

Staphylococcal Exotoxins and Nasal Polyposis: Analysis of Systemic and Local Responses

Anju Tripathi, M.D.,* Robert Kern, M.D.,# David B. Conley, M.D.,# Kristin Seiberling, M.D.,# Julie C. Klemens, M.D.,* Kathleen E. Harris, B.S.,* Lydia Suh, B.S.,* Jie Huang, Sc.D.,§ and Leslie C. Grammer, M.D.*

ABSTRACT

Background: Staphylococcal exotoxins have been implicated in the pathogenesis of several chronic inflammatory diseases including atopic dermatitis (AD), asthma, and, most recently, chronic rhinosinusitis with nasal polyposis (CRS/NP). In severe AD, these toxins act both as superantigens (SAg), triggering massive T-cell activation, and as conventional allergens, triggering toxin-specific immunoglobulin E (IgE) in the serum. In CRS/NP, evidence for both processes has been reported but it is unclear whether these processes are linked. The aim of this study was to correlate SAg activity as inferred by staphylococcal-specific T-cell receptor (TCR) V- β expansion in the polyp and blood of CRS/NP patients with staphylococcal-specific anti-IgE antibodies in the serum.

Methods: IgE antibodies to staphylococcal exotoxin A (SEA), staphylococcal exotoxin B (SEB), and toxic shock syndrome toxin (TSST) 1 were measured in the serum of 12 individuals with CRS/NP before functional endoscopic sinus surgery. Flow cytometry was used to analyze the SEA, SEB, and TSST-1–

specific TCR V- β domains on the T cells from the polyp and blood of these patients.

Results: Serum SEA-, SEB-, and TSST-1–specific IgE antibodies were detected in 0/12 (0%), 6/12 (50.0%), and 9/12 (75%) of CRS/NP patients, respectively. Evidence of SAg effect in the polyp lymphocytes (TCR V- β expansion in both CD4⁺ and CD8⁺ subsets) was noted in 7/12 (58.3%) patients. Five of 6 CRS/NP patients had overlapping evidence of a systemic IgE response and TCR V- β expansion, suggestive of exposure to the same exotoxin. No patients had evidence a SAg effect in blood lymphocytes. Nine of 12 subjects also had coexistent asthma.

Conclusion: These results provide evidence for a local SAg effect in 7/12 (58.3%) polyp patients and establish a positive correlation of V- β expansion with the presence of corresponding toxin-specific IgE in the serum. (American Journal of Rhinology 19, 327–333, 2005)

From the *Division of Allergy-Immunology, #Department of Otolaryngology–Head and Neck Surgery, and §Department of Preventive Medicine, Northwestern University Feinberg School of Medicine, Chicago, Illinois

Supported by the Ernest S. Bazley Grant to Northwestern Memorial Hospital and Northwestern University

Address correspondence and reprint requests to Anju Tripathi, M.D., Division of Allergy-Immunology, 676 North Saint Clair, No. 14020, Chicago, IL 60611

Copyright © 2005, OceanSide Publications, Inc., U.S.A.

Recent literature has suggested a potential role for staphylococcal exotoxins in the etiology and pathogenesis of chronic rhinosinusitis with nasal polyposis (CRS/NP).^{1–4} In other chronic inflammatory disorders such as asthma and atopic dermatitis (AD), these exotoxins have a more established role in the disease process.^{5–8} These toxins have a dual mode of action in disease pathogenesis, having the capacity to act as both conventional allergens and superantigens (SAg). As SAg, staphylococcal exotoxins have the capacity to activate large populations (5–20%) of T cells by bypassing normal antigen processing in antigen-presenting cells (APCs).⁹ In contrast to conventional antigens, which are processed inside the APCs and presented to T cells on the peptide groove on the major histocompatibility complex (MHC), SAg are not processed inside APCs, binding MHC

outside of the peptide binding groove. In addition, SAg bind the β -domain of the variable region (V- β) of selected T-cell receptor (TCR) on the surface of the lymphocytes, resulting in an effective "clamp" between the APC and the T cell.^{9,10} Because the number of distinct V- β domains is limited to ~50 and most SAg can bind more than one, up to 30% of a patient's T cells can be stimulated. Distinct SAg only bind certain V- β domains; therefore, a characteristic "clonal signature" of lymphocyte proliferation has been assigned to each SAg.¹¹ Because SAg activation of lymphocytes is not classically MHC restricted, both CD4⁺ and CD8⁺ T cells can proliferate in response to SAg binding. Therefore, evidence for SAg activity is most convincing when SAg-specific TCR V- β expansion occurs in both CD4⁺ and CD8⁺ T cells.^{9,12,13} In addition to classic SAg effects, SAg may act as conventional allergens, inciting a specific immunoglobulin E (IgE) response in the serum.^{6,12,14,15} The presence of serum IgE to these SAg is associated with higher severity of disease in AD and asthma, because the conventional allergic reaction is believed to augment the local SAg-mediated inflammation.^{6,7,8,16}

