
Correlation of plasma complement split product levels with allergic respiratory disease activity and relation to allergen immunotherapy

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Background: Allergens, including dust mite and grass pollen, and mast cell tryptase are known to generate the complement split products (CSPs) C5a and C3a, which can then trigger allergic inflammation. The relation of these anaphylatoxin levels to clinical allergic disease responses is not known.

Objective: To evaluate the relationship of plasma CSP levels to allergic respiratory disease variables in an adult cohort.

Methods: A cross-sectional survey was used to assess the association of plasma C5a desArg and C3a desArg levels with clinical allergic respiratory disease variables. Furthermore, a time course of the effect of routine allergen immunotherapy on plasma CSP levels and cutaneous and pulmonary responses was determined.

Results: Adult plasma C5a desArg levels correlate with asthma severity as determined by a physician ($P = .01$) and by Asthma Quality of Life Questionnaire scores ($P < .01$). Change in plasma C5a desArg levels 1 hour after immunotherapy is associated with baseline rhinoconjunctivitis symptom severity ($P = .03$), change in total mean wheal diameter ($P = .05$), and total dust mite dosage ($P = .04$). Change in plasma C3a desArg levels 3 hours after immunotherapy correlates with change in total mean wheal diameter induced by dust mite ($P = .01$). Change in plasma CSP levels after immunotherapy did not correlate with change in spirometric outcome.

Conclusions: Plasma C5a desArg levels reflect allergic respiratory disease severity as assessed by physicians and correlate with Asthma Quality of Life Questionnaire scores. Changes in CSP levels after immunotherapy reflect cutaneous allergic responses, especially to dust mite allergen.

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INTRODUCTION

The role of innate immune responses to allergen involving complement in the pathogenesis of allergic disease has yet to be fully determined. The complement split product (CSP) proteins C5a and C3a, long recognized as anaphylatoxins, are cleaved from C5 and C3, respectively, by allergens,¹ tissue-based phagocyte-derived proteases,² and human mast cell β -tryptase.³ Levels of these CSPs are increased in bronchoalveolar lavage fluid after segmental allergen provocation.⁴ Elevated levels of C5a/C5a desArg have been detected in induced sputum from asthmatic patients and patients with chronic obstructive pulmonary disease.⁵ Airway activation of complement by allergens may potentiate airway responses in asthma and may contribute to clinical disease. Studies by Fregonese and colleagues⁶ have shown increased expression of anaphylatoxin receptor levels in the lung

tissue of individuals with fatal asthma. There is a paradoxical protective effect demonstrated in murine models in which C5a also protects against allergen sensitization through its effects on dendritic cells.^{7–9} Once generated, C5a and C3a are rapidly cleaved by a C-terminal carboxypeptidase to C5a desArg and C3a desArg,¹⁰ respectively, the more stable and measurable end products.

There has been limited investigation of the association of complement levels with clinical allergic disease. Nakano et al¹¹ determined that asthmatic patients admitted to the hospital for exacerbation of their lung disease have elevated C3a levels that subsequently decrease after inpatient treatment. Recent studies by Khodoun et al¹² demonstrated that C3a is generated by peanut and cashew extract and may contribute to anaphylactic shock.

In a cross-sectional survey performed in an outpatient setting, we determined in this pilot study whether plasma levels of C5a/C5a desArg and C3a/C3a desArg correlate with clinical allergy variables. We further determined whether cutaneous allergic inflammation resulting from allergen immunotherapy correlates with changes in CSP levels and how these changes relate to changes in spirometric measures.

METHODS

This protocol was approved by the institutional review board at State University of New York (SUNY) Downstate Medical

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Center. Patients signed informed consent or assent forms. Adults with asthma, allergic rhinoconjunctivitis, or both receiving care at SUNY were evaluated in 2 stages. The first stage was a cross-sectional survey of the relation of plasma CSP levels with parameters of allergic/asthma disease activity. The second stage evaluated change in plasma CSP levels after allergen immunotherapy and relation to induction of cutaneous allergic inflammation and other variables of allergic/asthma disease activity.

