

Original Article

Atopic Dermatitis Endotypes Based on Allergen Sensitization, Reactivity to *Staphylococcus aureus* Antigens, and Underlying Systemic Inflammation

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What is already known about this topic? Atopic dermatitis is a chronic inflammatory skin disease characterized by significant barrier disruption that permits allergen penetration and microbial invasion.

What does this article add to our knowledge? Stratification of patients with atopic dermatitis on the basis of allergen sensitization profiles reveals differential systemic inflammation profiles and anti-*Staphylococcus aureus* toxin antibody levels.

How does this study impact current management guidelines? This study highlights the need to further characterize the allergen-specific responses in patients with atopic dermatitis because more personalized approaches to therapeutic intervention may be needed to effectively treat the differential systemic inflammation and microbial responses.

BACKGROUND: Atopic dermatitis (AD) is a chronic inflammatory disease with significant local and systemic inflammation and barrier disruption. AD is associated with increased risk of allergen sensitization and skin colonization by *Staphylococcus aureus*. The heterogeneity of AD is unknown, and its complexity suggests its subdivision into several endotypes.

OBJECTIVE: To evaluate allergy-driven endotypic differences in patients with AD and identify proteomic signatures to distinguish between inflammatory responses. To perform proteomic

profiling of allergen sensitivity, antibody levels to *S aureus* antigens, and circulating inflammatory mediators to characterize AD subsets in 76 subjects with moderate to severe AD and 39 healthy controls (HCs).

METHODS: Sera were collected from 76 subjects with moderate to severe AD and 39 HCs with no history of skin disease. Serum was tested for levels of total serum immunoglobulin E (IgE) and allergen-specific IgE using a panel of 119 allergens as well as IgE antibodies against *S aureus* antigens, and was profiled for more than 1100 proteins by SOMAscan to detect differential expression of inflammatory mediators.

RESULTS: Total serum IgE levels were significantly ($P < .001$) elevated in subjects with AD versus controls. A greater percentage of subjects with AD were allergic compared with HCs, and patients with AD tested positive to a greater number of allergens than did HCs. IgE was upregulated across 4 allergen subsets (food, perennial, seasonal, and mixed), and each allergen subset was associated with a distinct inflammatory signature marked by a specific suite of upregulated proteins. Finally, IgE antibodies against *S aureus* toxic shock syndrome toxin-1 were significantly upregulated in subjects with seasonal allergy ($P = .0430$) and perennial allergy ($P = .00032$).

CONCLUSIONS: Overall, this study addresses the heterogeneity of AD by characterizing subsets of AD on the basis of allergen sensitization. It also demonstrates the differential systemic inflammation and *S aureus*-specific antibody responses associated with the allergenic endotypes. These unique proteomic signatures may be valuable for precise disease characterization and subsequent personalized treatment. © 2019 American Academy of Allergy, Asthma & Immunology (J Allergy Clin Immunol Pract 2019;■:■-■)

Key words: Allergy; Atopic dermatitis; Proteomics; Inflammation

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Conflicts of interest: J. Wang, L. Yu, H. Liu, L. Greenlees, R. McPhee, A. Ruzin, and M. D. Howell were full-time employees and shareholders in AstraZeneca/MedImmune at the time of this research. H. Liu and M. D. Howell are currently employed at Incyte Corporation and R. McPhee is currently employed by GlaxoSmithKline. E. Guttman-Yassky is an employee of Mount Sinai and has received research funds (grants paid to the institution) from Abbvie, Celgene, Eli Lilly, Janssen, MedImmune/AstraZeneca, Novartis, Pfizer, Regeneron, Vitae, Glenmark, Galderma, Asana, Innovaderm, Dermira, and UCB; and is also a consultant for Sanofi Aventis, Regeneron, Stiefel/GlaxoSmithKline, MedImmune, Celgene, Anacor, AnaptysBio, Dermira, Galderma, Glenmark, Incyte, Novartis, Pfizer, Vitae, Leo Pharma, Abbvie, Eli Lilly, Kyowa, Mitsubishi Tanabe, Asana Biosciences, and Promius. The rest of the authors declare that they have no relevant conflicts of interest.

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Abbreviations used

AD- Atopic dermatitis
 AT- Alpha toxin
 CCL17- Chemokine (C-C motif) ligand 17
 CCL18- Chemokine (C-C motif) ligand 18
 CCL22- Chemokine (C-C motif) ligand 22
 DT- Delta toxin
 HC- Healthy control
 IgE- Immunoglobulin E
 IL- Interleukin
 MDC- Macrophage-derived chemokine
 PARC- Pulmonary and activation-regulated chemokine
 TSST-1- Toxic shock syndrome toxin-1

INTRODUCTION

Atopic dermatitis (AD) is a chronic inflammatory skin disease that affects up to 7% to 10% of adults.¹ Widespread lesions and intense pruritus characterize patients with moderate to severe AD, with a profound impact on their quality of life. Approximately 80% of patients with AD present with increased serum immunoglobulin E (IgE) levels and sensitization to environmental/food allergens ("extrinsic AD"), whereas 20% have normal IgE levels and lack other atopic manifestations ("intrinsic AD").^{2,3}

Characterization of AD in both skin tissue⁴⁻⁶ and blood compartments demonstrated key roles for T_H2 (interleukin (IL)-4 and IL-13) and T_H22/IL-22 cytokines⁷⁻⁹ in driving disease pathogenesis through the disruption of the epidermal barrier and impairing innate immune response.¹⁰⁻¹³ As a result of these barrier abnormalities, allergens have better ability to penetrate, rendering patients with AD more susceptible to recurrent bacterial and viral skin infections.¹⁴

Although current treatment guidelines include allergen-specific immunotherapy in selected patients with AD with concomitant allergen sensitivity,¹⁵ there is little information indicating how this information can assist in the overall management of AD.

Staphylococcus aureus colonization, infection, and production of superantigens are hypothesized to play a role in the pathogenesis of AD.^{14,16} *S aureus* colonizes approximately 90% of patients with AD, and superantigen-producing strains of *S aureus* are found in more than 50% of these patients.¹⁷ Skin colonization in patients with AD can rapidly advance to superinfection with 10⁷ bacterial cells per square centimeter, whereas healthy subjects show a much lower bacterial burden.¹⁸ Up to 60% of *S aureus* colonizing in patients with AD are toxin-producing strains.¹⁹ In addition, patients with AD colonized by *S aureus* may show greater allergen sensitization and barrier dysfunction compared with *S aureus*-negative patients.²⁰ *S aureus* produces several toxins, including alpha toxin (AT), delta toxin (DT), and toxic shock syndrome toxin-1 (TSST-1), all of which induce significant inflammation.²¹ AT provokes significant epithelial damage and inflammation in AD skin.²² DT activates mast cells, resulting in AD-like inflammation in skin,²³ and TSST-1 is a predominant toxin produced by *S aureus* in patients with AD.²⁴

The present study investigated the relationship between allergen sensitization, subsequent systemic inflammation, and

TABLE I. Patients' demographic and baseline characteristics

Measurement	HCS (N = 39)	Patients with AD (N = 76)	P value
Sex			
Female	43.6%	47.4%	.8436
Male	56.4%	52.6%	
Race			
Black	38.5%	9.2%	<.0001
Hispanic	12.8%	18.4%	
White	48.7%	64.5%	
Other	0.0%	7.9%	
Age*	44 (21-68)	46 (18-81)	.1439
Percentage of skin affected*	NA	27% (15%-85%)	NA

NA, Not applicable/available.

For categorical variables, summary statistics of frequency (%) and exact *P* value of Pearson χ^2 test are provided; for continuous variables, summary statistics of median and range and *P* value of Wilcoxon rank sum test are provided.

*Data presented as median years of age (range).

toxin-specific IgE responses in patients with moderate to severe AD and healthy volunteers with the goal of establishing more granular subclassifications of patients with AD.

METHODS

Subjects and assessments

Subjects included 39 adult (>18 years) healthy controls (HCs) with no history of skin disease and 76 adult (>18 years) subjects with moderate to severe AD (affected body surface area > 10%). Subjects were allowed to use emollients only and avoided systemic immunomodulators and phototherapy within the previous 4 weeks and topical steroids or calcineurin inhibitors within the previous week. Detailed characteristics are presented in Table I.

Blood samples were collected from all subjects using BD Vacutainer Plus plastic serum tubes. After collection, blood was allowed to clot by leaving undisturbed at room temperature for 15 to 30 minutes. Tubes were centrifuged within 3 hours of collection (1500g for 10 minutes at room temperature). Sera were transferred to cryotubes and stored at -80°C until analysis.

