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Superantigens and chronic rhinosinusitis: Skewing of T-cell receptor V*β*-distributions in polyp-derived CD4+ and CD8+ T cells

David B. Conley, M.D.[*], **Anju Tripathi, M.D.**[#], **Kristin A. Seiberling, M.D.**[*], **Robert P. Schleimer, Ph.D.**[#], **Lydia A. Suh, B.S.**[#], **Kathleen Harris, B.S.**[#], **Mary C. Paniagua, B.S.**[§], **Leslie C. Grammer, M.D.**[#], and **Robert C. Kern, M.D.**[*]

[*]Department of Otolaryngology–Head and Neck Surgery, Northwestern University Feinberg School of Medicine, Chicago, Illinois

[#]Division of Allergy and Immunology–Department of Medicine, Northwestern University Feinberg School of Medicine, Chicago, Illinois

[§]Robert H. Lurie Comprehensive Cancer Center, Northwestern University Feinberg School of Medicine, Chicago, Illinois

Abstract

Background—*Recent studies have suggested that* Staphylococcus aureus secrete superantigenic toxins that play a role in the etiology of chronic rhinosinusitis with nasal polyposis (CRSwNP). Twenty S. aureus superantigens (SAg's) have been identified, each of which bind the Vβ-region of the T-cell receptor (TCR) outside the peptide-binding site. Approximately 50 distinct Vβ-domains exist in the human repertoire, and distinct SAg's will bind only particular domains generating a pattern of Vβ-enrichment in lymphocytes dependent on the binding characteristics of a given toxin. The aim of this study was to analyze the pattern of Vβ-expression in polyp-derived lymphocytes from CRSwNP patients.

Methods—Polyps were harvested from 20 patients with CRSwNP and 3 patients with antrochoanal polyps. Flow cytometry was used to analyze the Vβ-repertoire of polyp-derived $CD4^+$ and $CD8^+$ lymphocytes. Data were analyzed in light of the known skewing associated with SAg exposure in vivo *and* in vitro. Skewing was defined as a percentage of Vβ-expression >2 SD of that seen in normal blood.

Results—Seven of 20 subjects exhibited skewing in Vβ-domains with strong associations with S. aureus SAg's. The three antrochoanal polyps failed to show any significant Vβ-skewing.

Conclusion—*This study establishes evidence of* S. aureus SAg–T-cell interactions in polyp lymphocytes of 35% of CRSwNP patients. Although these results are consistent with intranasal exposure of polyp lymphocytes to SAg's, additional study is necessary to establish the role of these toxins in disease pathogenesis.

The relationship between infectious agents and the pathogenesis of chronic rhinosinusitis with nasal polyposis (CRSwNP) remains unclear. Recent studies have implicated the presence of fungi or toxigenic Staphylococcus aureus in the nose with the development of the eosinophilic, lymphocytic tissue infiltration characteristic of CRSwNP.1 The fungal hypothesis proposes that patients with CRSwNP mount a non-IgE–mediated hypersensitivity response to fungal

Address correspondence and reprint requests to David B. Conley, M.D., Department of Otolaryngology–Head and Neck Surgery, Northwestern University Feinberg School of Medicine, 303 East Chicago Avenue, Searle 12–561, Chicago, IL 60611 dbcentmd@northwestern.edu.

antigens, triggering eosinophil recruitment and activation in the sinus mucosa and lumen.2 The superantigen (SAg) hypothesis proposes that the local action of one or more staphylococcal toxins triggers a similar histological picture.[3] The molecular pathways of either hypothesis remain vague.

