The IgG response against *Staphylococcus aureus* is associated with severe atopic dermatitis in children

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ABSTRACT

Background

An altered immune response against *Staphylococcus (S.) aureus* might contribute to inflammation and barrier damage in atopic dermatitis (AD).

Objectives

To profile IgG antibodies against 55 *S. aureus* antigens in sera of children with mild-to-severe AD and to evaluate the association between IgG levels and disease severity.

Methods

In this cross-sectional study, we included children with AD from two interventional study cohorts, the Shared Medical Appointment (SMA) cohort (n = 131) and the older DAVOS cohort (n = 76). AD severity was assessed using the Self Administrated-Eczema Area and Severity Index (SA-EASI) and levels of thymus and activation-regulated chemokine (TARC) in serum. IgG antibody levels against 55 *S. aureus* antigens were quantified simultaneously using a Luminex assay. Pair-wise correlations were calculated between the 55 IgG levels using the Spearman rank correlation test. Linear regression analysis was performed to test for associations between 55 IgG levels and SA-EASI and TARC, adjusting for age, sex and *S. aureus* colonization.

Results

In the SMA cohort 16 antigens were associated with SA-EASI and 12 antigens were associated with TARC (10 overlapping antigens; *P*-values 0.001 to 0.044). The associated IgG antibodies targeted mainly secreted proteins with immunomodulatory functions. In the DAVOS study, IgG levels against only four and one *S. aureus* antigen(s) were associated with SA-EASI and TARC, respectively (no overlap).

Conclusions

In young children, severity of AD is associated with an IgG response directed against *S. aureus* antigens with mainly immunomodulatory functions. These findings encourage further evaluation of the role of *S. aureus* in the pathogenesis of AD.

INTRODUCTION

Staphylococcus (S.) aureus is involved in the multifactorial pathogenesis of atopic dermatitis (AD).[1] Approximately 70% of the skin lesions in AD are colonised with *S. aureus*, and bacterial density was found to be associated with the severity of AD.[2] The exact mechanisms through which *S. aureus* causes inflammation are not fully understood, but the bacterium expresses different virulence factors that can trigger T-cell immune responses in AD and contribute to the inflammatory response.[3] For example, staphylococcal enterotoxins (SE) have the ability to act as superantigens via direct stimulation of T cells.[4] Colonization with staphylococcal strains that produce these virulence factors, including SEA, SEB, SEC and SED, is thought to be related to AD severity.[5,6] To date, the role of other staphylococcal antigens in AD has barely been investigated.[7]

There is increasing interest in understanding the immune response against *S. aureus* in AD as an altered immune response might contribute to inflammation and barrier damage. The current literature focuses on IgE antibody titers directed against some of the *S. aureus* antigens. Increased IgE-specific antibodies against *S. aureus* antigens, mainly SEA and SEB, have been described in patients with AD vs. healthy controls. Furthermore, an association between IgE levels and AD severity has been confirmed in some studies.[8-10] Although IgG is known for its involvement in the neutralization and elimination of microbes, little is known about anti-*S. aureus* IgG antibody patterns in patients with AD.[11] Previous studies measured IgG against two antigens, exfoliative toxin A and SEB, and reported higher IgG levels in patients vs. controls (significant for SEB).[12,13] Other antigens were not studied. Two studies performed detailed IgG subclass analysis and found an IgG2 deficiency against SEC1 and an elevated IgG4 against SEB in patients with AD.[14,15] Although studies are limited in number and focus on a few single antigens, they emphasize the possible relevance of IgG in the response against *S. aureus* in AD.

To gain more insights into the IgG-mediated immune response against *S. aureus* in patients with AD, we profiled IgG antibodies against 55 *S. aureus* antigens in sera of children with mild-to-severe AD, using a Luminex assay.[16] Additionally, we evaluated the association between IgG levels and disease severity.