These studies were conducted according to the Declaration of Helsinki and were approved by the institutional review boards at TR Bio and Icahn School of Medicine at Mount Sinai. All subjects gave written informed consent before enrollment.

Serological measurement of antibody responses and allergen reactivity

Serum samples were analyzed for total serum IgE and allergen sensitivity to a panel of 104 environmental and food allergens using the ImmunoCAP Solid-Phase Allergen Chip (ISAC; TFS, Uppsala, Sweden). Serum levels of IgE antibodies against *S aureus* TSST-1, AT, and DT were analyzed at Meso Scale Discovery (Rockville, Md).

On the basis of the number of allergen-specific IgE responses within panels of perennial inhalants, seasonal inhalants, or food allergens, subjects were further classified as nonallergic, perennial, seasonal, food, or mixed (subjects classified into mixed allergen showed equal levels of reactivity in more than 1 allergen category).

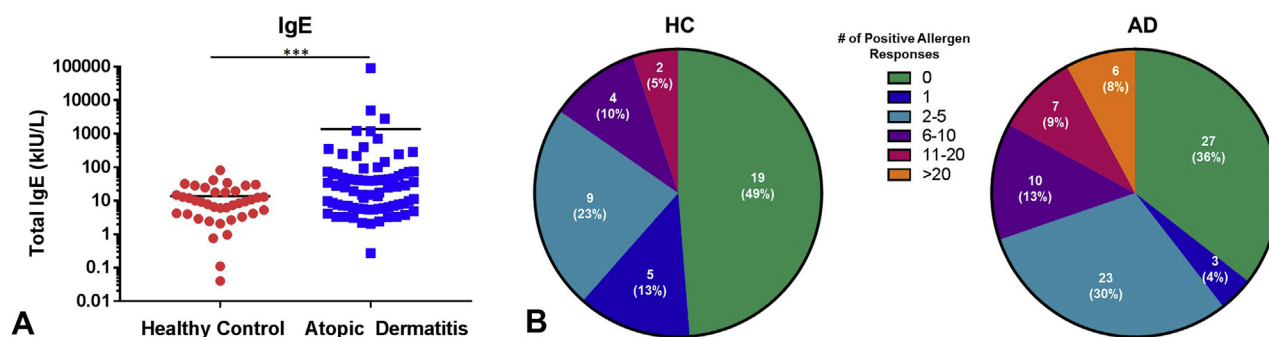


FIGURE 1. Total IgE levels and the number of sensitized allergens is increased in patients with AD compared with HCs. (A) Scatter plot showing the expression of total IgE levels in HCs and patients with AD. *** $P < .001$. (B) Pie chart illustrating the percentage of HCs and patients with AD who reacted to a given number of allergens.

SOMAscan assay

Sera were evaluated using the previously described SOMAscan proteomic assay (SOMAscan Assay 1.1k), which detects levels of 1129 proteins simultaneously.⁸ Briefly, DNA-based Slow Off-rate Modified Aptamers that specifically bind to natively folded proteins were designed. These were mixed with serum samples to allow binding. Sample concentrations of 40%, 1%, and 0.005% were used to allow measurement of protein concentrations at approximately 8 log range. Protein-bound Slow Off-rate Modified Aptamers were eluted and quantified using a DNA microarray. Protein concentrations in sera were transformed into corresponding signature of DNA aptamer concentrations followed by a fluorescence signal on the microarray. Values are reported in relative fluorescence units.

Statistical analysis

Statistical differences in IgE between groups were determined using a nonparametric Mann-Whitney test. In cases in which multiple groups were compared (allergen levels or protein expression) with one another, data were analyzed by a 1-way ANOVA and significant differences between exposure groups were determined by a Tukey-Kramer test. Statistical differences in anti-*S aureus* toxin antibody levels were determined using the 1-way ANOVA model with heterogeneous within-group variance applied for comparison of mean IgE levels relative to the healthy nonallergic group. Statistical analysis was conducted using GraphPad Prism (GraphPad Software, La Jolla, Calif), R (www.r-project.org), and SAS (SAS Institute, Inc, Cary, NC).

Before analysis, relative fluorescence unit values from SOMAscan assays were normalized against hybridization control sequences to correct for any systematic effects introduced during hybridization. Median normalization was performed across samples within an array. Calibration was performed using samples in each array to allow comparison across runs.²⁵ To reduce heteroscedasticity, values were log2-transformed. Protein expression was modeled using a linear model with age as numerical covariate and disease as factor covariate; parameters were estimated using the empirical Bayes method available in the *Limma* package in R.²⁶ Default parameters were used, except that the “proportion” parameter was set to 0.05. Between-group comparisons were assessed using contrast, and the P values of the moderated t test were adjusted by the Benjamini-Hochberg procedure, which

TABLE II. Allergen subclassification

Subjects	No allergen reactivity	Perennial	Seasonal	Food	Mixed	Total
HCs	19	11	7	1	1	39
Subjects with AD	27	32	9	2	6	76

Number of HCs or subjects with AD within each allergen classification based on serological responses to 104 allergens.

controls the false-discovery rate. Differentially expressed proteins were defined as those with fold change greater than or equal to 1.5 and false-discovery rate—adjusted P value of .05 or less. Correlation analysis was performed with Pearson correlation test or Spearman correlation test (when normal distribution of the data cannot be guaranteed).

RESULTS

AD is characterized by higher serum IgE levels and greater allergen sensitization than those in HCs

Seventy-six patients with moderate to severe AD (body surface area $> 10\%$) and 39 HCs were included (Table I). Age and sex were comparable across groups. A lower percentage of blacks was noted among the AD group versus the control group ($P < .0001$). Median body surface area involvement of AD was 27% (15%-85%).

Total serum IgE levels were measured in all subjects. Patients with AD had significantly higher (mean, 1372.52 ± 1185.07 kU/L; $P < .001$; median, 25.52 kU/L) IgE levels compared with controls (mean, 13.67 ± 2.44 kU/L; median, 9.13 kU/L) (Figure 1, A). To better understand the primary allergen reactivity of the cohort, subjects with AD and controls were further classified by allergen-specific IgE testing to a large panel of 104 allergens (see Table E1 in this article's Online Repository at www.jaci-inpractice.org). Overall, a greater percentage of subjects with AD showed allergen sensitization compared with controls. Approximately 61% (46 of 76) of subjects with AD tested positive on at least 2 allergen tests, and 8% (6 of 76) tested positive on more than 20 tests (Figure 1, B). HCs had a lower allergen response rate, with only 39% (15 of 39) reacting to 2 or more allergen tests. No controls tested positive to more than 20

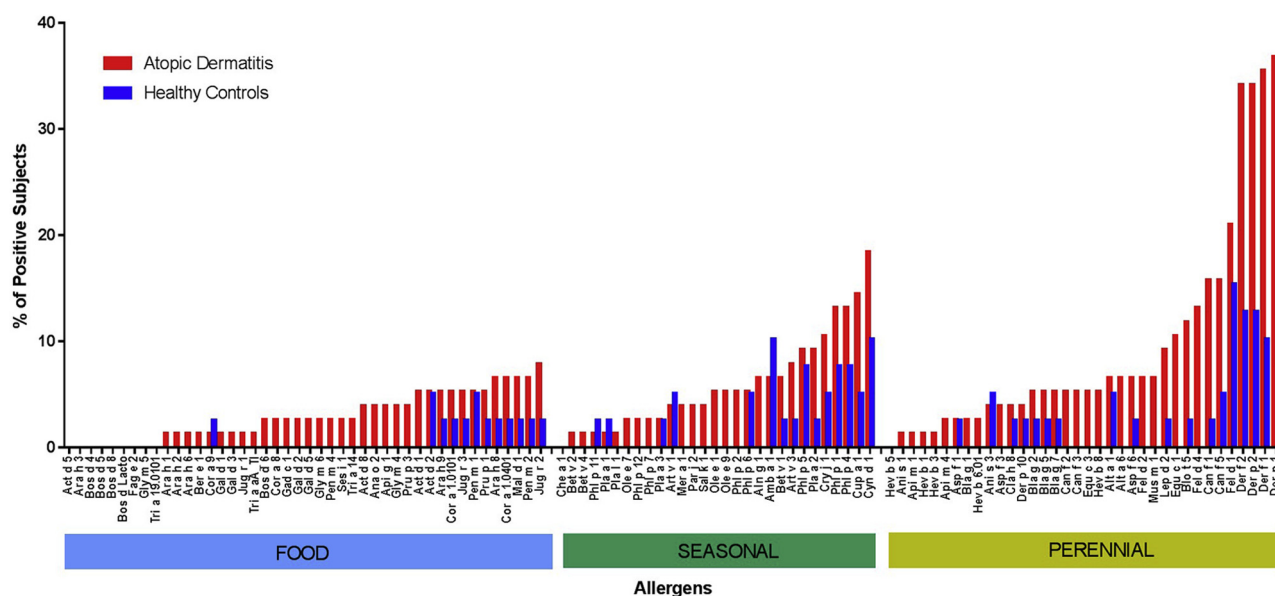


FIGURE 2. A greater percentage of subjects with AD exhibit specific IgE responses to allergens. Sera from all subjects were analyzed for allergen-specific reactivity using the ISAC ImmunoCap assay. Allergens were further separated into subsets of food, seasonal, and perennial. The percentage of subjects with AD who tested positive is indicated by the red lines, whereas the blue lines indicate the percentage of HCs.

tests, and indeed, only 5% (2 of 39) tested positive to between 11 and 20 allergen tests. Nearly half (46%; 18 of 39) the controls showed no reaction to allergens at all.