The role of staphylococcus in sinonasal inflammatory disease initially was suggested by the high rate (35%) of nasal cultures positive for this organism in patients with CRS.[4] Furthermore, as many as 55% of these strains were found to secrete SAg's.5 SAg's are proteinaceous exotoxins and thus far 20 have been associated with *S. aureus*.6 These exotoxins have been implicated in several chronic inflammatory diseases, including atopic dermatitis (AD) and asthma.[7] SAg's bypass the conventional immunologic response by binding directly to the T-cell receptor (TCR) and the major histocompatibility complex (MHC) of the antigen-presenting cell outside the peptide binding groove, resulting in T-cell proliferation and cytokine release. 6,8 SAg's bind to the Vβ-region of the TCR and ~50 distinct Vβ-domains exist in the human repertoire.[6] Distinct SAg's will bind only particular domains generating a characteristic pattern or "signature" of Vβ-enrichment in lymphocytes at the site of exposure.[8] Current theory and observations would indicate that both T-helper ($CD4^+$) and T-cytotoxic/suppressor ($CD8^+$) cells should be affected by SAg stimulation.[9]

The pattern of Vβ-expansion or signature associated with each SAg has been suggested by *in vitro* and *in vivo* studies.[9] Typically, a given SAg will bind multiple Vβ-domains with a range of avidity, and stronger binding will trigger greater proliferation of cells bearing that domain.[10-13] Of the ~50 Vβ-domains in the human TCR repertoire, 24 represent major Vβ-types, 7 of which have been identified as having strong associations with particular staphylococcal SAg's (Table 1).[6] This study uses flow cytometric analysis of polyp lymphocytes from CRSwNP patients to detect evidence of skewing of these "strong" Vβ-domains. Increased levels in both $CD4^+$ and $CD8^+$ subpopulations for these seven domains is indirect evidence of local SAg exposure in the nose.[9]

METHODS

Twenty patients with CRSwNP undergoing endoscopic sinus surgery were sequentially selected for this study. All patients had obvious sinonasal polyposis confirmed by office nasal endoscopy and CT scanning and each patient met criteria for chronic sinusitis as defined by the Sinus and Allergy and Health Partnership.[1] All 20 patients had failed medical therapy including antibiotics, nasal steroids, and oral steroids. An additional three patients with antrochoanal polyps were included to serve as control specimens. The diagnosis was suggested by physical exam and CT scans and confirmed by intraoperative findings. Informed consent was given before the procedure and the study was approved by the Northwestern University Medical School Institutional Review Board Committee. Polyp tissue was collected in saline and processed according to the following protocol.

Cell Processing and Staining

Nasal polyp tissue was placed in RPMI medium and mechanically dissociated by mincing with a scalpel and then put through a steel mesh to mechanically dissociate the cells. Alternative, enzymic methods of tissue dissociation were attempted in an effort to maximize the numbers of lymphocytes extracted from the tissue. These methods were abandoned as the enzymes (collagenase) altered the results, probably by enzymically digesting the surface proteins on the T-cell surfaces (unpublished data from our laboratory). Consequently, only mechanical dissociation was used. Unfortunately, mechanical dissociation alone does not permit lymphocyte extraction from nonpolypoid CRS tissue (CRSsNP) or normal sinus mucosa (unpublished data from our laboratory). Hence, these tissues were not used in this study.

Mechanical dissociation can be used to extract cells from antrochoanal polyps and these samples were included for subsequent analysis.

After mechanical dissociation, the cell suspension was transferred to a conical tube, allowed to settle, and then transferred to another conical tube and centrifuged for 5 minutes at 1500 rpm. After discarding the supernatant as waste, 5 mL of 1.5 M of 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 were then centrifuged for 5 minutes at 1500 rpm. After discarding the supernatant, the cell pellet was suspended in 5 mL of PBS–2% FBS solution and centrifuged for 5 minutes at 1500 rpm. The wash procedure was repeated after a cell count was performed. Cells were resuspended in PBS–2% FBS at a final cell concentration of between 5 and 10×10^6/mL. The cell suspension was aliquoted into tubes at 1×10^6 cells/tube and stained with conjugated antibodies using a commercially available TCR Vβ-kit (IO Test Beta Mark; Immunotech/Beckman Coulter, San Jose, CA). CD3 was labeled with $CD3^+$ antigen-presenting cells (Immunotech/Beckman Coulter). CD4 was labeled with $CD4^+$ ECD (Beckman Coulter, Miami, FL). CD8 was labeled with $CD8^+$ Percp (Becton Dickinson, San Jose, CA). 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 cells with 0.5 mL of 0.5% paraformaldehyde.