MATERIALS AND METHODS

Study design and population

This cross-sectional study was embedded in two interventional studies: the Shared Medical Appointment (SMA) study and the DAVOS study.[17,18] SMA included patients with mild-to-severe AD, aged between 0 and 18, between November 2009 and December 2011. The DAVOS study included children with difficult-to-treat eczema, aged 8-18 years, between January 2011 and June 2015. Both studies were conducted at the Wilhelmina Children's Hospital in The Netherlands and were approved by the University Medical Centre Utrecht's medical and ethical review board (09-192/K, 08-368/K). Written informed consent was obtained from all patients. Serum samples, microbial swabs, eczema severity scores and patient characteristics, obtained at baseline in both the SMA and DAVOS studies, were analysed in this study. In both studies, AD was diagnosed according to the UK Working Party criteria.[19-21] Severity was assessed by the parents using the Self-Administrated Eczema Area and Severity Index (SA-EASI).[22] Apart from the SA-EASI, the levels of thymus and activation-regulated chemokine (TARC) in serum were used as a marker for AD severity.[23,24] Age of AD onset, treatment history and diagnosis of asthma and allergic rhinitis were based on clinical history. Food allergy was diagnosed based on convincing clinical history in the SMA study and/or double-blind food provocation test in the DAVOS study.

Microbial samples

Skin microbial samples were taken from the nose and lesional skin according to a standardized procedure using a sterile swab (Sterile Dryswab™) moistened with NaCl 0.9%. Skin samples were collected from lesions at the antecubital fold or the popliteal fossa. Bacterial cultures for *S. aureus* were performed using routine diagnostic culture procedures using *S. aureus*-specific Mannitol salt agar plates.

Measurement of antibodies against *S. aureus*

Antigens and coupling procedure

In a pilot experiment, the IgG antibody titers against a set of 57 *S. aureus* antigens were measured in sera of 23 AD patients. It was decided to include 55 antigens in the present study as the signals for two antibodies (ESAT-6 secretion system extracellular A and SA2097) were very low in the pilot study (data not shown). The 55 antigens used in this study were divided into four categories based on their biological function: household enzymes, immune modulators (superantigens and nonsuperantigens), cell membrane-damaging molecules and microbial surface components recognizing adhesive matrix molecules (MSCRAMMs) (table S1). All used antigens were recombinant proteins, expressed with a histone tag in the *Escherichia coli* XL1-blue strain and purified under denaturing conditions with nickelnitrilotriacetic acid agarose. They were coupled to the SeroMAP carboxylated beads (Luminex Corporation) as described previously.[25,26] The final bead concentration was adjusted to 3000 beads/μL and they were stored at 6°C in the dark. As a negative control, the coupling procedure was performed in absence of any antigen.

Luminex assay

Serum samples were stored at -80°C until quantification of IgG antibody levels against *S. aureus* antigens with a fluorescent bead-based flow cytometry technique (xMapâ, Luminex).[16] In the wells of a 96-well filter microtiter plate (Millipore Corporation), 50 µL bead mix (containing the different antigen-coupled beads each at a concentration of 3000 beads/µL) was mixed with 50 µL 1:100 diluted patient serum. Follow-up steps have previously been described in detail.[27] Each measurement lasted 1 minute; during this time a minimum of 100 beads had to be analysed for each antigen-coupled bead, otherwise the data were excluded from further analysis. IgG antibody levels against *S. aureus* antigens were expressed as median fluorescence intensity (MFI). A without non-protein-coupled control bead was included to determine nonspecific antibody binding. The nonspecific MFI values were subtracted from the results.[26] The MFI values of the two independent experiments, reflecting semi-quantitative antibody levels, were averaged. The coefficient of variation (CV) was calculated for the duplicate experiments. Measurements were excluded if the CV value was >25% and average MFI values were >1000. Failures of the Luminex per well were defined as missing values.

Statistical analysis

For this study, a convenience sample was obtained from the SMA and the DAVOS studies. As the DAVOS and SMA studies used different inclusion criteria regarding AD severity and age, the study cohorts were analysed separately. For further analysis the IgG data were preprocessed by replacing negative and zero MFI values (resulting from correction for nonspecific binding) by 1. Absolute IgG levels per antigen are presented as median [interquartile range (IQR)]. The IgG data were log-transformed to obtain a parametric distribution, and standardised using a zero mean unit variance method. Pairwise correlations were calculated between the 55 IgG levels using the Spearman rank correlation test. A hierarchical clustering analysis with the 55 antigens was carried out to identify main antibody clusters, but no robust clusters were identified (data not shown). Therefore, for further analysis the 55 antibodies were analysed separately.