On the basis of the number of allergen-specific IgE responses within panels of allergens, subjects were further clustered into 1 of the following 5 groups: no allergen reactivity, perennial inhalants, seasonal inhalants, food allergens, and mixed allergen categories (subjects classified into mixed allergen showed equal levels of reactivity in more than 1 allergen category) (Table II). Among patients with AD, 27 showed no allergenic reactivity, whereas 32, 9, 2, and 6 showed reactivity to perennial, seasonal, food, and mixed allergen groups, respectively.

To get a sense of the most common allergens for which subjects tested positive, we grouped the allergens by classification and sorted them by frequency of allergen positivity (Figure 2). Dust mite (Der p 1, Der p 2, Der f 1, and Der f 2), cat (Fel d 4), and horse (Equ c 1) sensitization was observed in more than 20% of subjects with AD compared with less than 15% of controls. In addition, a greater percentage of subjects with AD reacted to timothy grass (Phl p 1 and Phl p 4), Bermuda grass (Cyn d 1), and various tree pollens (Cry j 1, Cyn d 1, and Pla a 2) in comparison with HCs. Of note, patients with AD did not show significantly increased sensitivity to egg, peanut, wheat, or milk compared with controls.

Using the raw values (ISAC Standardized Units) for Der p 1, Der p 2, Der f 1, Der f 2, Fel d 1, and Equ c 1, we compared the levels of allergen sensitization in each endotype for subjects with AD as well as HCs (Figure 3, A-F). As expected, no significant increases in allergen sensitization were observed for the different endotypes within the HCs. Interestingly, the perennial allergy cohort showed overall the highest reactivity for the subjects with AD followed by the food-reactive cohort. Specifically, the perennial AD cohort had significantly ($P < .01$) higher levels of Der p 1, Der p 2, Der f 1, and Der f 2 when compared with

either of the nonallergic endotypes. Although the mean levels for the Fel d 4 and Equ c 1 allergens were numerically elevated in the perennial AD cohort, they did not reach statistical significance. No significant increases in these allergens were observed for the AD seasonal or AD mixed endotypes; in addition, the data for the food endotypes should be interpreted with caution because of the limited group size.

Proteomic analysis in blood reveals common and unique markers in patients with AD subclassified by allergen sensitization

To determine whether the various allergen subgroups were associated with a particular inflammatory skewing, we performed SOMAscan to test a panel of 1300 proteins in the serum of subjects with AD and HCs. Figure 4, A, illustrates a heatmap of the top differentially expressed proteins delineating our allergen subgroups. A total of 98 proteins showed significant upregulation (fold change ≥ 1.5) in at least 1 allergy subset (Figure 4, A and B) and 28 proteins showed significant downregulation (fold change ≥ 1.5) (Figure 4, C).

In subjects with AD, IgE was upregulated across all 4 allergen subsets (Figure 4, B, and Figure 5, A), consistent with our previous reports showing that IgE is a unique marker in the AD signature.⁸ No protein was downregulated across all allergen subsets (Figure 4, C). Each allergen subset was associated with a distinct inflammatory signature marked by a specific suite of upregulated proteins, as detected by SOMAscan. The food AD cohort, for example, showed significant upregulation of 56 distinctive inflammatory markers, including periostin, chemokine (C-C motif) ligand 3 (CCL3)/macrophage inflammatory protein 1 α , CCL27/cutaneous T-cell-attracting chemokine, kynureninase, and matrix metalloproteinase 13 (Table III). Mixed allergy displayed 10 uniquely upregulated markers, including glucocorticoid-induced TNFR family related, IL-4R, and matrix metalloproteinase 10.

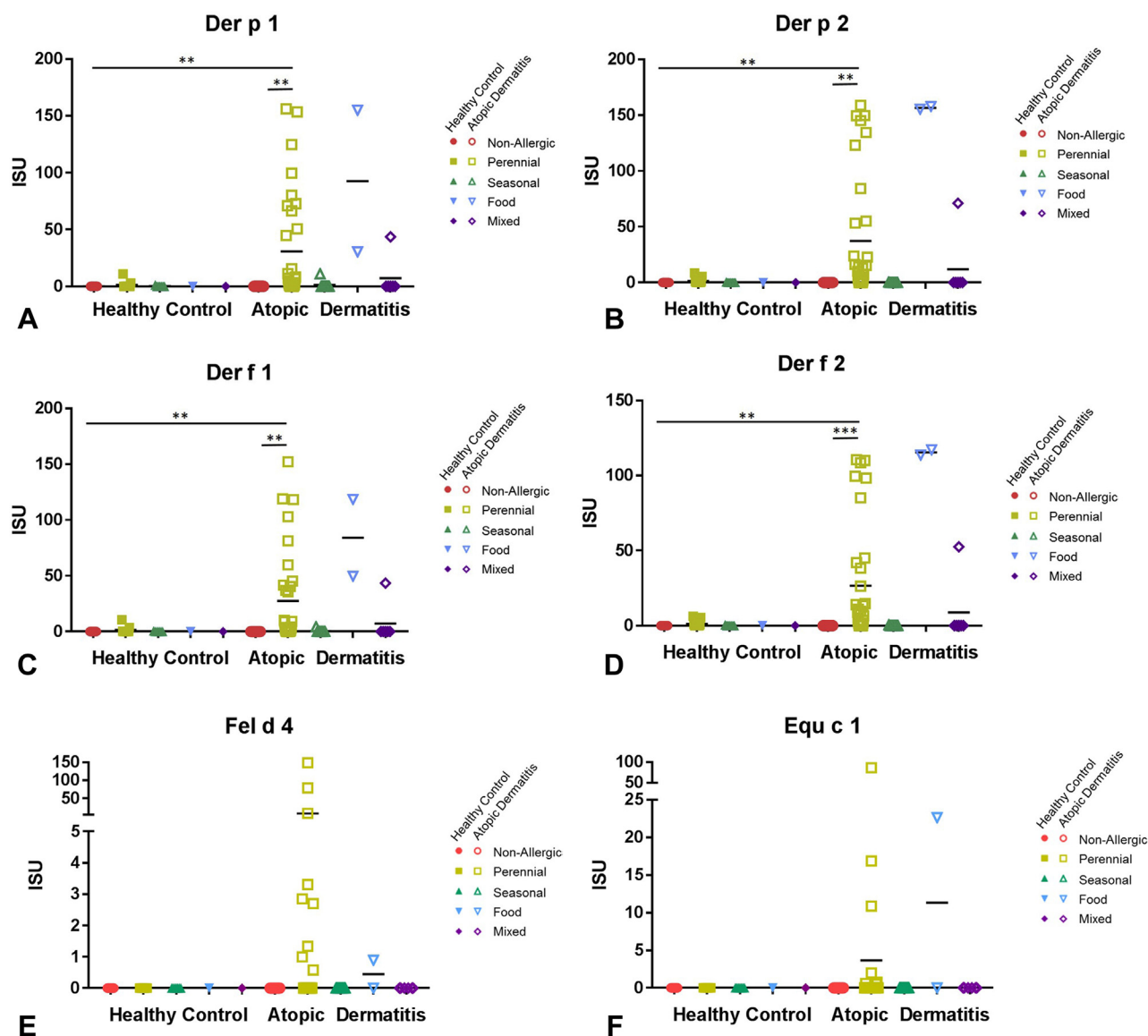


FIGURE 3. The perennial and food AD cohorts exhibit greater allergen sensitization. Scatter box plots illustrate the allergen-specific IgE levels (ISAC Standardized Units [ISUs]) for each cohort against Der p 1 (A), Der p 2 (B), Der f 1 (C), Der f 2 (D), Fel d 4 (E), and Equ c 1 (F). ** and *** indicate statistical significance of $P < .01$ and $P < .001$, respectively.