Flow Cytometry

The samples were analyzed on a Beckman Coulter Elite ESP flow cytometer (Beckman Coulter) using laser excitation at 488 and 633 nm. 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. The analysis software used was Elite 4.0. T cells from polyp tissue were assessed for evidence of clonal expansion of Vβ-domains 1, 2, 3, 4, 5.1, 5.2, 5.3, 7.1, 7.2, 8, 9, 11, 12, 13.1, 13.2, 14, 16, 17, 18, 20, 21.3, 22, and 23. Cells were first gated on lymphocytes by forward/side scatter plus $CD3^+$ cells versus side scatter. These two gates were applied to the Vβ-data for $CD3^+$ and then an additional gate of $CD4^+$ or $CD8^+$ was applied to obtain the subset Vβ-information. All fluorescence data were displayed on a log scale.

Data Analysis

The results were compared with normative data on the expected distribution of specific Vβ-domains in healthy control patients (IOTest Beta Mark PN IM3497, 5/2000, Beckman Coulter). This is necessary because the distinct Vβ-domains are not equally represented under baseline conditions. Vβ-expansion was defined as a percentage of cells >2 SD above the mean percent present in the blood of normal subjects.

RESULTS

T cells derived from polyp tissue were analyzed for Vβ-skewing in $CD4^+$ and $CD8^+$ populations (Table 2). Of the 24 Vβ-domains evaluated in this study, 16 have been identified as having “strong” or “weak” associations with particular *S. aureus* SAg’s (Table 1). The Vβ-domains 2, 3, 5.1, 5.3, 8, 12, and 14 have established strong associations with S. *aureus* SAg’s and were included in this study. These seven Vβ-domains are related to the following SAg’s: Toxic Shock Syndrome Toxin-1 (TSST-1) and staphylococcal Enterotoxins (B, C1, I, A, D, E, G) (SEB, SEC1, SEI, SEA, SED, SEE, and SEG). At present, the remaining *S. aureus* SAg’s are not known to possess a strong association with known Vβ-domains.[6,11-13]

Table 3 ranks both the strong and the weakly associated Vβ-domains that occurred simultaneously in both $CD4^+$ and $CD8^+$ subsets. Seven of the 20 subjects (35%) had skewed flow cytometric data consistent with exposure to an SAg with a strong Vβ-association. Of these

seven patients, two had skewing of more than one strongly associated Vβ, indicating the possibility of more than one SAg acting in these two patients (Table 4). The remaining five subjects had responses suggestive of a single SAg effect. An additional eight patients had skewing of Vβ-domains in both $CD4^+$ and $CD8^+$ with only weak associations.

Polyp tissues obtained from three patients with antrochoanal polyps also were subjected to flow cytometric analysis. In marked contrast to polyps from CRSwNP tissue, skewing of Vβ-domains for both $CD4^+$ and $CD8^+$ was completely absent. Specifically, there was no sign of any staphylococcal SAg effects, either strong or weak, in antrochoanal polyp tissue.

For purposes of quality control, the 24 individual Vβ-percentages were totaled for each subject by $CD4^+$ and $CD8^+$ subsets. Normative blood distributions total 72.3% for the 24 $CD4^+$ Vβ -lymphocyte subsets and 66.6% for the 24 $CD8^+$ Vβ-lymphocyte subsets in this assay (IOTest Beta Mark PN IM3497, 5/2000; Beckman Coulter). This compared favorably with the average of these 20 subjects: 71.1% in $CD4^+$ and 67.6% in $CD8^+$. All individual totals were <100% for $CD4^+$ or $CD8^+$ subsets.