Survey

A cross-sectional survey was administered to adults receiving allergy and asthma care during a single study visit at SUNY. Plasma samples were obtained to compare CSP levels with other variables of allergic/asthmatic disease activity, including physician-assessed and subject-evaluated disease severity, spirometric measures, exhaled nitric oxide levels (Instrument NOA 280; Sievers/Ionics, Boulder, Colorado), and total serum IgE levels. Concurrent asthma severity was also determined using the 2002 National Heart, Lung, and Blood Institute guidelines: a score of 0 represents no asthma; 1, mild intermittent asthma; 2, mild asthma; 3, moderate asthma; and 4, severe persistent asthma.

Patient symptoms were evaluated using the Asthma Quality of Life Questionnaire (AQLQ)¹³ and the Rhinoconjunctivitis Quality of Life Questionnaire (RQLQ).¹⁴ The AQLQ is a validated asthma-specific instrument. Total scores were used in statistical analysis. Lower total scores are associated with increased asthma severity. The RQLQ has a similar design except that higher scores are associated with increased symptoms.

Exhaled nitric oxide levels were measured online (Instrument NOA 280) according to American Thoracic Society guidelines.¹⁵ Subsequently, spirometry was performed (KoKo Spirometer model 313100; Ferraris CardioRespiratory, Louisville, Colorado). Total serum IgE levels were measured in the Diagnostic Immunology Laboratory at SUNY using a fluoroenzyme immunoassay (Unicap System; Pharmacia Upjohn, Uppsala, Sweden).

Time Course Evaluation of the Effect of Allergen Immunotherapy on Plasma CSP Levels

This evaluation was designed to determine the relationship of induced cutaneous allergic inflammation resulting from allergen immunotherapy to plasma CSP levels and spirometry findings.

The immunotherapy study cohort consisted of patients aged 13 to 75 years who received individualized standard allergen immunotherapy for allergic rhinoconjunctivitis, asthma, or both. Patients had initiated allergen immunotherapy after demonstration of positive epicutaneous skin prick test reactivity for a specific IgE was found to correlate with suspected triggers and patient exposures and after the failure of avoidance measures and medication therapy. Antihistamine use was discontinued for 24 hours before immunotherapy. Individual allergens administered (according to sensi-

Table 1. Demographic Characteristics From the Cross-sectional Survey of 29 Adult Outpatients With Allergic Rhinoconjunctivitis, Asthma, or Both^a

Characteristic	No. (%) of patients
Female sex, No. (%)	18 (62)
Age, mean (SD), y	48.8 (15.1)
Allergic rhinoconjunctivitis only, No. (%)	5 (17)
Allergic rhinoconjunctivitis and asthma, No. (%)	24 (83)
Ethnicity, No. (%)	
Black	17 (59)
White	9 (31)
Hispanic	3 (10)

^a Data are presented as number (percentage) of patients unless otherwise indicated.

zation) included the following: dust mite (*Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, or both) (Hollister-Stier, Spokane, Washington), American cockroach, ragweed (short and tall), 5-grass mix (timothy, orchard, June, red top, and sweet vernal), English plantain, 10-tree mix A (ash, beech, birch, elm, hickory, maple, oak, poplar, sycamore, and alder), *Cladosporium sphaerospermum*, *Alternaria*, dog epithelium, and cat pelt (Alk-Abello, Round Rock, Texas). Individual allergen injections were placed in the subcutaneous area of the upper arm using Becton-Dickinson 26G3/8 syringes (Becton-Dickinson, Franklin Lakes, New Jersey). C5a desArg and C3a desArg levels were obtained at baseline before immunotherapy and 1 and 3 hours after immunotherapy. Total serum IgE levels were measured at baseline.

Cutaneous reactivity tracings were recorded using clear adhesive tape measurements of indurations from each injection site taken 15 minutes, 1 hour, and 3 hours after the injections. The mean orthogonal diameter of wheals resulting from each allergen injection site was calculated and was used in the analysis.