Perennial allergy showed 2 unique markers (sialic acid-binding immunoglobulin-type lectin-14 and peptidase inhibitor 3/elafin), whereas seasonal allergy had 9 markers, including IL-8/CXCL8, signal transducer and activator of transcription 3, and sialic acid-binding immunoglobulin-type lectin-6. Proteins uniquely down-regulated in allergy subtypes are outlined in Table IV.

Of the 98 proteins upregulated in at least 1 of the AD allergy subsets, only chemokine (C-C motif) ligand 17 (CCL17)/thymus and activation-regulated chemokine (TARC), chemokine (C-C motif) ligand 18 (CCL18)/pulmonary and activation-regulated chemokine (PARC), and chemokine (C-C motif) ligand 22 (CCL22)/macrophage-derived chemokine (MDC), all associated with T_H2 pathogenesis, were upregulated across food, perennial, and seasonal allergy groups, representing 3.1% (3 of 98) of the upregulated markers (Figure 4, A and B). When separated by allergen, CCL17/TARC and CCL22/MDC showed highest levels

in the food allergy group followed by both the seasonal and perennial groups (Figure 5, B and C). Proteins common to food and perennial groups were the T_H2 marker IL-22 and the cell adhesion protein desmocollin 3 (Figure 5, D and E). Finally, CCL18/PARC showed significant upregulation in the food and perennial allergen subsets (Figure 5, F).

Subjects with allergic AD exhibit significant increases in IgE levels against *S aureus* toxins

Serum levels of IgE antibodies against the *S aureus* toxins including AT, DT, and TSST-1 were measured and calculated for each subject by comparing the geometric mean of the antibody level relative to HCs. Levels were then subdivided by antibody, disease profile, allergen sensitization, and toxin type (Figure 6; see Table E2 in this article's Online Repository at www.jaci-inpractice.org). IgE antibodies against *S aureus* toxin

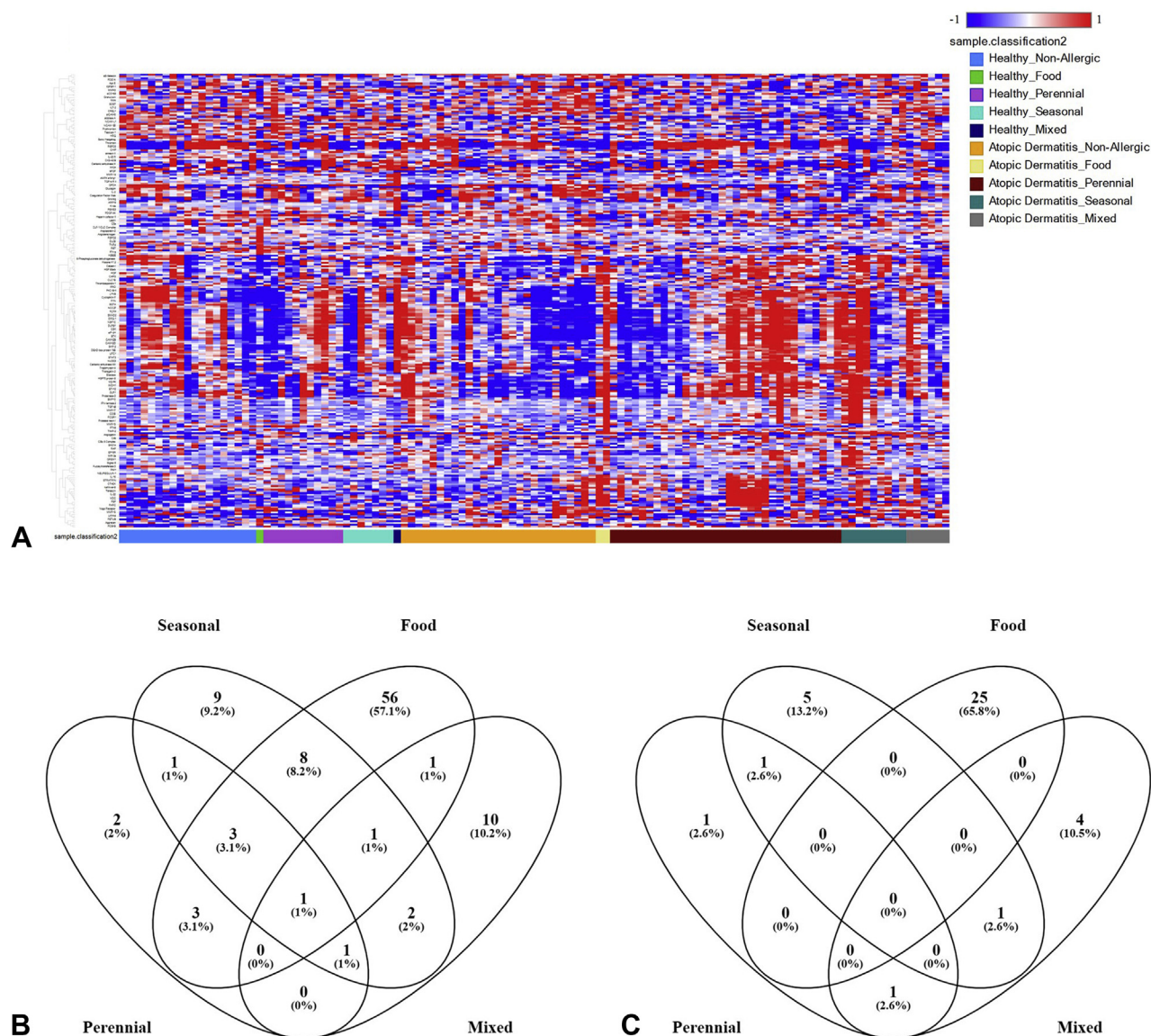


FIGURE 4. Protein targets with significant expression change in 4 allergen subsets of subjects with AD and HCs. **(A)** Heatmap showing expression of proteins with significant upregulation and downregulation per allergen subset ($FCH \geq 1.5$ and $FDR \leq 0.05$). Red indicates high expression, and green indicates low expression. **(B)** Venn diagram showing overlap of proteins with significant upregulation in subjects with AD with perennial, seasonal, food, and mixed allergy, compared with healthy nonallergic subjects, based on serological responses to 104 allergens. **(C)** Venn diagram showing overlap of proteins with significant downregulation in subjects with AD with perennial, seasonal, food, and mixed allergy, compared with healthy nonallergic subjects, based on serological responses to 104 allergens. *FCH*, Fold change; *FDR*, false-discovery rate.

TSST-1 were significantly upregulated in subjects with seasonal allergy ($P = .0430$) and perennial allergy ($P = .00032$).

DISCUSSION

AD is a heterogeneous inflammatory skin disease characterized by significant barrier disruption and bacterial colonization. This permits enhanced allergen penetration and potentiates systemic inflammation. The present study characterized the heterogeneity of AD by initially evaluating allergen sensitization profiles and then assessing corresponding circulating inflammatory responses.

Subjects in the HC and AD groups were age- and sex-matched to ensure consistency between populations; however, there were significantly more blacks in the HC group (Table 1). Previous studies have demonstrated similar T_H2/T_H22 skewing in black and European American patients with AD²⁷; however, levels of T_H1/T_H17 differ on the basis of ethnicity. Therefore, additional studies are needed with matched race and ethnicities to confirm the observations in this study.

Approximately 80% of patients with AD have evidence of sensitivity to 1 or more environmental or food allergens.³ Accordingly, determination of the atopic status of patients with