DISCUSSION

The current results indicate a positively skewed distribution of specific TCR Vβ-domains with a strong SAg association in 35% (7 of 20) of CRSwNP patients. The results were obtained under a conservative definition of skewing. Positive results were defined as a TCR Vβ-percentage >2 SD above normal mean for blood in both $CD4^+$ and $CD8^+$ subsets. The simultaneous increase in both $CD4^+$- and $CD8^+$-specific TCR Vβs in a given subject is most consistent with an SAg response. Of these seven patients, two had skewing of more than one strongly associated Vβ, indicting the probability of more than one SAg acting in these two patients (Table 4). The remaining five patients had responses suggestive of a single SAg effect.

Twenty *S. aureus*-related SAg's have been identified, but only TSST, SEB, SEC1, SEI, SEA, SED, SEE, and SEG have an established strong Vβ-domain.[6,10-13] Local effects of the remaining 12 SAg's can not, therefore, be readily detected using the foregoing methodology. It is possible that other Vβ-domain skewing in $CD4^+$ and $CD8^+$ lymphocytes detected in this study (Table 2) may reflect activity of these other SAg's. An additional eight patients had skewing of Vβ-domains in both $CD4^+$ and $CD8^+$ with no known strong association (Table 2). Although these results are suggestive of additional "SAg" effects, the significance is currently unclear. It also should be remembered that the establishment of strong associations between individual SAg's and clonal expansion of particular Vβ-domains is based primarily on *in vitro* assays. The pattern observed *in vivo* may reflect the superimposed effects of multiple SAg's at various concentrations present in unique temporal sequences. Changes in the domain profile of either $CD4^+$ or $CD8^+$ alone, however, were interpreted as insufficient evidence of an SAg effect in keeping with the accepted action of these toxins on lymphocyte populations.[9]

Clonal expansion of Vβ-domains has been observed primarily in immunologic diseases with a suspected role for SAg's in the pathogenesis (toxic shock syndrome, AD, asthma, and rheumatoid arthritis), as well as in the setting of neoplastic disease such as lymphoma.[14-16] Consequently, the demonstration of Vβ-skewing in both $CD4^+$ and 8^+ lymphocyte populations derived from the polyps of CRSwNP patients is interpreted as indicative of SAg exposure in the nasal mucosa. However, it should be remembered that this skewing of the lymphocyte profile in CRSwNP was based on a comparison with established normative peripheral blood Vβ-distributions rather than normative tissue distributions. For technical reasons, this study can not analyze the Vβ-profile expressed in lymphocytes from the normal nasal mucosa or from the mucosa of CRSsNP (see Methods section). Specifically, the use of flow cytometry

on nonpolypoid ethmoid mucosa requires enzymic rather than mechanical tissue digestion, which alters the results (unpublished data from our laboratory). However, it is possible to use standard flow cytometry on antrochoanal polyp tissue because no enzymic digestion is necessary. Interestingly, lymphocytes derived from antrochoanal polyps failed to establish any Vβ-domain skewing indicative of SAg exposure.

The current results support the hypothesis that staphylococcal SAg's play a role in the pathogenesis of CRSwNP. However, results on antrochoanal polyp tissue showed no evidence of SAg effects. A recent biochemical study from our laboratory detected staphylococcal SAg's in the mucus and tissue homogenates of 8 of 21 CRSwNP patients, 0 of 5 normal patients, and 0 of 3 CRSsNP.[17] In addition, specific IgE directed against staphylococcal SAg's has been shown in a much higher percentage of nasal (polyp) tissue derived from CRSwNP patients in comparison with either nasal tissue obtained from normal patients or CRSsNP.[18] Taken together with the present findings, these results support the hypothesis of SAg effects in tissues of at least a portion of CRSwNP patients, with no clear evidence of SAg effects in other diseases of the sinonasal mucosa.