The first study suggesting a role for staphylococcal toxins in CRS/NP patients showed toxin-specific IgE in polyp homogenates of 10 of 20 patients.² Later results indicated specific IgE in the serum of a high percentage of CRS/NP patients.^{4,17} In parallel with these later studies came the first indications of a classic SAg effect in the lymphocytes of polyp patients.^{1,18} These reports showed expansion of toxin-specific TCR V- β domains in the polyp lymphocytes of CRS/NP patients. Although these studies collectively support a role for staphylococcal SAg in CRS/NP, it remains unclear whether the conventional allergen response and the classic SAg response are both present in the same patient and whether this concordance correlates with disease severity.

In this study, we used flow cytometry to analyze the staphylococcal toxin-specific TCR V- β repertoire from polyps and blood of CRS/NP patients to assess differences between these lymphoid compartments. This phenotypic analysis serves as a surrogate for systemic and local SAg responses to specific toxins. In addition, the presence of toxin-specific IgE in the serum was concurrently assessed using enzyme-linked immunosorbent assay. The expansion of lymphocytes bearing SAg-specific V- β domains in polyp tissue and peripheral blood of CRS/NP patients then was correlated with the presence of toxin-specific anti-IgE antibodies in serum. We observed a correlation between systemic and local responses of specific SAg in CRS/NP patients.

METHODS

Patients

Twelve patients with CRS/NP undergoing functional endoscopic surgery were included in the study. CRS was defined based on the guidelines established by the American Academy of Otolaryngology-Head and Neck

Surgery Task Force for Defining Adult Chronic Rhinosinusitis.¹⁹ All subjects underwent endoscopy and sinus computed tomography scan confirming CRS/NP. Blood was collected from these individuals on the day of the surgical procedure. Details of patients' characteristics are included in Table I. All individuals were skin tested to pollens (tree, grass, and ragweed), dust mites, cats, dogs, and molds with Hollister-Stier Canada Co. (Toronto, Ontario) extracts. A positive skin test response was defined by a wheal of 3 mm or greater in size than that produced by the saline control. Histamine was used as a positive control. Atopic individuals had at least one positive skin reaction to the extracts with the prick-puncture technique. Nine of the individuals had asthma requiring inhaled corticosteroids and as-needed albuterol. Asthma severity ranged from mild to severe disease. None of the subjects had AD. Informed consent was given by the patients before the procedure, and the study was approved by the Institutional Review Board of Northwestern University Feinberg School of Medicine.

Toxin-Specific Anti-IgE Antibodies

IgE to staphylococcal exotoxin A (SEA), staphylococcal exotoxin B (SEB), and toxic shock syndrome toxin (TSST) 1 in the serum was determined by Toxin Technology, Inc. (Sarasota, FL) as previously reported.^{14,20-22}

Preparation and Staining of Polyp and Blood Lymphocytes for V- β Flow Cytometric Analysis

To prepare a cell suspension from the polyp tissue, Roswell Park Memorial Institute (RPMI) was added to the tissue. The tissue was minced into small pieces using a scalpel blade to dissociate the cells. The cell suspension was transferred to a conical tube where the larger tissue pieces were allowed to settle to the bottom. Then, the cell

TABLE I

Clinical Characteristics of Subjects with CRS/NP

Patient	Age	Sex	Allergic Rhinitis	Asthma	Aspirin Sensitivity
1	60	M	No	Yes	No
2	35	F	Yes	Yes	No
3	55	F	Yes	Yes	No
4	58	F	No	Yes	Yes
5	44	M	Yes	No	No
6	40	F	Yes	Yes	Yes
7	40	M	Yes	Yes	No
8	65	M	Yes	No	No
9	36	F	No	Yes	Yes
10	36	M	Yes	Yes	No
11	41	F	No	Yes	No
12	54	M	Yes	No	No

