate neuronal regeneration and the process of neurogenesis, both of which can cause transient or permanent loss of functional neurons. These effects have been previously reported for an array of cytokines that include tumor necrosis factor α (TNF-α), IL-6, and interferon γ (IFN-γ).[38–40] The potential adverse effects of TNF-α and IFN-γ on olfactory neuron function and/or survival have been confirmed in recent animal studies.[41–46] Likewise, Henkin et al.[47] found that patients with olfactory loss due to multiple etiologies had elevated plasma, saliva, and nasal mucus levels of IL-6. However, these are all nonspecific or Th-1–associated cytokines that are not closely correlated with eosinophilic inflammation. In a recent study by Schlosser et al.,[48] elevated mucus levels of IL-5 were found to be associated with reduced objective olfactory function in CRS patients. IL-5 is a well-known survival factor for eosinophils and elevated tissue levels of IL-5 are more commonly associated with CRSwNP than CRSsNP. Thus it is conceivable that the association between tissue eosinophil counts and olfactory function in CRSwNP patients could simply be a reflection of higher local levels of IL-5. Future studies that correlate olfactory function with both tissue eosinophil counts and local levels of pro-inflammatory cytokines will likely be needed to clarify the etiology of eosinophil-associated olfactory loss.

The strengths of the current study include its prospective design and use of objective olfactory testing. We excluded subjects who had received systemic corticosteroids prior to surgery and also performed olfactory testing on the day of tissue collection. We chose to use the validated SIT for olfactory testing, given its close correlation with threshold testing ($r = 0.8$ or greater) and the ability for testing to be easily administered on the day of surgery.[49] While threshold testing may have identified a small minority of patients with decreased threshold and normal identification, we feel it likely would not have significantly altered our results and likely would have required olfactory testing and evaluation of surgical tissue to be performed at different times. As decreased olfactory threshold has recently been associated with eosinophilic inflammation[50] and CRS in general,[51] inclusion of threshold testing may be considered in future studies.

Although we chose to use tissue eosinophil counts as a measure of eosinophilic inflammation, it remains possible that this assessment may not accurately reflect activated or degranulating eosinophils. For example, a previous study assessed tissue eosinophilia in CRS patients via histopathology for EG2, an antibody that recognizes eosinophil granule proteins.[52] However, many eosinophil granule proteins are not specific to eosinophils, and there is currently no consensus regarding which eosinophil granule protein may best reflect active eosinophilic inflammation. Future studies that assess both eosinophil counts, eosinophil markers, and tissue cytokine levels may help further this line of research.

Conclusion

CRSwNP and eCRS are associated with decreased objective olfactory function, and tissue eosinophil counts are directly associated with olfactory function in CRSwNP, independent of disease severity. More study is needed to further explore the role of eosinophil granule proteins or eosinophil-associated cytokines in CRS-associated olfactory loss.

Acknowledgments

The contents of this work are solely the responsibility of the authors and do not necessarily represent official views of the National Institutes of Health (NIH) or the National Center for Advancing Translational Sciences (NCATS).

References

1. Soler ZM, Mace J, Smith TL. Symptom-based presentation of chronic rhinosinusitis and symptom-specific outcomes after endoscopic sinus surgery. *Am J Rhinol*. 2008;22:297–301.
2. Thompson CF, Kern RC, Conley DB. Olfaction in endoscopic sinus and skull base surgery. *Otolaryngol Clin North Am*. 2015;48:795–804.
3. Soler ZM, Smith TL, Alt JA, et al. Olfactory-specific quality of life outcomes after endoscopic sinus surgery. *Int Forum Allergy Rhinol*. 2016;6:407–413.
4. Yee KK, Pribitkin EA, Cowart BJ, et al. Neuropathology of the olfactory mucosa in chronic rhinosinusitis. *Am J Rhinol Allergy*. 2010;24:110–120.
5. Epstein VA, Bryce PJ, Conley DB, Kern RC, Robinson AM. Intranasal *Aspergillus fumigatus* exposure induces eosinophilic inflammation and olfactory sensory neuron cell death in mice. *Otolaryngol Head Neck Surg*. 2008;138:334–339.
6. Snidvongs K, Lam M, Sacks R, et al. Structured histopathology profiling of chronic rhinosinusitis in routine practice. *Int Forum Allergy Rhinol*. 2012;2:376–385.
7. Soler ZM, Sauer DA, Mace J, Smith TL. Relationship between clinical measures and histopathologic findings in chronic rhinosinusitis. *Otolaryngol Head Neck Surg*. 2009; 141:454–61.
8. Soler ZM, Sauer DA, Mace J, Smith TL. Impact of mucosal eosinophilia and nasal polyposis on quality-of-life outcomes after sinus surgery. *Otolaryngol Head Neck Surg*. 2010;142:64–71.
9. Tosun F, Arslan HH, Karslioglu Y, et al. Relationship between postoperative recurrence rate and eosinophil density of nasal polyps. *Ann Otol Rhinol Laryngol*. 2010;119:455–459.
10. Zhang N, Gevaert P, van Zele T, et al. An update on the impact of *Staphylococcus aureus* enterotoxins in chronic sinusitis with nasal polyposis. *Rhinology*. 2005;43:162–168.