The nasal mucosa of over 60% of CRSwNP patients is colonized with *S. aureus*.[18] Because most *S. aureus* strains carry at least one SAg gene, the mucosa of the majority of CRSwNP patients is likely exposed to SAg's to some degree, although the amount and duration of exposure are likely highly variable. Results indicate that only a portion of CRSwNP patients show evidence of local SAg exposure, however. Toxin-specific IgE was shown in the polyps of 50% of CRSwNP patients,[19] and in this study, evidence of *S. aureus* SAg–T-cell interactions in polyp lymphocytes was established in only 7 of 20 CRSwNP subjects. This indicates that individual reactions to SAg's are likely modulated by factors other than simple exposure *and* that not all nasal polyps can be associated with SAg effects. Recent studies have indicated that the genetically determined MHC class II isotypes and MHC class II polymorphisms can significantly affect the binding affinity of the TCR–SAg–MHC class II complex.[20,21] This has been hypothesized to at least partly explain the variability in virulence or disease severity seen among individuals exposed to SAg.[22] Although definitive studies are lacking, it seems plausible that this variation in immunogenetic background may be a factor in the development of SAg-mediated sinus disease. Theoretically, MHC class II haplotypes may be a risk factor for the development of severe chronic inflammation in response to these relatively ubiquitous bacterial toxins. Individual variations also may exist in the ability of SAg to access the acquired immune system. Specifically, the mucosa of some individuals may be more susceptible to the degrading effects of proteases secreted by colonizing microorganisms such as bacteria and fungi.[23] SAg's may have heightened effects in this setting in that the mucosal barrier would be deficient, allowing increased exposure of SAg to lymphocytes.

The mechanism whereby SAg-mediated lymphocyte expansion can trigger nasal polyposis has not been fully elucidated. Specifically, it remains unclear how SAg's will trigger eosinophil recruitment and activation. Initial studies have suggested that SAg's trigger a specific, local IgE response in polyps in association with marked tissue eosinophilia and increased IL-5.[3,19] Although not studied in CRSwNP, *in vitro* exposure of SEB to peripheral blood mononuclear cells from allergic rhinitis and AD patients resulted in IL-5 production that does not occur in controls.[24,25] Taken together, these studies suggest the hypothesis that SAg's may trigger nasal polyposis by the superantigenic stimulation of lymphocytes to secrete mediators that stimulate the recruitment, activation, and persistence of eosinophils. A superimposed and interrelated conventional chronic allergic response also may augment tissue eosinophilia. The relationship between the conventional IgE response to SAg and classic SAg actions on T cells likely will be particularly significant in the study of effects of these toxins in CRSwNP. Although this study provides evidence of a local SAg effect on polyp lymphocytes, validation of the "SAg hypothesis" of CRSwNP will require additional study.

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Table 1

S. aureus SAg's and the related V*β*s

***S. aureus* SAg**	**V*β*-Association**	
	Strong	**Weak**
SEA	5.3	1.1 , 9, 23
SEB	3	1.1 , 2, 17
SEC1	3	12
SEC2		12, 13.1, 14, 17, 20
SEC3		5.1, 12
SED	5.3, 12.1	1.1, 8.1
SEE	8.1	5.1
SEG	14	3, 12, 13.1, 13.2
SEI	5.1	1.1, 5.3, 23
SEK		5.1, 5.2
SEQ		2.1, 5.1, 21.3
TSST1	2	

Associations shown are based on published papers and reviews of the literature.[6,10-13] Strong associations indicate a large subset expansion in T cells after SAg stimulation and weak associations represent a small number of lymphocyte polyclonal expansions.

SE(A–Q) = Staphylococcal Enterotoxin (A–Q); TSST1 = Toxic Shock Syndrome Toxin-1.