M = male; F = female.

suspension was aspirated carefully to another conical tube and centrifuged for 10 minutes at 1500 rpm. After discarding the supernatant as waste, 5 mL of 1× ammonium chloride lysing solution was added to the cell pellet to lyse the red blood cells by incubation for 10 minutes at room temperature. The cells then were centrifuged for 5 minutes at 1500 rpm. After discarding the supernatant, the cell pellet was suspended in 5 mL of phosphate-buffered saline (PBS)–2% fetal bovine serum (FBS) solution and centrifuged for 5 minutes at 1500 rpm. The wash procedure was repeated a second time after a cell count was performed. Cells were then resuspended in PBS–2% FBS at a final cell concentration of between 5 and 10 × 10⁶/mL. The cell suspension was aliquoted into test tubes at 1 million cells (100 μL) per tube and stained with conjugated antibodies using a commercially available TCR V-β kit (IO Test, Beta Mark; Immunotech/Beckman Coulter, San Jose, CA). The 1 × 10⁶ cells were aliquoted into each tube and vortexed after conjugated antibody was added per the manufacturer instructions. The antibody/cell suspension was incubated for 30 minutes at room temperature in the dark. Excess antibody was removed by washing twice with PBS–2% FBS solution before fixing the cell with 0.5 mL of 0.5% paraformaldehyde.

A similar protocol for staining whole blood was followed. One hundred microliters of whole blood was aliquoted into individual test tubes and stained with antibody according to the manufacturer's instructions. The blood/antibody suspension was incubated for 15 minutes at room temperature in the dark. Two milliliters of 1× ammonium chloride solution was added to each tube to lyse the red blood cells. The tubes were incubated for 10 minutes at room temperature in the dark. The tubes were centrifuged for 5 minutes at 1500 rpm and the supernatant was decanted. After washing twice with 1 mL of PBS–2% FBS solution as previously described, the cells were resuspended in 0.5 mL of 0.5% paraformaldehyde.

The samples were assayed for antibody activity using a Beckman Coulter Elite ESP flow cytometer using laser excitation at 488 and 633 nm (beams spatially separated in time). The optical filter setup for fluorescence detection used 550, 600, and 645 nm dichroic long pass filters, and 525, 575, 620, and 675 nm band pass filters. Analysis software used was Elite 4.0 (Coulter, Miami, FL). T cells from blood and polyp tissue were assessed for evidence of clonal expansion of V-β domains 1, 2, 3, 5.3, 12, 17, 20, and 23 because these are known to be associated with SEA, SEB, and TSST-1 (Table II).^{9,10,13} Cells were first gated on light scatter on a CD3 marker and then further gated by CD4 and CD8 markers. The results were compared with normative data on the expected distribution of specific V-β domains in healthy control patients (IOTest Beta Mark PN IM3497, 5/2000; Beckman Coulter, Miami, FL). V-β expansion was defined as a percentage of cells >2 SD above the normal mean.

TABLE II

Staphylococcal Toxins and Their Respective V-β Motifs Assessed in the Study

Staphylococcal Toxin	Associated V-β(s)
SEA	1, 5.3, 23
SEB	1, 3, 12, 17, 20
TSST-1	2

Statistical Analysis

We computed the Spearman's correlation between the staphylococcal toxin-specific IgE antibodies in the serum and proliferation of V-β domains in the polyp and blood lymphocytes. The corresponding 95% CIs were constructed.

RESULTS

Serum IgE against the Staphylococcal Toxins

SEA-, SEB-, and TSST-1-specific IgE antibodies in the serum were detected in 0, 6 (50.0%), and 9 (75%) patients, respectively (Table III).

Blood

TCR V-β Repertoire by Flow Cytometry and Correlation to Serum Toxin Specific-Anti-IgE Antibodies. Four of 12 (33.3%) patients had expansion of SEA-, SEB-, and/or TSST-1-specific TCR V-β-bearing T-cell subsets in their peripheral blood by flow cytometry, which were increased above the range of normal control subjects (more than or equal to the mean + 2 SD). However, of these four patients none had elevation of a given V-β subset in both the CD4⁺ and the CD8⁺ populations (Table III). In all cases, an elevation was present in either the CD4⁺ or the CD8⁺ subset, but not for both, suggesting a conventional antigen response rather than an SAg-driven proliferation.^{9,12,13}

Of the two patients with the expansion of SEB-specific V-β domains in the CD4⁺ subset from the blood, both had detectable staphylococcal toxin-specific IgE to SEB in the serum. The expansion of V-β subsets of T cells was positively correlated with the presence of staphylococcal-specific IgE antibodies in the serum with the Spearman correlation coefficient of 0.53 (95% CI = 0.18, 0.88). Similarly, the expansion of TSST-1 specific V-β domains in the CD4⁺ subset from the blood and the presence of IgE to TSST-1 in the serum were positively correlated. (Spearman's correlation coefficient of 0.36 (95% CI = 0.01, 0.71)).