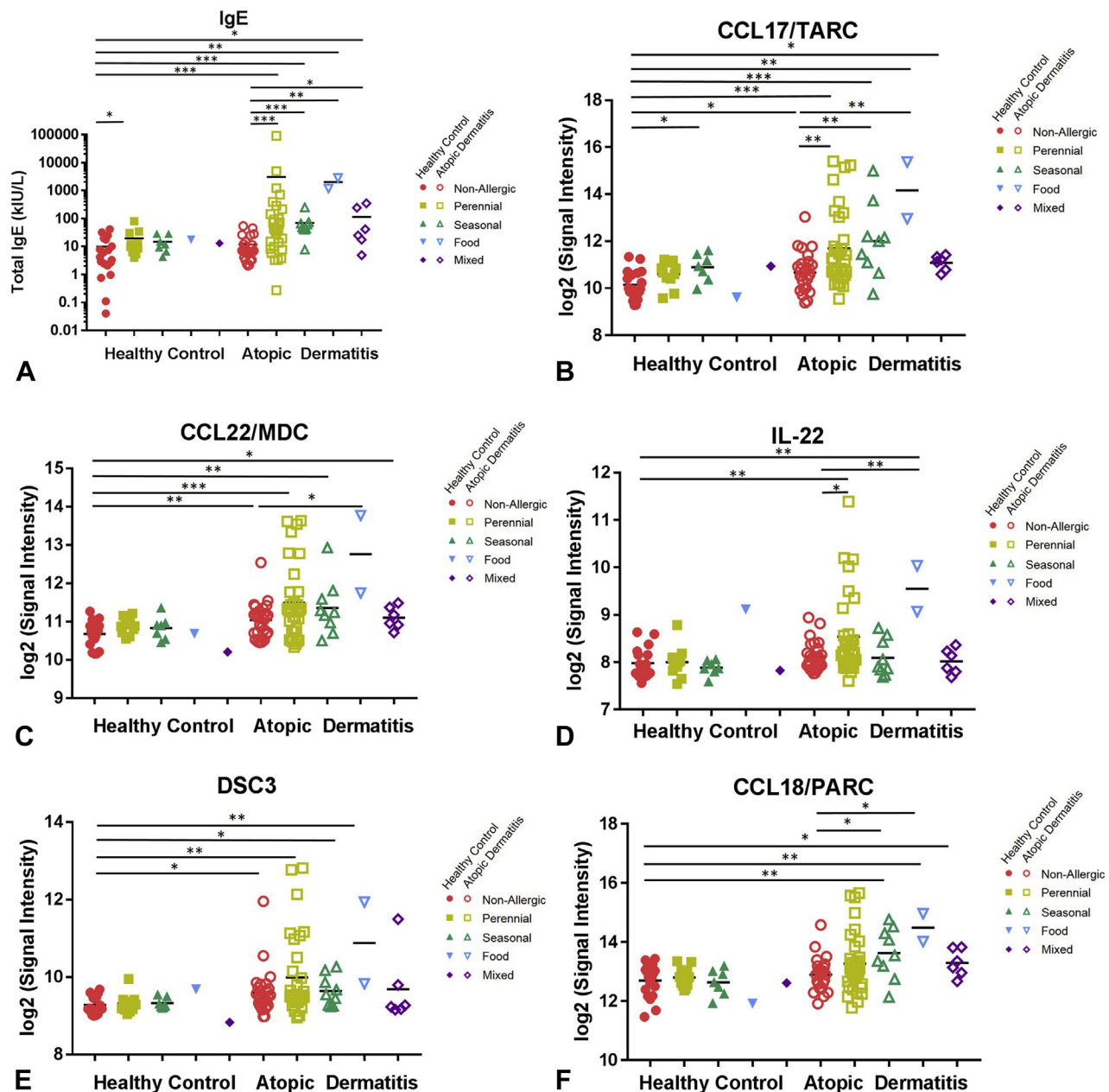


FIGURE 5. Differential expression of markers across the different endotypes of HCs and subjects with AD. Scatter dot plots illustrating the expression of (A) IgE, (B) CCL17/TARC, (C) CCL22/MDC, (D) IL-22, (E) DSC3, and (F) CCL18/PARC across the different HC and AD cohorts. In graphs B to F, the y-axis indicates log₂-transformed signal intensity from SOMAscan assays (relative fluorescence units). DSC3, Desmocollin 3; MDC, macrophage-derived chemokine; TARC, thymus and activation-regulated chemokine. Significant *P* values were designated as ****P* < .001, ***P* < .01, or **P* < .05.

AD through measurement of total serum IgE level and allergen sensitivity is frequently performed in clinical practice; however, testing is typically conducted with a representative panel of allergens. Although current treatment recommendations include the use of allergen-specific immunotherapy in selected patients with AD with concomitant allergen sensitivity,²⁸ there is little information indicating how this information could otherwise be used to assist in the overall management of AD. Furthermore, determination of atopic status, including sensitivity to allergens, would be an important advancement in the potential understanding of disease phenotypes. As expected, total serum IgE

levels were significantly greater in patients with AD than in HCs. Interestingly, levels within the AD cohort differed depending on the allergenic classification. The highest mean IgE levels were observed in perennial allergen-sensitized subjects with AD (*n* = 32) followed by food (*n* = 2), mixed (*n* = 6), and seasonal allergens (*n* = 9). In contrast, no significant differences in total IgE levels were observed across the different allergenic classifications of the HC group.

Of 104 allergens, a greater, and statistically significant (*P* < .05), prevalence was observed for dust mite (Der p 1, Der p 2, Der f 1, and Der f 2), cat (Fel d 4), and horse (Equ c 1)

TABLE III. Proteins significantly ($P < .05$) upregulated in allergic endotypes of subjects with AD in comparison to subjects with nonallergic AD

Group	Protein	Fold change	P value
Perennial (n = 32)	IgE	11.25	<.0001
	CCL17/TARC	2.9287	<.0001
	Siglec-14	1.8418	.0118
	CCL22/MDC	1.7553	<.0001
	C3a	1.663	.0181
	IL-9	1.6463	.0024
	DSC3	1.6371	.0006
	PAPP-A	1.5099	.0119
	PI3/Elafin	1.5089	.0006
	CCL18/PARC	1.4928	.0073
	IL-22	1.4706	.0005
Seasonal (n = 9)	IgE	14.0385	<.0001
	CCL17/TARC	3.6271	<.0001
	C3a	2.1818	.0097
	CCL18/PARC	1.9158	.002
	PAPP-A	1.8438	.0076
	CATE	1.7821	<.0001
	ATPO	1.7791	.0024
	GREM1	1.7626	<.0001
	PKC-Z	1.7195	.0002
	STAT3	1.7174	.0003
	C3b	1.6713	.0024
	LKHA4	1.6274	.0024
	CCL22/MDC	1.6046	.0112
	IL-8/CXCL8	1.6027	.0005
	RBM39	1.5982	.0006
	Siglec-6	1.579	<.0001
	MMP-17	1.5774	<.0001
	ARI3A	1.5756	.0002
	CCL13/MCP-4	1.5436	.0003
	Annexin I	1.5364	.0114
	Bcl-2	1.4983	.0004
	Ephrin-B3	1.4971	<.0001
	CSK	1.4864	.0396
	PCSK9	1.481	.0228
	EP15R	1.4754	.0012
	CD30	1.4585	.0001
Food (n = 2)	IgE	114.294	<.0001
	CCL17/TARC	16.159	<.0001
	Hemoglobin	9.8738	.0035
	Mesothelin	7.2881	<.0001
	Phosphoglycerate mutase 1	5.2562	.0034
	CCL22/MDC	4.2434	<.0001
	PTK6	3.937	<.0001
	Cathepsin V	3.725	<.0001
	CCL18/PARC	3.4763	.0013
	IGFBP-1	3.2929	.0446
	IL-9	3.2896	.0046
	DSC3	3.0271	.0025
	CgA	3.0186	.0294
	IL-22	2.967	.0001
	STRATIFIN	2.9616	.0017

(continued)

TABLE III. (Continued)

Group	Protein	Fold change	P value
	XTP3A	2.7322	<.0001
	Elastase	2.5503	.0002
	EDAR	2.517	.0133
	Tenascin	2.4921	.004
	Proteinase-3	2.3851	.0303
	MMP-13	2.3007	<.0001
	CCL13/MCP-4	2.2204	.0003
	Fucosyltransferase 3	2.2151	.0416
	HSP 90a/b	2.215	.0003
	M2-PK	2.2051	.0032
	OLR1	2.1974	.0435
	CCL3/MIP-1a	2.1971	.0092
	PSP	2.17	.0343
	Azuocidin	2.1147	.0422
	Alpha enolase	2.0982	.0026
	C4b	2.0796	.027
	Myeloperoxidase	2.039	.0164
	CD30	2.0322	.0001
	HSP 90b	2.0172	.0006
	PKC-Z	2.0033	.0077
	LKHA4	1.9239	.0247
	MMP-17	1.9208	.001
	BSP4	1.9164	.0063
	HSP70 protein 8	1.9011	.0006
	CTACK	1.9005	<.0001
	TIMP-2	1.8955	.0001
	B7-H2	1.8926	.0195
	PIM1	1.8468	<.0001
	Protease nexin I	1.8016	.0025
	BID	1.8004	.0407
	hnRNP A/B	1.7757	.0403
	MAPK14	1.7571	.001
	RBM39	1.7384	.0245
	14-3-3 protein zeta/delta	1.7294	.0118
	ER	1.7197	.0021
	KYNU	1.6905	.0137
	Thrombospondin-1	1.6835	.0134
	Kallikrein 8	1.6793	.0151
	NAGK	1.678	.0436
	Bcl-2	1.6688	.0132
	PHI	1.6539	.0398
	Ephrin-B3	1.6513	.0072
	GREM1	1.6372	.0332
	GPC2	1.6218	.0154
	DLL4	1.621	.0148
	NCAM-L1	1.6195	.0066
	Periostin	1.6044	.033
	CLC1B	1.5989	.0104
	FCGR1	1.5698	.0208
	TGF-b3	1.5544	.0074
	IFN-lambda 2	1.5525	.0268
	EGF	1.552	.0479
	CAPG	1.5466	.049
	Kallikrein 11	1.5346	.0096