Linear regression analysis

Severity scores and patient age were tested for normal distributions with the one-sample Kolmogorov–Smirnov test and transformed when necessary, to obtain a normal distribution. Multivariable linear regression analyses were carried out using the standardized IgG levels (described above) for each of the 55 *S. aureus* antigens against the SA-EASI score as a main predictor, adjusted for age, sex and colonization with *S. aureus* on skin and/or in nose (*S. aureus* present, 'yes' or 'no'). The multivariable linear regression was repeated in a separate model using TARC as a main predictor instead of SA-EASI to validate our results with an intrinsic marker for AD severity. For antibodies that did not follow linear distribution after transformation, bootstrapping (iteration 1000) was used to obtain regression coefficients and 95% confidence intervals (CIs). Given the exploratory nature of this study and the observed correlations between the 55 tested antibody levels, we used a *P*-value <0.05 to indicate significant associations between antibody levels and SA-EASI/ TARC. Analyses were performed using SPSS version 23.0 for Windows (IBM, Armonk, NY, U.S.A.).

RESULTS

Population characteristics

We included 136 and 76 children from the SMA and DAVOS studies, respectively (figure S1). The median age of the children from the SMA cohort was 2 years (IQR 1-5). The DAVOS population consisted of older children with a median age of 13 years (IQR 11-15). Eczema severity measured with the SA-EASI gave a median of 27 (IQR 16-42) in the SMA cohort and 24 (IQR 12-42) in the DAVOS cohort. Median TARC values were also higher in the SMA cohort [1441 in pg/ml (IQR 713-2794) vs. 1119 (IQR 696-2400). Skin and nasal colonization with *S. aureus* was found in 36% and 34% of the children in the SMA cohort, respectively, and in 47% and 67% of the children in the DAVOS cohort. Detailed baseline characteristics, including use of medication, are given in table 1.

Antibody characteristics

Median IgG levels against 55 *S. aureus* antigens measured in the sera of the children from both study cohorts are presented in table S1. In the DAVOS cohort, the medians of the absolute antibody levels were higher and showed less variation than the SMA study cohort. Figure 1 shows that the IgG antibody responses do not clearly differ between the four main biological groups of antigens (immune modulators (superantigens and non-superantigens), household enzymes, cell-membrane-damaging molecules and MSCRAMMs). A Spearman correlation test showed correlations between the IgG levels of the staphylococcal superantigen-like (SSL) proteins 3, 5, 9 and 10 (coefficients >0.7). High correlations (>0.7) were also identified between leukotoxin (Luk) E, LukD, LukS and between extracellular fibrinogen-binding protein and alanine transaminase 2. Additionally, some enterotoxins were correlated: SEB with SEC (>0.7), SEI with SEM (>0.8), SEN with SEI (0.69) and SEA with SEE (0.69) (figure 2).

Association between antistaphylococcal IgG levels and atopic dermatitis severity measured with SA-EASI and TARC

We found significant associations between IgG levels and AD severity in the SMA cohort. Sixteen antigens were associated with SA-EASI and 12 with TARC (table 2). Ten of the