Table 2

TCR Vβ-skewing in $CD4^+$ and $CD8^+$ T cells from nasal polyps

Subject	$CD4^+$										$CD8^+$							
1	18										2	5.2	5.3	13.1	17	22		
2	5.1	5.3	11	13.6	18	21.3	23				1	5.1	5.2	13.1	16	18	21.3	
3	5.2	16	21.3	23							5.3	21.3	23					
4	4	11	13.1	14														
5	1	2	5.3	7.1	13.6						1	8	21.3					
6	2	4	5.3	13.1	16	17	21.3				13.1	16	17	21.3	22			
7	3	4	7.1	13.1	13.6	17					13.6	17						
8	2	4	5.2	5.3	13.6	17					2	4	5.2	5.3	13.6	8	17	
9	5.2	5.3	12	13.6							4	5.1	5.2	12	13.1	20	13.6	
10	7.1	16	20	22							7.1	8	14	20				
11	8	14									1	5.2	8	13.6				
12	2	4	5.1	8	13.6	23					8	12	13.1					
13	4	5.1	8	11	12	13.6	14	16	21.3	23	4	13.1	16	18	23			
14	1	5.1	7.1	13.1	14	21.3	22				13.6	16	21.3	23				
15	8	13.6	17								4	7.1	13.1	17				
16	3	5.1	13.1								3	5.1	11	13.1	14	17	22	
17	3	13.6									3							
18	4	5.1	12	13.6	14	16	18	23			1	2	5.3	7.1	11	12	13.6	18
19	12	16									8	12	16					
20	1	7.1	8	13.6	21.3						13.1	17						

Skewing was defined as >2 SD higher than the established prevalence in blood.

Table 3

Ranking of the incidence of simultaneous skewing in $CD4^+$ and $CD8^+$ T-cell subsets

Vβ	Number of Subjects Expressing Both $CD4^+$ and $CD8^+$ Skewed Vβs	Association to *S. aureus* SAg's	
		Strong	Weak
17	4 (20%)		SEB, SEC2
12	3 (15%)	SED	SEC1, SEC2, SEC3, SEG
21.3	3 (15%)		SEQ
8	2 (10%)	SEE	SED,
23	2 (10%)		SEA, SEI
3	2 (10%)	SEB, SEC1	SEG
5.2	2 (10%)		SEK
13.1	2 (10%)	SEC2	SEG
5.1	2 (10%)	SEI	SEC3, SEE, SEK, SEQ
5.3	1 (5%)	SEA, SED	SEI
2	1 (5%)	TSST1	SEB, SEQ
1	1 (5%)		SEA, SEB, SED, SEI
20	1 (5%)		SEC2
14	0	SEG	SEC2
13.2	0		SEG
9	0		SEA

Table depicts the ranking of simultaneous skewing in 16 of 24 specific Vβs analyzed based on frequency. Both strong and weak S. aureus SAg associations are shown. Some subjects expressed more than one Vβ-skewing. In these 20 subjects, 7 (35%) had skewed distributions for one or more Vβ related to a strong SAg effect.

SE(A–Q) = Staphylococcal Enterotoxin (A–Q); TSST1 = Toxic Shock Syndrome Toxin-1.

Table 4

Incidence of Vβ skewing in $CD4^+$ and $CD8^+$ T cells for strongly associated *S. aureus* SAg effects

Subject	Vβ	*S. aureus* SAg
1		
2	5.1	SEI
3		
4		
5		
6	13.1	SEC2
7		
8	2, 5.3	TSST1, SEA, SED
9		
10		
11	8	SEE
12	8	SEE
13		
14		
15		
16	3, 5.1, 13.1	SEB, SEC1, SEI, SEC2
17	3	SEB, SEC1
18		
19		
20		

SE(A–I) = Staphylococcal Enterotoxin (A–I); TSST1 = Toxic Shock Syndrome Toxin-1.