(continued)

TABLE III. (Continued)

Group	Protein	Fold change	P value
Mixed (n = 6)	IL-16	1.5255	<.0001
	Cadherin-12	1.5209	.0285
	Resistin	1.5153	.0454
	BMP10	1.5129	.0005
	IgE	11.7994	.0003
	IGFBP-1	2.7437	.0074
	PAPP-A	2.1624	.0038
	C3b	2.0855	.0002
	LKHA4	1.8851	.0007
	NEUREGULIN-1	1.7347	.0055
	PDE1A	1.707	.011
	PBEF	1.688	.0108
	IL-4 sR	1.6541	.008
	GITR	1.5811	.0034
	HCC-1	1.5735	.0057
	AMPK a2b2g1	1.5704	.0053
	PCSK9	1.5604	.0258
	MMP-10	1.5143	.0275
	SH21A	1.5022	.0114
	DLC8	1.4879	.0197

ATPO, ATP synthase subunit O; AMPK a2b2g1, protein kinase AMP-activated catalytic subunit alpha; ARI3A, AT-rich interactive domain-containing protein 3A; B7-H2, inducible T cell costimulator ligand; Bcl-2, B-cell lymphoma 2; BID, BH3 interacting domain death agonist; BMP10, bone morphogenetic protein 10; BSSP4, brain-specific serine protease 4; C3a, complement 3a; C3b, complement 3b; C4b, complement 4b; CAPG, macrophage-capping protein; CD30, cluster of differentiation 30; CgA, glycoprotein hormones alpha chain; CLC1B, C-type lectin domain family 1 member B; CSK, C-terminal src kinase; CTACK, cutaneous T-cell-attracting chemokine; DSC3, desmocollin-3; DLC8, dynein light chain 8; DLL4, delta-like 4; EDAR, ectodysplasin-A receptor; EGF, epidermal growth factor; EP15R, epidermal growth factor receptor substrate 15-like 1; ER, leucine-rich receptor-like protein kinase family protein; FCGR1, Fc gamma RI; GITR, glucocorticoid-induced TNFR family related; GPC2, glypican-2; GREM1, gremlin-1; HCC-1, hemofiltrate CC chemokine-1; HSP, heat shock protein; IFN-lambda 2, interferon lambda 2; IGFBP-1, insulin-like growth factor-binding protein 1; IL-4 sR, interleukin 4 soluble receptor; KYNu, kynurine; LKHA4, leukotriene A-4 hydrolase; M2-PK, pyruvate kinase PKM; MAPK14, p38α mitogen-activated protein kinase; MCP-4, monocyte chemoattractant protein 4; MIP-1a, macrophage inflammatory protein-1 alpha; MMP, matrix metalloproteinase; NAGK, N-acetyl-D-glucosamine kinase; NCAM-L1, L1 cell adhesion molecule; OLR1, oxidized low density lipoprotein receptor 1; PAPP-A, pappalysin-1; PBEF, pre-B cell-enhancing factor; PCSK9, proprotein convertase subtilisin/kexin type 9; PDE1A, phosphodiesterase 1A; PI3, peptidase inhibitor 3; PIMI, proto-oncogene serine/threonine-protein kinase; PKC-Z, protein kinase C - zeta; PSP, phosphoserine phosphatase; PTK6, protein tyrosine kinase 6; RBM39, RNA binding motif protein 39; SH21A, SH2 domain-containing protein 1A; Siglec, sialic acid-binding immunoglobulin-like lectin; STAT3, signal transducer and activator of transcription 3; TGF-β3, transforming growth factor, beta 3; TIMP-2, tissue inhibitor of metalloproteinases 2.

allergens in all patients with AD. Interestingly, we did not observe significantly increased allergen sensitivity to egg, peanut, wheat, or milk in this cohort of subjects with AD and only 2 subjects with AD had dominant food allergen sensitization. Importantly, IgE levels for each of the aforementioned allergens were elevated to a greater degree in perennial allergen-sensitized subjects with AD, corresponding with the overall increased total serum IgE in this population. Although not significant, the total IgE levels and allergen-specific IgE levels (Der p 1, Der p 2, Der f 1, Der f 2, Fel d 4, and Equ c 1) in perennial sensitized HCs trended toward significance, suggesting a dominant role for perennial allergen sensitization in the IgE-mediated pathogenesis of AD. Previously, we demonstrated a significant correlation

TABLE IV. Proteins significantly ($P < .05$) downregulated in allergic endotypes of subjects with AD in comparison to subjects with nonallergic AD

Group	Protein	Fold change	P value
Perennial (n = 32)	CK-MB	-1.8461	.0016
	bFGF	-1.7405	.015
	GPDA	-1.694	.016
Seasonal (n = 9)	Thrombin	-2.4594	.0206
	TFF1	-1.9589	.007
	bFGF	-1.9359	.0373
	RUXF	-1.8227	.0454
	ApoM	-1.5838	.0014
Food (n = 2)	HGFA	-1.5272	.0331
	Cathepsin V	-1.4775	.0054
	Haptoglobin; Mixed type	-19.9601	<.0001
	Thrombin	-6.2046	.0108
	Carbonic anhydrase III	-3.7706	.0088
	Carbonic anhydrase I	-3.3407	.0277
	CCL28	-2.6499	.0188
	MICA	-2.4396	.0332
	Prothrombin	-2.2272	.0124
	Apo B	-2.1649	.0029
	Granulysin	-2.0362	.0115
	SOST	-2.0106	.0192
	NOTC2	-1.9868	.0247
	PTN	-1.8659	.0093
	MMP-7	-1.8617	.0024
	RSPO3	-1.8002	.0361
	ILT-4	-1.7636	.0014
	MP2K4	-1.7025	.0365
	NPS-PLA2	-1.681	.0237
	MFGM	-1.6588	.0385
	Sonic Hedgehog	-1.6414	.0214
	Annexin II	-1.604	.003
	Heparin cofactor II	-1.6001	.0037
	CKAP2	-1.5917	.0007
	IL-23 R	-1.572	.0157
	FLRT3	-1.5663	.0258
	Apo E3	-1.5361	.0347
	SLPI	-1.5046	.0033
Mixed (n = 6)	CK-MB	-2.0501	.0209
	BMP-14	-2.0268	<.0001
	6-Phosphogluconate dehydrogenase	-1.9269	.0349
	CK2-A2:B	-1.795	.0124
	Cathepsin V	-1.5857	.0046
	PPID	-1.5262	.0069

Apo B, Apolipoprotein B-100; Apo E3, apolipoprotein E; ApoM, apolipoprotein M; bFGF, fibroblast growth factor 2; BMP-14, growth/differentiation factor 5; CK2-A2:B, casein kinase 2 alpha 2; CKAP2, cytoskeleton-associated protein 2; CK-MB, creatinine kinase; FLRT3, fibronectin leucine rich transmembrane protein 3; GPDA, NAD+-dependent glyceraldehyde phosphate dehydrogenase; HGFA, hepatocyte growth factor activator; IL-23 R, interleukin 23 receptor; ILT-4, leukocyte immunoglobulin-like receptor subfamily B member 2; MFGM, lactadherin; MICA, MHC class I polypeptide-related sequence A; MMP-7, matrix metalloproteinase-7; MP2K4, mitogen-activated protein kinase kinase 4; NOTC2, neurogenic locus notch homolog protein 2; NPS-PLA2, phospholipase A2; PPID, peptidylprolyl isomerase D; PTN, pleiotrophin; RSPO3, R-spondin-3; RUXF, small nuclear ribonucleoprotein F; SLPI, antileukoproteinase; SOST, sclerostin; TFF1, trefoil factor 1.