Polyp Tissue

TCR V-β Repertoire by Flow Cytometry and Correlation to Serum Staphylococcal-Specific Anti-IgE Antibodies. Staphylococcal toxin-specific TCR V-β expansions were observed in 9 of 12 (75%) and 11 of 12 (91.7%) of the CD4⁺ or CD8⁺ subsets, respectively. Evidence of superan-

TABLE III

Presence of IgE to SEA, SEB, and TSST-1 in the Serum of Patients with CRS/NP and V- β Clonal Expansion in the Blood and Polyp T Cells

Patient No.	IgE to SEA	IgE to SEB	IgE to TSST-1	Polyp CD8 (V- β)	Polyp CD4 (V- β)	Blood CD8 (V- β)	Blood CD4 (V- β)
1	—	—	+	A5.3, B17, T2	—	—	—
2	—	—	—	A1 B1	A23, A5.3	—	—
3	—	—	—	A5.3 A23	A23 —	—	—
4	—	—	+	—	—	B1, A1	—
5	—	+	+	A1 B1	A1, A5.3 B1, T2	—	—
6	—	—	+	B17	A5.3, B17, T2	—	T2
7	—	+	—	B17	B3, B17	—	B17
8	—	+	+	A5.3, B17, T2	A5.3, B17, T2	—	—
9	—	+	+	A5.3, B12	B12	—	—
10	—	+	+	B20	B20	—	—
11	—	+	+	A1, B1	—	—	B3
12	—	—	+	B12	A23, T2	—	—

The V- β domains associated with the particular toxins are prefixed with "A" for SEA, "B" for SEB, and "T" for TSST-1.

TABLE IV

Correlation of SEB-Specific TCR V- β Expansion in the Polyp CD4⁺ and CD8⁺ Subsets with the Presence of the SEB-Specific Anti-IgE Antibody in the Serum

	TCR V- β CD4 ⁺		TCR V- β CD8 ⁺		TCR V- β CD4 ⁺ and CD8 ⁺	
	Positive (No. of Patients)	Negative (No. of Patients)	Positive (No. of Patients)	Negative (No. of Patients)	Positive (No. of Patients)	Negative (No. of Patients)
SEB IgE ⁺	5	1	6	0	5	1
SEB IgE ⁻	1	5	4	2	1	5
Spearman's Correlation Coefficient (95% CI)	0.67 (0.24, 1.00)		0.45 (0.13, 0.77)		0.67 (0.24, 1.00)	

tigenic stimulation (expansion in both CD4⁺ and CD8⁺ cells for the same V- β domain) was observed in 7 of 12 (58.3%) individuals. Five of the six patients with SEB-specific TCR V- β in both CD4⁺ and CD8⁺ subsets (superantigenic stimulation) showed concomitant presence of SEB-specific IgE in the serum. This was positively correlated with the Spearman's correlation coefficient of 0.67 (0.24, 1.00). Only one subject had TSST-1-specific TCR V- β expansion in both CD4⁺ and CD8⁺ subsets in the polyp tissue. In this individual, TSST-1-specific IgE was positive in the serum. This was positively correlated with the Spearman's correlation coefficient of 0.17 (−0.02, 0.37).

Of the six patients with the expansion of SEB-specific V- β domain in the CD4⁺ subset from the polyp, five

patients had detectable staphylococcal toxin-specific IgE in the serum. This was positively correlated with the Spearman's correlation coefficient of 0.67 (95% CI = 0.24, 1.00). Six of the 10 individuals (60%) with SEB-specific TCR V- β expansion in the CD8⁺ subsets had the corresponding IgE in the serum. This was positively correlated as well (Table IV). Similarly, TSST-1-specific TCR V- β expansion was positively correlated with the presence of TSST-1-specific IgE in the serum in both CD4⁺ and CD8⁺ subsets (Table V).