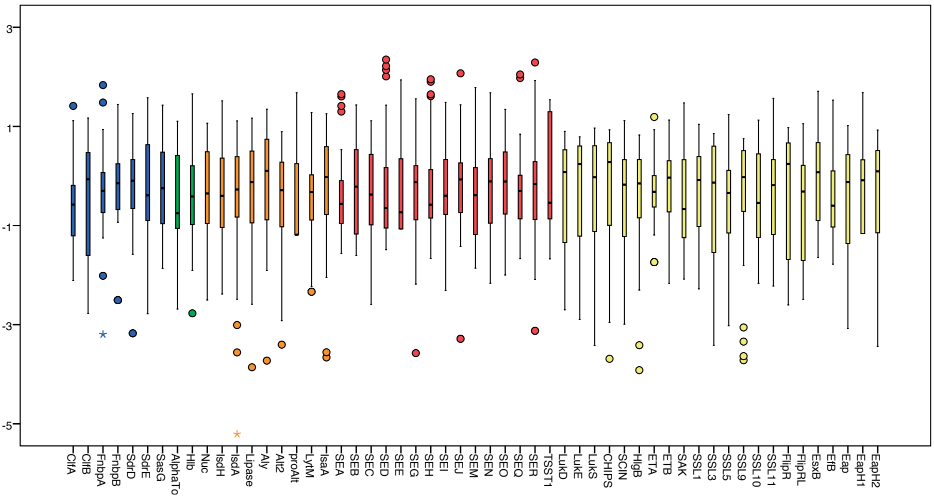
Table 1. Baseline characteristics

	SMA cohort (n=131)	DAVOS cohort (n=76)
Age in years; median (IQR)	2 (1-5)	13 (11-15)
Sex (male)	63 (48.1)	39 (51.3)
Ethnicity		
Dutch	95 (72.5)	54 (71.1)
Other ethnicity	19 (14.5)	22 (28.9)
Missing	17 (13.0)	0 (0)
Age of onset AD		
0-<2 years	106 (80.9)	66 (86.8)
2-<6 years	6 (4.6)	8 (10.5)
Missing	19 (14.5)	2 (2.6)
Atopy		
Food allergy	53 (40.4)[1]	49 (64.5)[1]
Allergic asthma	40 (30.5)[2]	59 (77.6)
Allergic rhinoconjunctivitis	36 (27.5)[2]	67 (88.2)
SA-EASI; median (IQR)	27.00 (16.00-42.20)[3]	24.00 (11.95-41.75)[2]
TARC pg/mL; median (IQR)	1441 (713-2794)	1119 (696-2400)[2]
Corticosteroid treatment		
Topical corticosteroid	101 (77.1)	70 (92.1)
Systemic corticosteroid	0 (0)	3 (3.9)
Neoral	0 (0)	7 (9.2)
Antibiotic treatment		
Topical antibiotic	11 (8.4)	2 (2.6)
Systemic antibiotic	3 (2.3)	1 (1.3)
***Staphylococcus aureus* positive** (> 10 CFU)		
Skin	47 (35.9)[4]	36 (47.4)[3]
Nose	45 (34.4)[2]	51 (67.1)[3]

Data are n (%) unless otherwise indicated. SMA, Shared Medical Appointment; IQR, interquartile range; AD, atopic dermatitis; SA-EASI, Self-Administered Eczema Area and Severity Index; TARC, thymus and activation-regulated chemokine; CFU, colony-forming unit.
Missings SMA: [1] = 4 (3.1%); [2] = 2 (1.5%); [3] = 41 (31.3%); [4] = 3 (2.3%)
Missings DAVOS: [1] = 2 (2.6%); [2] = 3 (3.9%); [3] = 6 (7.9%)

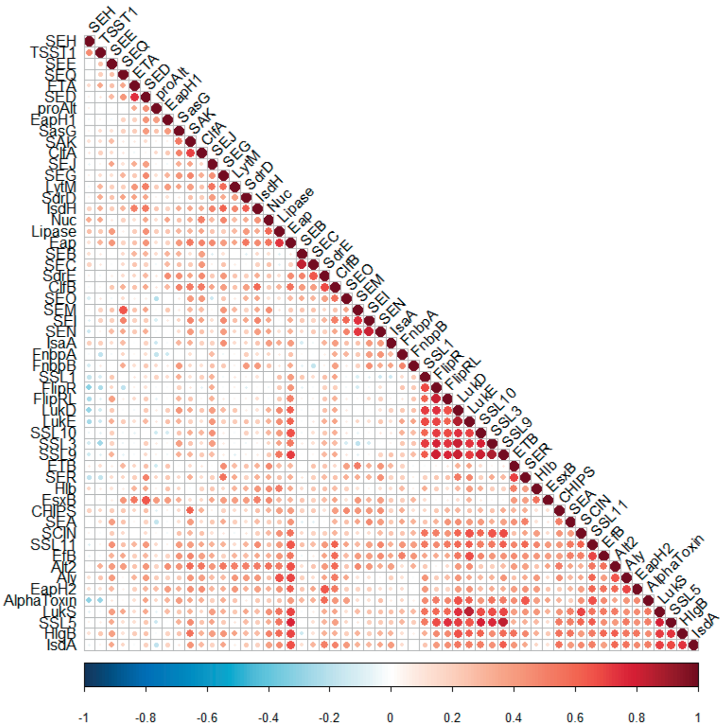
12 antigens associated with TARC were also associated with SA-EASI (*P*-values 0.001 to 0.044). The associated IgG antibodies targeted mainly secreted proteins with immuno-modulatory functions (e.g. LukD and LukE; table 2). The described associations between antigen levels and AD severity were independent of age, sex and colonization of the skin and/or nose with *S. aureus*. In the DAVOS study, IgG levels against only four and one *S. aureus* antigen(s) were associated with SA-EASI and TARC, respectively, and there was no overlap between the two markers for AD (tables S2).