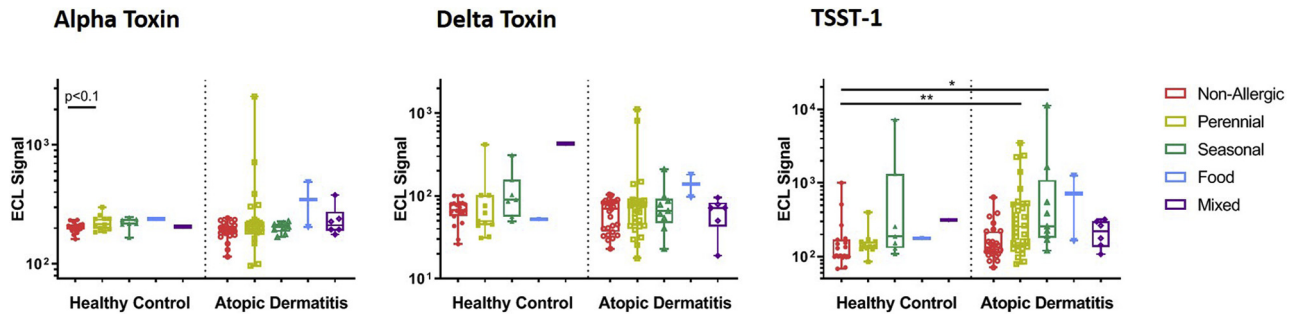


FIGURE 6. IgE antibody levels against *S aureus* AT, DT, and TSST-1. Level of IgE antibody against *S aureus* toxins in patients with AD and HCs, separated by allergen sensitization. Significant *P* values were designated as ***<.001, **<.01, or *<.05.

between disease severity and IgE levels⁸; however, the present study further clarified which allergens are driving the expansive IgE production in a representative AD population.

As mentioned previously, the overall increase in allergen sensitivity in patients with AD presented here is consistent with previous studies, suggesting that T_H2 pathway activation plays a central role in the pathogenesis of AD by activating inflammatory pathways in multiple cell types, impairing epidermal barrier structure, and inducing allergen sensitization.^{2,29} These findings support the hypothesis that determination of atopic status by measuring total serum IgE and allergen sensitivity in patients with AD may allow identification of patient subsets, which may have implications for future therapeutic approaches. Clinical studies evaluating therapeutics in patients with moderate to severe AD currently use allergen sensitization and disease extent as independent variables for inclusion and evaluation of efficacy.^{30–32} This study demonstrates that further analyses of AD subgroups may provide insights into the effectiveness of therapeutics across the heterogeneous spectrum of AD and also increase our understanding of key factors driving biomarkers associated with disease severity.

The present study investigated the relationship between allergen sensitization and subsequent toxin-specific IgE responses in patients with AD and HCs. Using healthy, nonallergic individuals as baseline controls, Figure 6 illustrates the effect that allergic sensitization in the setting of AD has on *S aureus* toxin-specific IgE responses. Interestingly, IgE antibody responses in patients with nonallergic AD were similar to those in healthy, nonallergic controls. In contrast, allergic sensitization corresponded with IgE increases for both healthy subjects and subjects with AD. These data demonstrate a positive relationship between allergen reactivity and *S aureus* toxin-specific responses. Specifically, we demonstrate clear separation of toxin-specific IgE antibody responses between allergic subclasses and nonallergic subjects.

Using broad proteomic analysis, we identified unique proteomic signatures for the different AD endotypes when compared with the nonallergic HC subjects. Interestingly, several proteins were elevated in more than 1 allergenic endotype of subjects with AD. Specifically, IgE was elevated across all AD endotypes, whereas CCL18/PARC, CCL17/TARC, and CCL22/MDC were elevated in the food, perennial, and seasonal AD endotypes. We previously described the significant elevation of these 3 proteins⁸ as a potential signature that distinguishes AD from other inflammatory skin diseases with similar barrier disruption

and overlapping epidermal inflammation. In addition, we observed increased IL-22 and desmocollin 3 in the food and perennial allergen endotypes. Interestingly, an IL-22 mAb demonstrated a therapeutic benefit in subjects with AD with more disease,³³ suggesting potential utility in subsets of subjects with AD. Additional investigation is needed to explore the relationship between IL-22 in circulation, expression in the active lesion, and allergen sensitization. Desmocollin 3 is a transmembrane protein of the cadherin family that is primarily required for cell adhesion and desmosome formation, and is compromised in pemphigus.³⁴ CCL18/PARC is a dendritic cell–derived chemokine upregulated in AD that mediates skin homing of human memory T cells. Furthermore, a significant decrease in PARC was observed in AD after immunotherapy.³⁵

Despite overlapping inflammation, we observed distinct signatures for each of the AD endotypes, with 56 proteins uniquely upregulated in food-allergic AD, 2 proteins in perennial AD, 9 proteins in seasonal AD, and 10 proteins in the mixed endotype. Furthermore, we observed distinct signatures of downregulation as well with 25 proteins uniquely downregulated in food-allergic AD, 1 protein in perennial AD, 5 proteins in seasonal AD, and 4 proteins in the mixed endotype. These unique signatures are characterized by distinct differences in inflammatory mediator expression that may play a role in the differential sensitization profiles as well; however, the interpretations should be taken with some caution because of the limited sample sizes. Additional studies are needed with larger sample sizes and greater endotype representation to further investigate whether the aforementioned signatures are further modulated under differing pathophysiological states of the disease (flare, *S aureus* colonization, etc).

Given the emerging landscape of therapies for AD and the desire for more personalized approaches to treatments targeting the systemic inflammation, it will be important to assess the therapeutic response of each allergenic endotype within the clinical studies. Importantly, T_H2/T_H22 signaling has been shown to modulate barrier proteins such as FLG, loricrin, and involucrin, and destabilization of tight junctions,^{10,11,13} leading to the enhanced penetration of allergens. In addition, the T_H2 cytokines IL-4 and IL-13 have been shown to drive antibody class switching and the production of IgE in subjects with AD.^{3,36} Targeting the elevated IgE levels in AD provides a therapeutic benefit in a subset of subjects^{37–39}; however, additional investigation is necessary to further characterize those who

respond. Allergen immunotherapy has additionally been tested as a potential therapy in subjects with AD⁴⁰⁻⁴² with a goal of allergen desensitization and disease improvement. Although larger studies are needed to confirm the observations, initial studies were associated with reduced need for steroid rescue, improved skin disease, reduced allergen-specific IgE, and reduced systemic inflammatory mediators (ie, CCL17/TARC and CCL22/MDC).^{40,43}

This study further illustrates the heterogeneity of AD and addresses the opportunity to further characterize different endotypes of disease. Historically, it was estimated that 80% of patients with AD have elevated levels of serum IgE and allergen sensitization.³ This study further illustrated the diversity of allergen sensitization and the ability to segment subjects on the basis of dominant allergen classification. In addition, this study demonstrated differential systemic inflammation and *S aureus* antibody responses associated with the allergenic endotypes. These unique proteomic signatures may be valuable for correct disease characterization and personalized treatment. It will also be important to extend the analysis to larger patient populations and to perform mechanistic clinical studies to understand the function of these proteins in disease pathogenesis and in response to treatment.

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ONLINE REPOSITORY

TABLE E1. Allergen classification

Allergen	Abbreviation	Classification
<i>Alternaria alternata</i>	Alt a 1	Perennial
<i>Alternaria alternata</i>	Alt a 6	Perennial
<i>Aspergillus fumigatus</i>	Asp f 1	Perennial
<i>Aspergillus fumigatus</i>	Asp f 3	Perennial
<i>Aspergillus fumigatus</i>	Asp f 6	Perennial
<i>Blatella germanica</i> (cockroach)	Bla g 1	Perennial
<i>Blatella germanica</i> (cockroach)	Bla g 2	Perennial
<i>Blatella germanica</i> (cockroach)	Bla g 5	Perennial
<i>Blatella germanica</i> (cockroach)	Bla g 7	Perennial
<i>Blomia tropicalis</i> (mite)	Blo t 5	Perennial
<i>Canis familiaris</i> (dog)	Can f 1	Perennial
<i>Canis familiaris</i> (dog)	Can f 2	Perennial
<i>Canis familiaris</i> (dog)	Can f 3	Perennial
<i>Canis familiaris</i> (dog)	Can f 5	Perennial
<i>Cladosporium herarum</i>	Cla h 8	Perennial
<i>Dermatophagoides farinae</i> (dust mite)	Der f 1	Perennial
<i>Dermatophagoides farinae</i> (dust mite)	Der f 2	Perennial
<i>Dermatophagoides pteronyssinus</i> (dust mite)	Der p 1	Perennial
<i>Dermatophagoides pteronyssinus</i> (dust mite)	Der p 10	Perennial
<i>Dermatophagoides pteronyssinus</i> (dust mite)	Der p 2	Perennial
<i>Equus caballus</i> (horse)	Equ c 1	Perennial
<i>Equus caballus</i> (horse)	Equ c 3	Perennial
<i>Felis domesticus</i> (cat)	Fel d 1	Perennial
<i>Felis domesticus</i> (cat)	Fel d 2	Perennial
<i>Felis domesticus</i> (cat)	Fel d 4	Perennial
<i>Hevea brasiliensis</i> (latex)	Hev b 1	Perennial
<i>Hevea brasiliensis</i> (latex)	Hev b 3	Perennial
<i>Hevea brasiliensis</i> (latex)	Hev b 5	Perennial
<i>Hevea brasiliensis</i> (latex)	Hev b 6.01	Perennial
<i>Hevea brasiliensis</i> (latex)	Hev b 8	Perennial
<i>Lepidoglyphus destructor</i> (mite)	Lep d 2	Perennial
<i>Mus musculus</i> (mouse)	Mus m 1	Perennial
<i>Alnus glutinosa</i> (pollen)	Aln g 1	Seasonal
<i>Ambrosia artemisiifolia</i> (ragweed)	Amb a 1	Seasonal
<i>Artemisia vulgaris</i> (mugwort)	Art v 1	Seasonal
<i>Artemisia vulgaris</i> (mugwort)	Art v 3	Seasonal
<i>Anisakis simplex</i> (herring worm)	Ani s 1	Seasonal
<i>Anisakis simplex</i> (herring worm)	Ani s 3	Seasonal
<i>Apis mellifera</i> (honeybee)	Api m 1	Seasonal
<i>Apis mellifera</i> (honeybee)	Api m 4	Seasonal
<i>Betula verrucosa</i> (birch)	Bet v 1	Seasonal
<i>Betula verrucosa</i> (birch)	Bet v 2	Seasonal
<i>Betula verrucosa</i> (birch)	Bet v 4	Seasonal