DISCUSSION

This study evaluates two potential immunomodulatory mechanisms by which staphylococcal toxins may play a role in the pathogenesis of CRS/NP (Fig. 1). First, we assessed polyps and blood of CRS/NP patients for evidence

TABLE V

Correlation of TSST-1-Specific TCR V- β Expansion in Polyp CD4⁺ and CD8⁺ Subsets with the Presence of TSST-1-Specific Anti-IgE Antibody in the Serum

	TCR V- β CD4 ⁺		TCR V- β CD8 ⁺		TCR V- β CD4 ⁺ and CD8 ⁺	
	Positive (No. of Patients)	Negative (No. of Patients)	Positive (No. of Patients)	Negative (No. of Patients)	Positive (No. of Patients)	Negative (No. of Patients)
TSST-1 IgE ⁺	4	5	2	7	1	8
TSST-1 IgE ⁻	0	3	0	3	0	3
Spearman's Correlation Coefficient (95% CI)	0.41 (0.13, 0.69)		0.26 (0.04, 0.48)		0.17 (-0.02, 0.37)	

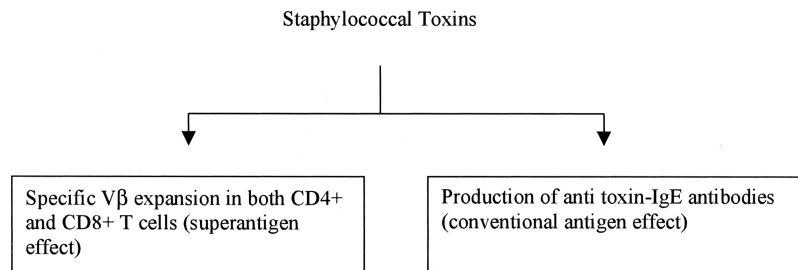


Figure 1. Two pathways by which staphylococcal toxins have been hypothesized to mediate inflammation in CRS/NP patients.

of a SAg response by measuring specific TCR V- β expansion. Second, we measured staphylococcal toxin-specific anti-IgE antibodies in the serum to assess for the conventional pathway of antigenic stimulation. In addition, we assessed the relationship between these two host responses to staphylococcal toxins. Specifically, we addressed the question of concordance of these two responses, systemic IgE and V- β , skewing for the same SAg.

The presence of staphylococcal-specific TCR V- β expansion was seen in 11 of 12 polyp specimens and 4 of 12 blood samples in this series. However, only 7 of 12 polyp samples and 0 of 12 blood samples showed expansion of both CD4⁺ and CD8⁺ lymphocytes subsets. Expansion of both T-cell subsets is considered the strongest evidence of a SAg response.^{9,12,13} Two earlier reports showed expansion of the corresponding TCR V- β region from polyp lymphocytes in CRS/NP patients but neither of these earlier reports documented expansion in both subsets.^{1,18} This study, using the more stringent criteria, shows evidence of an SAg effect in polyp lymphocytes of 58.3% of the CRS/NP patients. No strong evidence of a SAg effect is seen in the blood. The presence of TCR V- β skewing in polyp lymphocytes and the absence of this response in the peripheral blood suggests that the SAg exposure is occurring locally at the nasal and paranasal mucosa in this subset (7 of 12) of CRS/NP patients. Of the seven patients, one showed a signature profile suggestive of SEA, one patient showed a profile suggestive of TSST-1, and five patients showed a profile suggestive of SEB exposure.

Bacterial SAg-mediated chronic T-cell inflammation has been studied more extensively in AD and asthma. In AD, SAg facilitate homing of T cells to the site of inflammation in the skin by up-regulating expression of a skin homing receptor, cutaneous lymphocyte-associated antigen, expression on both CD4⁺ and CD8⁺ T cells.^{23,24} The number of cells with staphylococcal-related TCR V- β expression is increased in circulating cutaneous lymphocyte-associated antigen-positive lymphocytes as well as in T lymphocytes from affected skin lesions of AD patients, implying a staphylococcal SAg mechanism.^{5,23-25} Association of SAg and T-cell-mediated inflammation in asthma is suggested by the report of an increase in V- β domain 8 expression in T cells (both CD4⁺ and CD8⁺ subsets) in the bronchoalveolar lavage fluid of poorly controlled asthmatic patients.²⁶ In a mouse asthma model, intranasal SEB exposure induces inflammation of lower airways characterized by marked leukocyte infiltration.²⁷ Although not studied in CRS patients, the evidence for SAg-induced eosinophilic inflammation is supported by studies that show high levels of interleukin-5 and interleukin-13 secretion by SAg-stimulated skin T cells from AD patients.²⁸⁻³⁰ These cytokines are known to enhance eosinophil survival and suppress eosinophil apoptosis.²⁸⁻³⁰