Figure 1. Boxplots showing the levels of IgG against 55 antigens in the SMA study (normalized data)



NOTE: Blue indicates MSCRAMMs, green the membrane-damaging molecules, orange the housekeeping antigens, red the superantigens and yellow the immunomodulating proteins.

Figure 2. Spearman's rank correlation coefficients of the IgG values (MFI) against 55 antigens (SMA cohort).



NOTE: The size and intensity of the red dots reflects the height of the correlation coefficients, identifying high correlations for example between the SSL 3, 5, 9 and 10 antigens.

Table 2. List of *S. aureus* antigens of which the IgG levels were significantly associated with patient eczema severity, according to SA-EASI and TARC, *P*-value < 0.05)

Antigens	SMA study (n=131)						DAVOS study (n=76)					
	SA-EASI ^			TARC			SA-EASI			TARC		
	Regression coefficient (SE)	95% CI	*P*-value	Regression coefficient (SE)	95% CI	*P*-value	Regression coefficient (SE)	95% CI	*P*-value	Regression coefficient (SE)	95% CI	*P*-value
LukD	0.134 (0.042)*	0.054-0.219	0.003	0.379 (0.160)*	0.080-0.714	0.018	-	-	-	-	-	-
LukE	0.111 (0.048)*	0.016-0.213	0.033	0.396 (0.141)*	0.108-0.672	0.005	-	-	-	-	-	-
SSL3	0.145 (0.052)*	0.045-0.258	0.008	0.404 (0.144)*	0.150-0.700	0.006	-	-	-	-	-	-
SSL5	0.153 (0.044)*	0.070-0.242	0.001	0.379 (0.179)	0.024-0.734	0.036	-	-	-	-	-	-
SSL9	0.149 (0.048)*	0.053-0.243	0.004	0.309 (0.143)*	0.036-0.617	0.035	-	-	-	-	-	-
SSL10	0.127 (0.047)*	0.036-0.224	0.009	0.432 (0.151)*	0.162-0.747	0.004	-	-	-	-	-	-
FlipRL	0.120 (0.057)*	0.018-0.243	0.043	0.398 (0.183)*	0.047-0.768	0.036	-	-	-	-	-	-
SEA	0.177 (0.057)*	0.062-0.292	0.002	0.613 (0.194)	0.230-0.997	0.002	-	-	-	-	-	-
IsdA	0.203 (0.045)*	0.115-0.294	0.001	0.346 (0.135)*	0.094-0.624	0.022	-	-	-	-	-	-
Eap	0.146 (0.048)*	0.047-0.236	0.002	0.286 (0.142)*	0.018-0.582	0.044	-	-	-	-	-	-
LukS	0.113 (0.058)*	0.002-0.223	0.047	-	-	-	-	-	-	-	-	-
HlgB	0.116 (0.047)*	0.022-0.214	0.019	-	-	-	-	-	-	-	-	-
SSL1	0.134 (0.050)*	0.045-0.238	0.004	-	-	-	-	-	-	-	-	-
FlipR	0.144 (0.059)*	0.026-0.262	0.020	-	-	-	-	-	-	-	-	-
SdrE	-0.140 (0.062)*	-0.271-(-0.022)	0.036	-	-	-	-	-	-	-	-	-
IsaA	0.125 (0.047)*	0.023-0.215	0.008	-	-	-	-	-	-	-	-	-
SEE	-	-	-	0.550 (0.212)*	0.135-0.980	0.011	-	-	-	-	-	-
EfB	-	-	-	0.450 (0.178)	0.097-0.803	0.013	-	-	-	-	-	-
Hlb	-	-	-	-	-	-	0.155 (0.058)*	0.041-0.262	0.010	-	-	-
SEG	-	-	-	-	-	-	0.165 (0.069)*	0.050-0.326	0.019	-	-	-
FnbpB	-	-	-	-	-	-	0.151 (0.060)*	0.029-0.261	0.015	-	-	-
ClfA	-	-	-	-	-	-	0.142 (0.067)*	0.023-0.291	0.040	-	-	-
SAK	-	-	-	-	-	-	-	-	-	0.695 (0.302)*	0.084-1.285	0.027

^ N = 90 for SA-EASI analysis due to missing SA-EASI scores
* Regression coefficients and CI were obtained using bootstrapping iteration 1000
- = no significant association between the antigen and the severity parameter.
SMA, Shared Medical Appointment; AD, atopic dermatitis; SA-EASI, Self-Administered Eczema Area and Severity Index; TARC, thymus and activationregulated chemokine; SE, standard error; CI, confidence interval. Please find abbreviations of the antigens in table S1.