11. Bachert C, Gevaert P, Holtappels G, et al. Total and specific IgE in nasal polyps is related to local eosinophilic inflammation. *J Allergy Clin Immunol*. 2001;107:607–614.
12. Hirotsu M, Shiozawa A, Ono N, et al. Fungal extracts detected in eosinophilic chronic rhinosinusitis induced cytokines from the nasal polyp cells. *Laryngoscope*. 2014;124:E347–E353.
13. Orlandi RR, Marple BF, Georgelas A, Durtschi D, Barr L. Immunologic response to fungus is not universally associated with rhinosinusitis. *Otolaryngol Head Neck Surg*. 2009;141:750–756.
14. Shin SH, Ponihau JU, Sherris DA, et al. Chronic rhinosinusitis: an enhanced immune response to ubiquitous airborne fungi. *J Allergy Clin Immunol*. 2004;114:1469–1375.
15. Sakuma Y, Ishitoya J, Komatsu M, et al. New clinical diagnostic criteria for eosinophilic chronic rhinosinusitis. *Auris Nasus Larynx*. 2011;38:583-538.
16. Kim SY, Park JH, Rhee CS, Chung JH, Kim JW. Does eosinophilic inflammation affect the outcome of endoscopic sinus surgery in chronic rhinosinusitis in Koreans? *Am J Rhinol Allergy*. 2013;27:166–169.
17. Haruna S, Otori N, Moriyama H, Nakanishi M. Olfactory dysfunction in sinusitis with infiltration of numerous activated eosinophils. *Auris Nasus Larynx*. 2006;33:23–30.
18. Zuo K, Guo J, Chen F, et al. Clinical characteristics and surrogate markers of eosinophilic chronic rhinosinusitis in Southern China. *Eur Arch Otorhinolaryngol*. 2014;271:2461–2468.
19. Brode S, Farahi N, Cowburn AS, et al. Interleukin-5 inhibits glucocorticoid-mediated apoptosis in human eosinophils. *Thorax*. 2010;65:1116–1117.
20. Druilhe A, Létuvé S, Pretolani M. Glucocorticoid-induced apoptosis in human eosinophils: mechanisms of action. *Apoptosis*. 2003;8:481–495.
21. Fokkens WJ, Lund VJ, Mullol J, et al. European Position Paper on Rhinosinusitis and Nasal Polyps 2012. *Rhinol Suppl*. 2012;(23):3 p preceding table of contents, 1–298.
22. Orlandi RR, Kingdom TT, Hwang PH, et al. International Consensus Statement on Allergy and Rhinology: Rhinosinusitis. *Int Forum Allergy Rhinol*. 2016;6:S22–S209.
23. Hopkins C, Gillett S, Slack R, Lund VJ, Browne JP, et al. Psychometric validity of the 22-item Sinonasal Outcome Test. *Clin Otolaryngol*. 2009;111:447–454.
24. Lund VJ, Mackay IS. Staging in rhinosinusitus. *Rhinology*. 1993;31:183–184.
25. Soler ZM, Pallanch JF, Sansoni ER, et al. Volumetric computed tomography analysis of the olfactory cleft in patients with chronic rhinosinusitis. *Int Forum Allergy Rhinol*. 2015;5:846–854.
26. Doty RL, Shaman P, Kimmelman CP, Dann MS. University of Pennsylvania Smell Identification Test: a rapid quantitative olfactory function test for the clinic. *Laryngoscope*. 1984;94(2 Pt 1):176–178.
27. Barham HP, Osborn JL, Snidvongs K, Mrad N, Sacks R, Harvey RJ. Remodeling changes of the upper airway with chronic rhinosinusitis. *Int Forum Allergy Rhinol*. 2015;5:565–572.
28. Soy FK, Pinar E, Imre A, et al. Histopathologic parameters in chronic rhinosinusitis with nasal polyposis: impact on quality of life outcomes. *Int Forum Allergy Rhinol*. 2013;3:828–833.
29. Alt JA, Mace JC, Buniel MC, Soler ZM, Smith TL. Predictors of olfactory dysfunction in rhinosinusitis using the brief smell identification test. *Laryngoscope*. 2014;124:E259–E266.
30. Kim DW, Kim JY, Jeon SY. The status of the olfactory cleft may predict postoperative olfactory function in chronic rhinosinusitis with nasal polyposis. *Am J Rhinol Allergy*. 2011;25:e90–e94.
31. Vlaminck S, Vauterin T, Hellings PW, et al. The importance of local eosinophilia in the surgical outcome of chronic rhinosinusitis: a 3-year prospective observational study. *Am J Rhinol Allergy*. 2014;28:260–264.