In asthma and AD, evidence for the conventional pathway of allergic stimulation by staphylococcal toxins has been observed.^{8,14} Staphylococcal toxin-specific IgE antibodies have been shown to release histamine from basophils isolated from sensitized AD patients.¹⁴ In addition, staphy-

lococcal toxins augment IgE response against aeroallergens such as pollens. Hofer *et al.* have shown that staphylococcal toxins enhance inhalent-specific IgE production by B cells in atopic patients. TSST-1 can influence IgE regulation by inducing expression of B7.2, a costimulatory molecule important in IgE synthesis.¹⁵ The impact of staphylococcal toxins on IgE-mediated inflammation in AD suggests that analogous mechanisms can occur in CRS because these diseases share similar inflammatory mechanisms. Although CRS/NP can occur in individuals with and without aeroallergen sensitivity, studies have indicated that atopy is a risk factor for more extensive disease in CRS and higher radioallergosorbent scores are associated with worse disease on sinus computed tomography scans.^{31,32} Indirect evidence for involvement of staphylococcal toxins in IgE-mediated inflammation in CRS is provided by the studies that have established IgE to the specific toxins in the serum and polyp tissue.^{2,21} Bachert *et al.* have shown that eosinophilic inflammation is positively correlated with enterotoxin-specific IgE in the polyp tissue.² In another study, there was a trend, although not significant, toward an increase in numbers of eosinophils in the polyp tissue of individuals with staphylococcal-specific IgE in the serum.¹⁷ It may be that the extensive mast-cell degranulation observed in the nasal polyps is mediated *via* staphylococcal toxins, as observed in AD.³³ Although the exact mechanism is unclear, it appears that there is a correlation between the IgE antibodies to staphylococcal toxins and the eosinophilic inflammation seen in some individuals with polyps. Finally, serum IgE to *Staphylococcus aureus*-derived SAg is associated with higher severity of disease in AD and asthma; however, this has not been established in CRS.^{6,7,16}

The current data illustrate that CRS/NP patients may have staphylococcal specific-TCR V- β expansion in the polyp tissue, suggesting an SAg reaction as well as a systemic IgE-mediated conventional response. In this study, we found that the systemic IgE-mediated response to the SAg is positively correlated to the TCR V- β response observed in the polyps. This association suggests that the local T-cell inflammation is linked to the systemic IgE-mediated inflammatory process. However, there was no significant correlation between systemic TCR V- β expansion and TCR V- β expansion in the polyp lymphocytes. These observations suggest a higher level of T-cell activity at the site of the SAg exposure. Previously, others have correlated the presence of staphylococcal toxin-specific IgE antibodies in the polyp to local IgE production and eosinophilic inflammation in the polyp tissue.^{2,34} It has been hypothesized that locally produced IgE antibodies, without systemic IgE sensitization, drive the Th2-type inflammation observed in the sinus tissue.³⁵ SAg have been suggested as possible triggers for local IgE production. We now suggest that sinus inflammation may be correlated with systemic IgE sensitization to the staphylococcal toxins as well.

The portal of entry of the microbial toxins may determine whether the response is systemic or local. For example, in

AD, toxin release and skin injury from colonized *S. aureus* is believed to be responsible for the serum IgE response. In our patients, the source of the serum IgE is not apparent. A pulmonary source should be considered because Bachert *et al.* have previously established staphylococcal-specific IgE antibodies in the serum of asthmatic patients. Thus, coexistent asthma may promote the appearance of staphylococcal-specific IgE in the serum in our patients.⁸ Given the high concordance between asthma and CRS/NP, separate analysis of a subgroup with asthma alone was not conducted. However, in this study it is worth noting that two of three patients without asthma had staphylococcal-specific IgE in the serum. Others have also documented IgE antibodies to the staphylococcal toxins in patients with allergic rhinitis; therefore, it is plausible that the nasal passages are responsible for the systemic IgE response.³⁶