DISCUSSION

For the first time, IgG immune responses against a large panel of 55 *S. aureus* antigens were profiled in children with AD, showing that the children are exposed to the antigens and develop an IgG-mediated humoral immune response towards them. Additionally, AD severity was found to be associated with IgG antibodies directed against *S. aureus* antigens with mainly immunomodulatory functions. LukD and LukE are commonly expressed by strains of *S. aureus* and are involved in cell lysis of neutrophils.[28] SSL3, SSL5, SSL9 and SSL10 are variably expressed and are all involved in immunomodulation, for example by inhibiting complement activation.[29] Iron-responsive surface determinants A is a cell-surface protein that may function in both iron acquisition and adhesion.[30] SEA is more rarely expressed and has a strong immunostimulatory function. As a superantigen it can cause cytokine release and epithelial damage, but the literature also describes (anti-inflammatory) cytokine downregulating functions (interleukin-4).[28,31] *Staphylococcus aureus* formyl peptide receptor-like 1 inhibitor (FLIPr) and its homologue FLIPr-like are potent FcγR antagonists that inhibit IgG-mediated effector functions.[32]

Only Sohn *et al.* have studied the staphylococcal IgG response in relation to AD severity. They measured SEB only and found no correlation with AD severity, which corresponds with our findings (table 2).[13] The finding that the antibodies that were associated with AD severity in this study are known to target antigens with an immunomodulatory function suggests that *S. aureus* downregulates the immune system locally to help it maintain its colonization on the skin, a theory that was recently suggested by Biedermann *et al.*[33]

In contrast to the associations between AD severity and IgG levels for specific antigens found in the SMA study, few associations were found in the DAVOS cohort. In addition, there was no overlap between the antibodies associated with the SA-EASI and these associated with TARC in the DAVOS cohort, which suggests that these associations were false-positive results. The lack of associations found in the DAVOS cohort could be the result of the older age of the DAVOS participants, who may have been more chronically exposed to *S. aureus* (see tables 1 and S1) and therefore exhibited higher levels of IgG.[12,34] Furthermore, IgG antibody levels are known to increase with age (which is also true for patients with AD), reaching a plateau around adulthood.[34-36] Indeed, comparison between IgG levels of the SMA cohort and a sample of healthy adult pooled serum (appendix S1), showed higher levels for most of the tested IgG antibodies in the healthy pooled serum (figure S2). The DAVOS cohort had higher values than the healthy adult sample for a large part of the antibodies (figure S1). It could be argued that a plateau could have been reached in the older patients of the DAVOS cohort resulting in a lack of found associations. However, the DAVOS study included patients with difficult-to-treat (severe) eczema, most of whom were treated with topical corticosteroids or even systemic immunosuppressive therapy (table 1). Hence, the SA-EASI scores at baseline may have been biased towards lower scores, which could have hampered the associations. Most likely the lack of association is a combination of both biased scores and the older age of the cohort.

IgG levels against specific antigens showed correlations (Spearman's correlation test). SSL antigens and the superantigens SEI, SEM and SEN are known to be co-produced by *S. aureus*.[37,38] The combined presentation of these antigens to the immune system probably contributed to the observed correlations between IgG responses. However, other factors probably also drive the height and correlations of the IgG-specific immune responses.

This study has several limitations. Firstly, a control group of children without AD that would allow investigating the normal range of IgG antibodies was not available. Therefore, our conclusion focusses on a comparison between different severity phenotypes in a well-characterized cohort of children with AD. Due to the cross-sectional design we cannot conclude whether AD severity is the result of an altered IgG response. In addition, owing to the small sample size of this study, we could not perform multivariate analysis, such as unsupervised clustering, which would have allowed us to better understand the correlations found for different antibodies. Although we tested 55 different associations, we did not correct for multiple testing. Owing to the high correlations found between some of the antibodies, multiple testing correction is rather conservative. Because of this, and the hypothesis-generating nature of this study, our associations were kept nominally significant. Although cross-reactivity with other staphylococcal antigens cannot be completely ruled out, it is highly unlikely as *S. aureus* produces species-specific virulence factors that have not been found in *Staphylococcus epidermidis*.[39] Despite these limitations, the present study is the first to evaluate antibody responses against a broad panel of *S. aureus* antigens. Our study sheds light on the IgG-mediated immune response to *S. aureus* in children with AD and it highlights the relevance of other antigens (adhesins and immune modulators) next to the often studied superantigens. Further studies need to be conducted to validate the associations we found.