(continued)

TABLE E1. (Continued)

Allergen	Abbreviation	Classification
<i>Chenopodium album</i> (pollen)	Che a 1	Seasonal
<i>Cryptomeria japonica</i> (Japanese cedar)	Cry j 1	Seasonal
<i>Cupressus arizonica</i> (cypress)	Cup a 1	Seasonal
<i>Cynodon dactylon</i> (Bermuda grass)	Cyn d 1	Seasonal
<i>Mercurialis annua</i> (annual mercury)	Mer a 1	Seasonal
<i>Olea europaea</i> (olive)	Ole e 1	Seasonal
<i>Olea europaea</i> (olive)	Ole e 7	Seasonal
<i>Olea europaea</i> (olive)	Ole e 9	Seasonal
<i>Parietaria judaica</i> (wall pellitory)	Par j 2	Seasonal
<i>Phleum pratense</i> (timothy grass)	Phl p 1	Seasonal
<i>Phleum pratense</i> (timothy grass)	Phl p 11	Seasonal
<i>Phleum pratense</i> (timothy grass)	Phl p 12	Seasonal
<i>Phleum pratense</i> (timothy grass)	Phl p 2	Seasonal
<i>Phleum pratense</i> (timothy grass)	Phl p 4	Seasonal
<i>Phleum pratense</i> (timothy grass)	Phl p 5	Seasonal
<i>Phleum pratense</i> (timothy grass)	Phl p 6	Seasonal
<i>Phleum pratense</i> (timothy grass)	Phl p 7	Seasonal
<i>Platanus acerifolia</i> (maple leaf sycamore)	Pla a 1	Seasonal
<i>Platanus acerifolia</i> (maple leaf sycamore)	Pla a 2	Seasonal
<i>Platanus acerifolia</i> (maple leaf sycamore)	Pla a 3	Seasonal
<i>Plantago lanceolata</i> (plantain)	Pla l 1	Seasonal
<i>Salsola kali</i> (saltwort)	Sal k 1	Seasonal
<i>Actinidia deliciosa</i> (kiwi)	Act d 1	Food
<i>Actinidia deliciosa</i> (kiwi)	Act d 2	Food
<i>Actinidia deliciosa</i> (kiwi)	Act d 5	Food
<i>Actinidia deliciosa</i> (kiwi)	Act d 8	Food
<i>Anacardium occidentale</i> (cashew)	Ana o 2	Food
<i>Apium graveolens</i> (celery)	Api g 1	Food
<i>Arachis hypogaea</i> (peanut)	Ara h 1	Food
<i>Arachis hypogaea</i> (peanut)	Ara h 2	Food
<i>Arachis hypogaea</i> (peanut)	Ara h 3	Food
<i>Arachis hypogaea</i> (peanut)	Ara h 6	Food
<i>Arachis hypogaea</i> (peanut)	Ara h 8	Food
<i>Arachis hypogaea</i> (peanut)	Ara h 9	Food
<i>Bertholletia excelsa</i>	Ber e 1	Food
<i>Bos domesticus</i> (milk)	Bos d 4	Food
<i>Bos domesticus</i> (milk)	Bos d 5	Food
<i>Bos domesticus</i> (milk)	Bos d 6	Food
<i>Bos domesticus</i> (milk)	Bos d 8	Food
<i>Bos domesticus</i> (milk)	Bos d Lactoferrin	Food
<i>Corylus avellana</i> (hazelnut)	Cor a 1.0101	Food
<i>Corylus avellana</i> (hazelnut)	Cor a 1.0401	Food
<i>Corylus avellana</i> (hazelnut)	Cor a 8	Food
<i>Corylus avellana</i> (hazelnut)	Cor a 9	Food
<i>Fagopyrum esculentum</i> (buckwheat)	Fag e 2	Food
<i>Gadus morhua</i> (cod)	Gad c 1	Food
<i>Gallus domesticus</i> (egg)	Gal d 1	Food
<i>Gallus domesticus</i> (egg)	Gal d 2	Food

(continued)

TABLE E1. (Continued)

Allergen	Abbreviation	Classification
<i>Gallus domesticus</i> (egg)	Gal d 3	Food
<i>Gallus domesticus</i> (egg)	Gal d 5	Food
<i>Glycine max</i> (soy)	Gly m 4	Food
<i>Glycine max</i> (beta-conglycinin, soy)	Gly m 5	Food
<i>Glycine max</i> (glycinin)	Gly m 6	Food
<i>Juglans regia</i> (walnut)	Jug r 1	Food
<i>Juglans regia</i> (walnut)	Jug r 2	Food
<i>Juglans regia</i> (walnut)	Jug r 3	Food
<i>Malus domestica</i> (apple)	Mal d 1	Food
<i>Penaeus monodon</i> (tiger shrimp)	Pen m 1	Food
<i>Penaeus monodon</i> (tiger shrimp)	Pen m 2	Food
<i>Penaeus monodon</i> (tiger shrimp)	Pen m 4	Food
<i>Prunus persica</i> (peach)	Pru p 1	Food
<i>Prunus persica</i> (peach)	Pru p 3	Food
<i>Sesamum indicum</i> (sesame)	Ses i 1	Food
<i>Triticum aestivum</i> (wheat)	Tri a 14	Food
<i>Triticum spp</i> (wheat)	Tri a 19.0101	Food

List of allergens tested for IgE reactivity in the sera of both HCs and subjects with AD.

TABLE E2. The ratios of geometric mean levels of IgE antibody against *S aureus* toxins in patients with AD and HCs relative to HC nonallergic subjects along with 95% CIs and *P* values, separated by allergen sensitization

Group	Allergen subgroup	AT	DT	TSST-1
HCs	Perennial (n = 11)	1.10 (0.99-1.22) <i>P</i> = .0840	1.03 (0.62-1.71) <i>P</i> = .9142	1.05 (0.71-1.54) <i>P</i> = .8164
	Seasonal (n = 7)	1.08 (0.95-1.22) <i>P</i> = .1979	1.58 (0.88-2.85) <i>P</i> = .1081	2.61 (0.62-11.06) <i>P</i> = .1583
AD	Nonallergic (n = 27)	0.94 (0.87-1.01) <i>P</i> = .0999	0.90 (0.71-1.15) <i>P</i> = .3999	1.07 (0.74-1.55) <i>P</i> = .7115
	Perennial (n = 32)	1.13 (0.92-1.38) <i>P</i> = .2514	1.15 (0.82-1.61) <i>P</i> = .4059	2.12 (1.33-3.38) <i>P</i> = .0022
	Seasonal (n = 9)	1.00 (0.92-1.09) <i>P</i> = .9644	1.05 (0.65-1.71) <i>P</i> = .8185	3.21 (1.05-9.85) <i>P</i> = .0430
	Food (n = 2)	1.56 (0.01-363.79) <i>P</i> = .4894	2.07 (0.10-44.40) <i>P</i> = .2401	3.20 (0.00-4.33) <i>P</i> = .4553
	Mixed (n = 6)	1.12 (0.84-1.50) <i>P</i> = .3480	0.90 (0.49-1.67) <i>P</i> = .7049	1.42 (0.85-2.37) <i>P</i> = .1594

AT, Alpha toxin; DT, delta toxin; TSST-1, toxic shock syndrome toxin-1.

The food and mixed cohorts within the HCs were excluded from this analysis because they each had an n = 1.