In preparation for spirometric evaluation, patients receiving combination inhaled corticosteroid and long-acting β -agonist therapy were dose free for 12 hours; short-acting β -agonist use was limited to 6 hours before immunotherapy dosing. Patients with symptoms of asthma did not receive immunotherapy. Pulmonary reactivity to allergen immunotherapy was determined by means of spirometric measurements at baseline and 1 and 3 hours after allergen immunotherapy. Symptom scores (AQLQ and RQLQ) were obtained once at baseline. Total scores were used in the statistical analysis. The immunotherapy study was performed routinely between 1 and 5 PM.

CSP Measurements

Samples for plasma C5a desArg and C3a desArg levels were collected in tubes, with disodium EDTA as the anticoagulant. The samples were centrifuged within 1 hour for 15 minutes at 1,000g. Aliquots of plasma were stored at -70°C until the day of assay. The CSP levels were determined using the OptEIA enzyme-linked immunosorbent assay (ELISA) test

Table 2. Laboratory Profile From the Cross-sectional Survey of Adult Outpatients With Allergic Rhinoconjunctivitis, Asthma, or Both

	Reference range	Median	Minimum	Maximum	Mean	25th quartile	75th quartile	Standard deviation
Total IgE, IU/mL (n = 29)	<100	139.0	12.9	1,480.0	328.4	54.0	447.0	416.6
C3a desArg, ng/mL (n = 23)	449.4 ± 100.9	867.8	509.5	2,182.0	901.1	722.6	989.8	316.4
C5a desArg, ng/mL (n = 29)	3.92 ± 1.82	3.08	1.06	8.13	3.67	2.54	4.71	1.86

(Pharmingen, San Jose, California), a solid-phase sandwich ELISA, and KCjunior software (BioTek Instruments Inc, Winooski, Vermont). The sensitivities of the C3a desArg and C5a desArg ELISA assays are 0.7 and 47.0 pg/mL, respectively. Precision was determined based on 24 replicates each of 3 different levels of C3a desArg and C5a desArg, with the following coefficients of variation: intra-assay, 6.8 to 7.1 and 2.5 to 4.6, respectively; interassay, 7.1 to 9.4 and 5.1 to 7.9, respectively.

Statistical Analysis

Spearman correlation coefficients were calculated to determine whether 2 variables were significantly associated. The Wilcoxon 2-sample test was used to determine the 2-sided probability that the median difference between 2 groups for a variable was significant. Statistical significance was defined as $P < .05$.

RESULTS

Survey

Plasma C5a desArg and C3a desArg levels were determined for outpatient allergic adults (n = 29 and 23, respectively). The demographics of the study patients and their CSP and IgE levels are given in Tables 1 and 2, respectively. Whereas C5a desArg levels were within the reference range, levels of C3a desArg and serum IgE were elevated. Table 3 indicates that plasma C5a desArg levels correlated with physician-determined asthma severity ($r = 0.49$, $P = .02$) and AQLQ

scores ($r = 0.73$, $P < .01$) and with rhinoconjunctivitis symptom severity ($P < .01$). C3a desArg measurements inversely correlated with total serum IgE levels. Spirometric variables associated with CSP levels included forced expiratory flow between 25% and 75% with levels of C3a desArg ($P = .05$).

Time Course Evaluation of the Effects of Allergen Immunotherapy on Plasma CSP Levels

The demographic characteristics of the study patients, immunotherapy history, medication regimens, and profile of allergens given as immunotherapy are given in Table 4. Table 5 indicates changes in CSP levels after immunotherapy. As seen with the cross-sectional survey, plasma C5a desArg levels were within the reference range and C3a desArg levels were increased at baseline. There was no significant increase in plasma C5a desArg or C3a desArg levels after allergen immunotherapy. Only local pruritus and swelling from the injection sites were noted.