Interestingly, the association between IgG against *S. aureus* antigens and the severity of AD was independent of skin and nasal colonization with *S. aureus*. This suggests that the immune response against *S. aureus* might be altered, irrespective of the bacteria present on the skin at that time, and raises the question whether anti-*S. aureus* treatment should be guided by a positive culture. To further explore the significance of our findings, future studies should relate our findings to IgE and IgG subclass responses to *S. aureus* antigens in AD. These findings could also be related to *S. aureus* strain differences as different strains might have different ability to elicit immunological alterations in the host.[40] *In vitro* experiments should reveal the functional effect of the relevant *S. aureus* antigens on T-cell differentiation.

CONCLUSION

In a cohort of young children with AD, we identified significant associations between disease severity and IgG antibodies directed against *S. aureus* antigens with mainly immunomodulatory functions. The results of this study encourage more detailed evaluation of the role of *S. aureus* in the pathogenesis of AD.

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SUPPLEMENTARY MATERIAL

Appendix S1. Measurement of antibodies against *S. aureus* in human pooled serum.

Human pooled serum (HPS) is a mix of serum samples from 35 healthy adults (25% male, age between 19 and 61 years). The serum of all the AD patients included in this study (SMA=137 and DAVOS=76) was analysed for detection of IgG antibodies against 55 *S. aureus* antigens by Luminex assay. Detailed description of the Luminex assay is described in the methods section of the main manuscript. This analysis included three samples of the HPS mix, resulting in three HPS measurements per *S. aureus* antigen. After quality control as described the methods section of the main manuscript, the HPS IgG measurements were averaged per antigen for further analysis.