In conclusion we have observed significant TCR V- β expansion in the polyp T lymphocytes compared with circulating lymphocytes. The expansion in the polyp tissue may be secondary to persistent mucosal exposure to microbial agents such as *S. aureus*. Furthermore, there was significant correlation between TCR V- β expansion in the polyp lymphocytes and presence of serum IgE to the staphylococcal toxins. Our study is limited by the small number of patients. Future studies are needed to correlate changes in the TCR repertoire in the polyp tissue and the presence of serum and local staphylococcal-specific IgE antibodies. The use of objective measures of inflammation and disease severity in a larger cohort of patients will be helpful in the assessment of functional significance of these findings in CRS/NP.

REFERENCES

1. Bernstein JM, Ballou M, Schlievert PM, et al. A superantigen hypothesis for the pathogenesis of chronic hyperplastic sinusitis with massive nasal polyposis. *Am J Rhinol* 17:321–326, 2003.
2. 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* 107:607–614, 2001.
3. Schubert MS. A superantigen hypothesis for the pathogenesis of chronic hypertrophic rhinosinusitis, allergic fungal sinusitis, and related disorders. *Ann Allergy Asthma Immunol* 87:181–188, 2001.
4. Tripathi A, Conley DB, Grammes LC, et al. Immunoglobulin to staphylococcal and streptococcal toxins in patients with chronic sinusitis/nasal polyposis. *Laryngoscope*. 114(10):1822–1826, 2004.
5. Skov L, Olsen JV, Giorno R, et al. Application of staphylococcal enterotoxin B on normal and atopic skin induces up-regulation of T cells by a superantigen-mediated mechanism. *J Allergy Clin Immunol* 105:820–826, 2000.
6. Bunikowski R, Mielke M, Skarabs H, et al. Prevalence and role of serum IgE antibodies to the *Staphylococcus aureus*-derived superantigens SEA and SEB in children with atopic dermatitis. *J Allergy Clin Immunol* 103:119–124, 1999.
7. Bachert C, Gevaert P, and van Cauwenberge P. *Staphylococcus aureus* enterotoxins: A key in airway disease. *Allergy* 57:480–487, 2002.
8. Bachert C, Gevaert P, Howarth P, et al. IgE to *Staphylococcus aureus* enterotoxins in serum is related to severity of asthma. *J Allergy Clin Immunol* 111:1131–1132, 2003.