Changes in the sum diameter of injection site swelling after 1 hour correlated with changes in plasma C5a desArg levels 1 hour after immunotherapy ($P = .05$) (Table 6). However, although the total allergen immunotherapy dose did not correlate with changes in C5a desArg levels after 1 hour, it did correlate at 1 hour with the total dose of dust mite allergen extract administered ($P = .04$). There was no association of the individual dust mite species dosage, either *D. farinae* or *D. pteronyssinus*, with changes in C5a desArg levels. Changes in

Table 3. Correlations of the Clinical Variables With Plasma Levels of the Complement Split Products in the Adult Cohort Outpatient Survey

Variable	C5a desArg			C3a desArg		
	Spearman correlation coefficient	P value	No. of observations	Spearman correlation coefficient	P value	No. of observations
Age	0.14	.48	29	0.33	.13	23
Asthma severity	0.49	<.01	29	0.18	.41	23
AQLQ	-0.73	<.01	12	0.14	.66	12
RQLQ	0.59	<.01	19	-0.01	.97	19
IgE	0.14	.47	29	-0.52	.01	23
eNO	-0.25	.34	17	-0.06	.80	17
FEV ₁	0.05	.83	19	0.17	.48	19
FEV ₁ /FVC	0.14	.56	19	0.29	.23	19
PEFR	-0.35	.17	17	0.29	.25	17
FEF ₂₅₋₇₅	0.17	.49	19	0.46	.05	19

Abbreviations: AQLQ, Asthma Quality of Life Questionnaire; eNO, exhaled nitric oxide; FEF₂₅₋₇₅, forced expiratory flow between 25% and 75%; FEV₁, forced expiratory volume in 1 second; FVC, forced vital capacity; PEFR, peak expiratory flow rate; RQLQ, Rhinoconjunctivitis Quality of Life Questionnaire.

Table 4. Demographic Characteristics, Allergen Immunotherapy Profile, Medication Regimens, and Allergen Profile of 16 Adult Outpatients With Allergic Rhinoconjunctivitis, Asthma, or Both Receiving Allergen Immunotherapy^a

Variables	No. (%) of patients
Demographics	
Female	7 (44)
Age, mean (SD), y	36 (14)
Allergic rhinoconjunctivitis	14 (88)
Asthma	5 (31)
Ethnicity	
Black	9 (56)
White	7 (44)
Total serum IgE, mean (SD), IU/mL (reference range, <100 IU/mL)	497 (990)
Immunotherapy duration, mean (SD), y	2.6 (3.7)
Maintenance therapy	6 (44)
Medications	
Antihistamines	10 (63)
Nasal corticosteroids	6 (38)
Ocular antihistamines	1 (6)
Inhaled corticosteroids	0
Inhaled corticosteroids and LABAs	3 (19)
SABAs	4 (25)
Leukotriene antagonists	2 (13)
Allergens	
Dust mite	13 (81)
Tree pollen	6 (38)
Ragweed	6 (38)
Grass	5 (31)
<i>Alternaria</i>	3 (19)
Cat	2 (13)
<i>Cladosporium</i>	2 (13)
Cockroach	2 (13)
English plantain	1 (6)
Dog	1 (6)

Abbreviations: LABA, long-acting β -agonist; SABA, short-acting β -agonist.

^a Data are presented as number (percentage) of patients unless otherwise indicated.

C5a desArg levels 1 and 3 hours after immunotherapy correlated with total self-reported eye and nasal symptoms ($P = .03$ and $.05$, respectively). Total serum IgE levels did not correlate with changes in C5a desArg levels.

Table 7 indicates that changes in C3a desArg levels across 3 hours correlated with total skin swelling to dust mite after 3 hours ($P = .01$). The *D pteronyssinus* dosage also correlated with changes in C3a desArg levels across 3 hours ($P = .02$). There was no association of symptom scores with changes in C3a desArg levels. Total serum IgE levels correlated with changes in C3a desArg levels from 1 to 3 hours ($P = .04$). Changes in spirometric measures across the 3 time points did not correlate with changes in levels of C3a desArg or C5a desArg.