32. Acharya KR, Ackerman SJ. Eosinophil granule proteins: form and function. *J Biol Chem*. 2014;289:17406–17415.
33. Van Zele T, Claeys S, Cevaert P, et al. Differentiation of chronic sinus diseases by measurement of inflammatory mediators. *Allergy*. 2006;61:1280–1289.
34. Tomassen P, Vandeplas G, Van Zele T, et al. Inflammatory endotypes of chronic rhinosinusitis based on cluster analysis of biomarkers. *J Allergy Clin Immunol*. 2016;137:1449–1456.
35. Dennis SK, Lam K, Luong A. A review of classification schemes for chronic rhinosinusitis with nasal polyposis endotypes. *Laryngoscope Investig Otolaryngol*. 2016;1:130–134.
36. Akasheh N, Walsh MT, Costello RW. Eosinophil peroxidase induces expression of cholinergic genes via cell surface neural interactions. *Mol Immunol*. 2014;62:37-45.
37. Gu Q, Lim ME, Gleich GJ, Lee LY. Mechanisms of eosinophil major basic protein-induced hyperexcitability of vagal pulmonary chemosensitive neurons. *Am J Physiol Lung Cell Mol Physiol*. 2009;296:L453–L461.
38. Clarke DJ, Branton RL. A role for tumor necrosis factor alpha in death of dopaminergic neurons following neural transplantation. *Exp Neurol*. 2002;176:154–162.
39. Vallières L, Campbell IL, Gage FH, Sawchenko PE. Reduced hippocampal neurogenesis in adult transgenic mice with chronic astrocytic production of interleukin-6. *J Neurosci*. 2002;22:486–492.
40. Li L, Walker TL, Zhang Y, Mackay EW, Bartlett PF. Endogenous interferon gamma directly regulates neural precursors in the non-inflammatory brain. *J Neurosci*. 2010;30:9038–9050.
41. Lane AP, Turner J, May L, Reed R. A genetic model of chronic rhinosinusitis-associated olfactory inflammation reveals reversible functional impairment and dramatic neuroepithelial reorganization. *J Neurosci*. 2010;30:2324–2329.
42. Turner JH, May L, Reed RR, Lane AP. Reversible loss of neuronal marker protein expression in a transgenic mouse model for sinusitis-associated olfactory dysfunction. *Am J Rhinol Allergy*. 2010;24:192–196.
43. Turner JH, Liang KL, May L, Lane AP. Tumor necrosis factor alpha inhibits olfactory regeneration in a transgenic model of chronic rhinosinusitis-associated olfactory loss. *Am J Rhinol Allergy*. 2010;24:336–340.
44. Pozharskaya T, Liang J, Lane AP. Regulation of inflammation-associated olfactory neuronal death and regeneration by the type II tumor necrosis factor receptor. *Int Forum Allergy Rhinol*. 2013;3:740–747.
45. Pozharskaya T, Lane AP. Interferon gamma causes olfactory dysfunction without concomitant neuroepithelial damage. *Int Forum Allergy Rhinol*. 2013;3:861–865.
46. Sultan B, May LA, Lane AP. The role of TNF-α in inflammatory olfactory loss. *Laryngoscope*. 2011;121:2481–2486.
47. Henkin RI, Schmidt L, Vellicu I. Interleukin 6 in hyposmia. *JAMA Otolaryngol Head Neck Surg*. 2013;139:728–734.
48. Schlosser RJ, Mulligan JK, Hyer M, et al. Mucous cytokine levels in chronic rhinosinusitis-associated olfactory loss. *JAMA Otolaryngol Head Neck Surg*. 2016;142:731–737.
49. Doty RL, Shaman P, Dann M. Development of the University of Pennsylvania Smell Identification Test: a standardized microencapsulated test of olfactory function. *Physiol Behav*. 1984;32:489–502.
50. Lavin J, Min JY, Lidder AK, et al. Superior turbinate eosinophilia correlates with olfactory deficit in chronic rhinosinusitis patients. *Laryngoscope*. 2017 Mar 21. https://doi.org/10.1002/lary.26555. [Epub ahead of print]
51. Whitcroft JL, Cuevas M, Haehner A, et al. Patterns of olfactory impairment reflect underlying disease etiology. *Laryngoscope*. 2017;127:291–295.
52. Kountakis SE, Arango P, Bradley D, Wade ZK, Borish L. Molecular and cellular staging for the severity of chronic rhinosinusitis. *Laryngoscope*. 2004;114:1895–1905.