9. Bernal A, Proft T, Fraser JD, et al. Superantigens in human disease. *J Clin Immunol* 19:149–157, 1999.
10. Proft T, and Fraser JD. Bacterial superantigens. *Clin Exp Immunol* 133:299–306, 2003.
11. Llewelyn M, and Cohen J. Superantigens: Microbial agents that corrupt immunity. *Lancet Infect Dis* 2:156–162, 2002.
12. Herrmann T, Baschieri S, Lees RK, et al. In vivo responses of CD4+ and CD8+ cells to bacterial superantigens. *Eur J Immunol* 22:1935–1938, 1992.
13. Allouf JE, and Muller-Alouf H. Staphylococcal and streptococcal superantigens: Molecular, biological and clinical aspects. *Int J Med Microbiol* 292:429–440, 2003.
14. Leung DYM, Harbeck R, Bina P, et al. Presence of IgE antibodies to staphylococcal exotoxins on the skin of patients with atopic dermatitis. *J Clin Invest* 92:1374–1380, 1992.
15. Hofer MF, Harbeck RJ, Schlievert PM, et al. Staphylococcal toxins augment specific IgE responses by atopic patients exposed to allergen. *J Invest Dermatol* 112:171–176, 1999.
16. Breuer K, Wittmann M, Bosche B, et al. Severe atopic dermatitis is associated with sensitization to staphylococcal enterotoxin B (SEB). *Allergy* 55:551–555, 2000.
17. Conley DB, Tripathi A, Ditto AM, et al. Chronic sinusitis with nasal polyps: Staphylococcal exotoxin IgE and cellular inflammation. *Amer J Rhinol* 18(5):273–278, 2004.
18. Conley DB, Tripathi A, Grammer L, and Kern RC. Superantigens and chronic sinusitis II: Analysis of T-cell receptor V β domains in nasal polyps. American Rhinology Society Combined Otolaryngological Spring Meeting Program Abstracts 2004. p. 27.
19. Beninger MS, Ferguson BJ, Hadley JA, et al. Adult chronic rhinosinusitis: Definitions, diagnosis, epidemiology, and pathophysiology. *Otolaryngol Head Neck Surg* 129:S1–S32, 2003.
20. Miller BA, Reiser RF, and Bergdoll MS. Detection of staphylococcal enterotoxins A, B, C, D, and E in foods by radioimmunoassay using staphylococcal cells containing protein A as the immunoadsorbent. *Appl Environ Microbiol* 36:421–426, 1978.
21. Freed RC, Evenson ML, Reiser RF, et al. Enzyme-linked immunosorbent assay for detection of staphylococcal enterotoxins in foods. *Appl Environ Microbiol* 44:1349–1355, 1982.
22. Reiser RF, Jacobson JA, Kasworm EM, et al. Staphylococcal enterotoxin antibodies in pediatric patients from Utah. *J Infect Dis* 158:1105–1108, 1988.
23. Torres JM, Gonzlez FJ, Corzo JL, et al. Circulating CLA+ lymphocytes from children with atopic dermatitis contain an increased percentage of cells bearing staphylococcal-related T-cell receptor variable segments. *Clin Exp Allergy* 28:1264–1272, 1998.
24. Ha SJ, Lee HJ, Byun DG, et al. Expression of T cell receptor V beta chain in lesional skin of atopic dermatitis. *Acta Derm Venereol* 78:424–427, 1998.
25. Neuber K, Loliger C, Kohler I, et al. Preferential expression of T cell receptor V beta chains in atopic eczema. *Acta Derm Venereol* 76:214–218, 1996.
26. Hauk P, Wenzel SE, Trumble AE, et al. Increased T-cell receptor V(beta)8+ T cells in bronchoalveolar lavage fluid of subjects with poorly controlled asthma: A potential role for microbial superantigens. *J Allergy Clin Immunol* 104:37–45, 1999.
27. Herz U, Ruckert R, Wollenhaupt K, et al. Airway exposure to bacterial superantigen (SEB) induces lymphocyte-dependent airway inflammation associated with increased airway responsiveness—A model for non-allergic asthma. *Eur J Immunol* 29:1021–1031, 1999.
28. Akdis M, Simon HU, Weigl L, et al. Skin homing (cutaneous lymphocyte-associated antigen-positive) CD8+ T cells respond to superantigen and contribute to eosinophilia and IgE production in atopic dermatitis. *J Immunol* 163:466–475, 1999.
29. Heaton T, Mallon D, Venaille T, et al. Staphylococcal enterotoxin induced IL-5 stimulation as a cofactor in the pathogenesis of atopic disease: The hygiene hypothesis in reverse? *Allergy* 58:252–256, 2003.
30. Wedi B, Wiecezorek D, Stunkel T, et al. Staphylococcal exotoxins exert proinflammatory effects through inhibition of eosinophil apoptosis, increased surface antigen expression (CD11b, CD45, CD54, and CD69), and enhanced cytokine-activated oxidative burst, thereby triggering allergic inflammatory reactions. *J Allergy Clin Immunol* 109:477–484, 2002.
31. Hoover GE, Newman LJ, Platts-Mills T, et al. Chronic sinusitis: Risk factors for extensive disease. *J Allergy Clin Immunol* 100:185–191, 1997.
32. Ramadan HH, Fornelli R, Ortiz AO, et al. Correlation of allergy and severity of sinus disease. *Am J Rhinol* 13:345–347, 1999.
33. Drake-Lee A, and Price J. Mast cell ultrastructure in the inferior turbinate and stroma of nasal polyps. *J Laryngol Otol* 11:340–345, 1997.
34. Suh YJ, Yoon SH, Sampson AP, et al. Specific immunoglobulin E for staphylococcal enterotoxins in nasal polyps from patients with aspirin-intolerant asthma. *Clin Exp Allergy* 34:1270–1275, 2004.
35. van Cauwenberge P. Do local Th2-driven or local IgE-mediated diseases exist without systemic IgE sensitization? *Allergy* 57:373–374, 2002.
36. Rossi RE, and Monasterolo G. Prevalence of serum IgE antibodies to the Staphylococcus aureus enterotoxins (SAE, SEB, SEC, SED, TSST-1) in patients with persistent allergic rhinitis. *Int Arch Allergy Immunol* 133:261–266, 2004.