Whereas there was no correlation between spirometric outcomes and changes in C5a desArg or C3a desArg levels,

changes in spirometric outcomes correlated with changes in cutaneous responses to immunotherapy (Table 8). At 1 and 3 hours, there was a negative association between change in total mean wheal diameter from 15 minutes and peak flow rate ($P < .01$ and $P = .01$, respectively). Change in wheal size from dust mite immunotherapy inversely correlated with forced expiratory flow between 25% and 75% at 1 hour but not at 3 hours.

Spearman correlations generated for IgE levels with wheal size, severity scores, spirometric outcomes, and allergen dose were significant only for the association with change in total wheal diameter across 3 hours ($r = 0.65$, $P < .01$) (data not shown). These findings show that changes in blood CSP levels correlated with cutaneous allergic inflammation, especially in association with dust mite allergen, and with eye and nasal allergic symptoms. Changes in CSP levels did not correlate with spirometric outcomes.

DISCUSSION

We found that plasma C5a desArg levels correlate with allergic respiratory disease severity as evaluated by physicians and patients via AQLQ scores. Whereas plasma levels of C5a desArg and C3a desArg do not increase after allergen injections of immunotherapy, changes in CSP levels after immunotherapy correlate with the associated cutaneous allergic inflammation, especially in association with dust mite allergen, and with eye and nasal allergic symptoms.

This pilot study of complement levels in adult outpatients with allergic respiratory disease is limited by its small sample size, wide patient age range, varied ethnicities, and multiple sensitivities. Evaluation of a larger cohort would allow for these variables to be controlled for in a regression model. However, although there is literature that addresses urban allergen sensitization¹⁶ and the effect of age on skin test reactivity,¹⁷ there is a paucity of literature that addresses ethnicity, age, and range of allergen sensitivities with associated cutaneous responses to allergen immunotherapy.

This study demonstrates the link between complement protein levels and clinical allergic respiratory disease, most notably with asthma symptom severity. Preliminary measurements of CSP levels of adult asthmatic patients treated in the emergency department also correlate with AQLQ scores.^{18,19} Recent asthma guidelines^{20,21} include evaluation of asthma control using the Asthma Control Test and the Asthma Therapy Assessment Questionnaire. Preliminary studies²² of adult outpatients have shown a correlation between complement levels and asthma control, as evaluated by the Asthma Control Test²³ and the Asthma Therapy Assessment Questionnaire.²⁴

Because previous investigative work has shown that allergens directly activate complement,¹ the association of asthma severity with complement may be an indirect measure of the degree of allergen exposure or, more specifically, of the allergen load to which the patient has been exposed. The presence of C5 and C3 has been found in the airway in vivo and in vitro in human and murine models.

Table 5. Complement Split Product Levels After Allergen Immunotherapy

	Patients, No.	Median	Minimum	Maximum	Mean	25th quartile	75th quartile	SD
C5a desArg, ng/mL ^a								
Baseline	16	5.32	2.30	10.60	5.83	3.55	8.15	2.68
1 h	16	4.85	1.50	9.80	5.41	3.40	7.70	2.55
3 h	15	4.60	1.50	9.70	4.96	3.20	7.60	2.38
C3a desArg, ng/mL ^b								
Baseline	15	1,200.10	483.00	1,857.20	1,179.64	890.40	1,565.60	433.15
1 h	15	1,140.00	529.00	1,858.00	1,190.36	868.70	1,526.70	402.42
3 h	15	1,136.00	498.00	1,884.10	1,205.09	856.90	1,683.90	475.85

^a The mean (SD) reference range is 3.92 (1.82) ng/mL.^b The mean (SD) reference range is 449.4 (100.9) ng/mL.Table 6. Correlations Between Changes in Plasma C5a desArg Levels After Allergen Immunotherapy and Changes in Cutaneous Reactivity, Immunotherapy Dosage, Baseline Symptom Scores, and Changes in Spirometric Measures From Baseline to 1 Hour (<1 h), 1 to 3 Hours (1-3 h), and 3 Hours (Total) After Immunotherapy^a

Variable	Spearman correlation coefficient	P value	No. of observations
Total diameter (<1 h)	0.49	.05	16
Total diameter from dust mite (<1 h)	0.48	.11	12
Total diameter (1-3 h)	0.02	.95	15
Total diameter from dust mite (1-3 h)	-0.28	.41	11
Total diameter (total)	0.43	.11	15
Total diameter from dust mite (total)	0.42	.20	11
Total dose (<1 h)	0.40	.11	16
Total dose from dust mite (<1 h)	0.57	.04	13
Total dose (1-3 h)	-0.34	.21	12
Total dose from dust mite (1-3 h)	-0.77	<.01	12
Total dose (total)	0.27	.33	15
Total dose from dust mite (total)	0.30	.34	12
<i>Dermatophagoides farinae</i> (<1 h)	0.20	.53	12
<i>D farinae</i> (total)	0.29	.39	11
<i>D farinae</i> (1-3 h)	0.06	.87	11
<i>Dermatophagoides pteronyssinus</i> (<1 h)	0.43	.17	12
<i>D pteronyssinus</i> (total)	0.46	.15	11
<i>D pteronyssinus</i> (1-3 h)	0.11	.75	11
RQLQ (<1 h)	0.57	.03	14
AQLQ (<1 h)	0.30	.62	5
RQLQ (1-3 h)	0.27	.36	14
AQLQ (1-3 h)	0.40	.60	4
RQLQ (total)	0.53	.05	14
AQLQ (total)	0.40	.60	4
IgE (<1 h)	0.27	.31	16
FEV ₁ /FVC (<1 h)	-0.18	.51	16
IgE (total)	0.32	.24	15
FEV ₁ /FVC (total)	-0.08	.77	15
FEF ₂₅₋₇₅ (<1 h)	-0.34	.20	16
PEFR (<1 h)	-0.26	.34	16
FEF ₂₅₋₇₅ (1-3 h)	-0.21	.46	15
PEFR (1-3 h)	0.02	.95	15
FEF ₂₅₋₇₅ (total)	-0.28	.32	15
PEFR (total)	-0.38	.17	15

Abbreviations: AQLQ, Asthma Quality of Life Questionnaire; FEF₂₅₋₇₅, forced expiratory flow between 25% and 75%; FEV₁, forced expiratory volume in 1 second; FVC, forced vital capacity; PEFR, peak expiratory flow rate; RQLQ, Rhinoconjunctivitis Quality of Life Questionnaire.^a Baseline mean diameter was measured 15 minutes after immunotherapy.

Table 7. Correlations Between Changes in Plasma C3a desArg Levels After Allergen Immunotherapy and Changes in Cutaneous Reactivity, Immunotherapy Dosage, Baseline Symptom Scores, and Changes in Spirometric Measures From Baseline to 1 Hour (<1 h), 1 to 3 Hours (1-3 h), and 3 Hours (Total) After Immunotherapy^a

Variable	Spearman correlation coefficient	P value	No. of observations
Total diameter (<1 h)	-0.38	.16	15
Total diameter from dust mite (<1 h)	0.47	.14	11
Total diameter (1-3 h)	-0.07	.80	15
Total diameter from dust mite (1-3 h)	-0.25	0.33	0.72
Total diameter (total)	0.33	.23	15
Total diameter from dust mite (total)	0.72	.01	11
Total dose (<1 h)	-0.08	.78	15
Total dose from dust mite (<1 h)	-0.33	.29	12
Total dose (1-3 h)	0.05	.87	15
Total dose from dust mite (1-3 h)	0.14	.66	12
Total dose (total)	0.12	.67	15
Total dose from dust mite (total)	0.28	.38	12
<i>Dermatophagoides farinae</i> (<1 h)	-0.19	.57	11
<i>D farinae</i> (total)	0.50	.12	11
<i>D farinae</i> (1-3 h)	0.24	.49	11
<i>Dermatophagoides pteronyssinus</i> (<1 h)	-0.54	.09	11
<i>D pteronyssinus</i> (total)	0.67	.02	11
<i>D pteronyssinus</i> (1-3 h)	0.66	.03	11
RQLQ (<1 h)	-0.15	.63	13
AQLQ (<1 h)	0.60	.28	5
RQLQ (1-3 h)	0.04	.91	13
AQLQ (1-3 h)	-0.20	.75	5
RQLQ (total)	-0.02	.94	13
AQLQ (total)	0.60	.28	5
IgE (<1 h)	-0.04	.88	15
FEV ₁ /FVC (<1 h)	0.06	.84	15
IgE (1-3 h)	0.53	.04	15
FEV ₁ /FVC (1-3 h)	0.08	.78	15
IgE (total)	0.44	.10	15
FEV ₁ /FVC (total)	-0.26	.35	15
FEF ₂₅₋₇₅ (<1 h)	0.18	.53	15
PEFR (<1 h)	0.25	.38	15
FEF ₂₅₋₇₅ (1-3 h)	-0.08	.77	15
PEFR (1-3 h)	-0.02	.95	15
FEF ₂₅₋₇₅ (total)	-0.27	.33	15
PEFR (total)	-0.09	.74	15

Abbreviations: AQLQ, Asthma Quality of Life Questionnaire; FEF₂₅₋₇₅, forced expiratory flow between 25% and 75%; FEV₁, forced expiratory volume in 1 second; FVC, forced vital capacity; PEFR, peak expiratory flow rate; RQLQ, Rhinoconjunctivitis Quality of Life Questionnaire.

^a Baseline mean diameter was measured 15 minutes after immunotherapy.

Levels of both of these CSPs are increased in the bronchoalveolar lavage fluid of sensitized asthmatic patients after segmental allergen provocation.⁴ Increased C3 staining along airway epithelium and smooth muscle has been demonstrated in mouse models of ambient particulate-induced airway hyperreactivity.²⁵ Unstimulated human bronchial epithelial cells (BEAS-2B) generate C3.²⁶

The present finding that the dust mite immunotherapy dose is associated with changes in C5a desArg and C3a desArg levels is consistent with the results of Maruo and colleagues,¹ who showed that C5 and C3 are cleaved into their active split products by the inhalant allergen house

dust mite proteases Der p 3 and Der f 3.¹ Endotoxin, a known activator of the alternative pathway of complement,²⁷ is found in multiple extracts (including dust mite extracts) and may account for a portion of the association of dust mite immunotherapy dose with changes in CSP levels. Endotoxin levels are not routinely determined in allergen extracts used for allergen immunotherapy, and they vary greatly among allergens.²⁷ In the study by Trivedi et al,²⁸ *D farinae* extracts from multiple manufacturers were found to consistently have much more endotoxin than did *D pteronyssinus*. We found that changes in C3a desArg levels after dust mite immunotherapy are

Table 8. Correlations of Cutaneous Wheal Responses to Changes in Spirometric Measures (Percentage Predicted and Absolute Value) at 1 (<1 h) and 3 (Total) Hours After Allergen Immunotherapy

Variable	Δ Total diameter (<1 h)			Δ Total diameter from dust mite (<1 h)		
	Spearman correlation coefficient	P value	No. of observations	Spearman correlation coefficient	P value	No. of observations
Δ FEV ₁ /FVC (<1 h)	−0.16	.56	16	−0.25	.44	12
Δ FEF ₂₅₋₇₅ (<1 h)	−0.36	.17	16	−0.73	<.01	12
Δ PEFR (<1 h)	−0.67	<.01	16	−0.26	.41	12
Δ absFEF ₂₅₋₂₇ (<1 h)	−0.36	.17	16	−0.69	.01	12
Δ absPEFR (<1 h)	−0.68	<.01	16	−0.24	.44	12
Δ FEV ₁ /FVC (total)	−0.09	.74	16	−0.48	.11	12
Δ FEF ₂₅₋₇₅ (total)	−0.01	.96	16	−0.43	.17	12
Δ PEFR (total)	−0.60	.01	16	−0.31	.32	12
Δ absFEF ₂₅₋₇₅ (total)	−0.07	.81	16	−0.43	.17	.12
Δ absPEFR (total)	−0.68	<.01	16	−0.27	.40	12

Abbreviations: abs, absolute; FEF₂₅₋₇₅, forced expiratory flow between 25% and 75%; FEV₁, forced expiratory volume in 1 second; FVC, forced vital capacity; PEFR, peak expiratory flow rate; PEFR, peak expiratory flow rate.

associated with the *D pteronyssinus* dosage only. If endotoxin activation of the alternative complement pathway significantly contributed to these findings, the results of the study by Trivedi et al²⁸ suggest there should have been an association with *D farinae* therapy dosage. Furthermore, the difference in patterns of significant time course associations of dust mite immunotherapy and changes in C5a and C3a, namely, that total dust mite dose correlates with changes in C5a levels at 1 hour only and that *D pteronyssinus* dose is associated with changes in C3a desArg levels more than 3 hours after immunotherapy, suggests a differential activation in vivo, as might be expected with an enzymatic process. Thus, although endotoxin activation of complement could be invoked to explain the findings in part, the species-specific endotoxin predominance and time course factors suggest a more complex process.

The presence of CSP in airway tissues has multiple consequences. The anaphylatoxins include C5a, C3a, and C4a, which are generated when human serum is incubated with several different allergens.² C3a has been found to induce bronchoconstriction and edema formation and is involved in the attraction and activation of mast cells and eosinophils when it binds to its receptor in mouse models of asthma.^{3,4} Mice and guinea pigs that are deficient in either C3 or the C3a receptor have diminished airway hyperreactivity.^{5,6}

Whereas C3a is easily appreciated as a potentiator of airway bronchoconstriction and inflammation, the role of C5a is more complex. C5 deficiency has been associated with susceptibility to allergen-induced airway hyperresponsiveness in a murine model of asthma.⁷ The A/J strain develops airway hyperreactivity and pulmonary eosinophilia and has a T_H2 cytokine profile with associated antigen specific IgE. Genotyping studies have revealed that C5 on murine chromosome 2 may be a susceptibility gene for allergen-induced airway hyperreactivity. The susceptibility is inversely propor-

tional to C5 gene expression. The A/J mouse has a 2-base pair deletion at the 5' end of the gene. In addition to being a potent chemoattractant, C5a binds to its receptor on macrophages (CD88) and induces the formation of interleukin 12.⁷ Interleukin 12 promotes a T_H1 immune response, which steers the immune system away from IgE formation and allergic disease. Thus, the presence of C5a may promote T_H1 responses and may help prevent the development of T_H2 IgE-mediated hypersensitivity. Evaluation of levels of C5a and other complement proteins in humans with allergic diseases and correlation with standardized measures of allergic disease activity has not been previously performed in pediatric or adult populations.

Murine studies by Kohl et al⁸ determined the effect of C5a on the dendritic cell presentation of putative allergens to T cells. These mouse studies found that when C5a binds to its receptors on dendritic cells during the period of sensitization to allergens, it decreases their ability to invoke the T_H2 responses that result in allergic inflammation. Conversely, C5a is a known anaphylatoxin that potentiates allergic responses in established allergic disease. C5a receptors (protein and messenger RNA) have also been found on the bronchial smooth muscle cells of humans.²⁹ Therefore, it may be possible for C5a to directly cause bronchoconstriction without the intermediate step of mast cell activation with the release of histamine.

These findings raise the issue of whether plasma CSP levels could be used as biomarkers of clinical allergic disease and asthma. Current biomarkers available for quantifying the severity of asthma and affecting treatment include measurement of airway hyperreactivity with methacholine,³⁰ sputum eosinophils,³¹ and exhaled nitric oxide.^{32,33} Further confirmation of an association of plasma CSP levels with clinical allergic disease would result in an easily measurable blood biomarker for evaluating allergic disease activity.

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