Figure S1. Flowchart of the study population, SMA study and DAVOS study

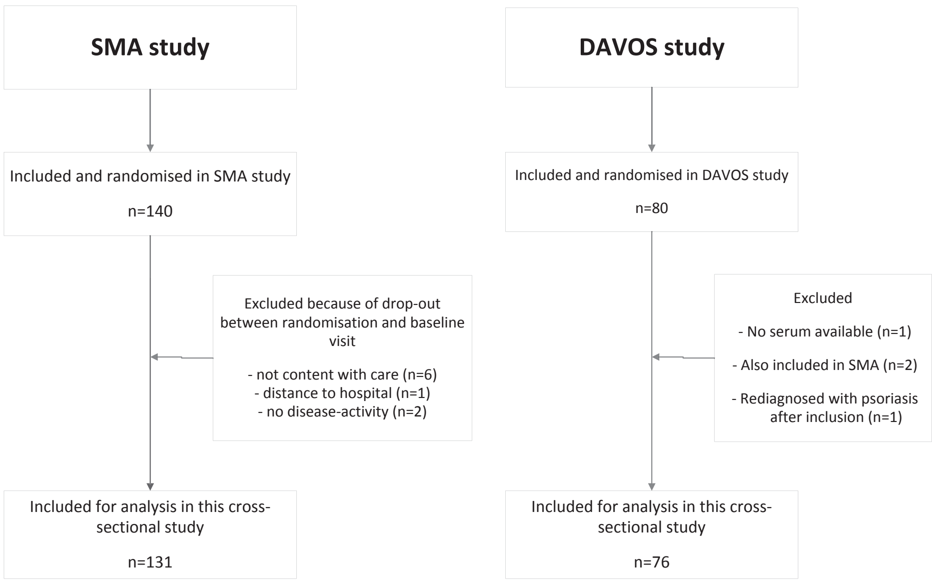

Figure S2. IgG levels of the 55 antigens in SMA, DAVOS and HPS

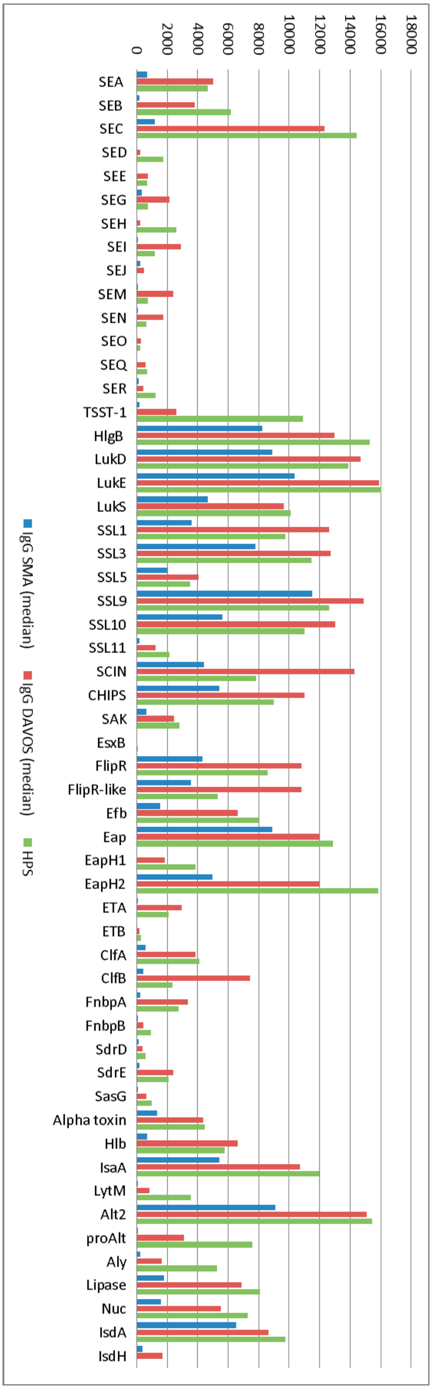

Figure S2 shows the absolute IgG levels of the 55 antigens for the SMA cohort (median), DAVOS cohort (median) and the human pooled serum (HPS). HPS signals for two antigens, SEJ and IsdH, were excluded according to the exclusion criteria described in the methods section of the main manuscript (e.g. coefficient of variation for duplicate experiment >25%). Overall, HPS levels are higher than the SMA levels for all 53 antigens. HPS levels are higher than the DAVOS samples for only 31 antigens. NOTE: as the IgG values are not standardized, the IgG levels between the different antigens cannot be compared. Figure 1 of the manuscript includes a boxplot with standardized IgG levels.

Table S1. Overview of 55 *S. aureus* antigens with function and MFI values

***S. aureus* antigen**	**Description**	**N**	**IgG SMA** (median, IQR)	**N**	**IgG DAVOS** (median, IQR)	***P*- value** *
Immune modulators (superantigens)						
SEA (Staphylococcal enterotoxin A)	Superantigen [1]	127	702.50 (228.50-4005.50)	63	5011.25 (931.00-11820.00)	< 0.001
SEB (Staphylococcal enterotoxin B)	Superantigen [1]	126	203.75 (9.25-3283.44)	62	3803.50 (780.25-13750.69)	< 0.001
SEC (Staphylococcal enterotoxin C)	Superantigen [1]	126	1179.38 (87.88-12018.94)	62	12330.63 (7764.56-14071.19)	< 0.001
SED (Staphylococcal enterotoxin D)	Superantigen [1]	119	24.00 (6.00-185.50)	68	222.50 (109.25-975.31)	< 0.001
SEE (Staphylococcal enterotoxin E)	Superantigeni [1]	119	12.50 (0.00-394.50)	67	764.00 (56.50-2279.00)	< 0.001
SEG (*St*aphylococcal enterotoxin G)	Superantigen [1]	125	323.00 (107.50-1410.13)	76	2156.5 (565.88-3468.13)	< 0.001
SEH (Staphylococcal enterotoxin H)	Superantigen [1]	120	30.50 (13.94-494.69)	69	232.75 (23.63-7071.25)	= 0.001
SEI (*St*aphylococcal enterotoxin I)	Superantigen [1]	120	95.63 (38.00-1012.75)	68	2920.38 (1034.13-5347.13)	< 0.001
SEJ (Staphylococcal enterotoxin J)	Superantigen [1]	119	223.50 (96.50-553.75)	68	490.50 (195.81-1071.88)	= 0.001
SEM (*St*aphylococcal enterotoxin M)	Superantigen [1]	127	72.50 (18.00-374.50)	63	2424.75 (480.75-4780.50)	< 0.001
SEN (Staphylococcal enterotoxin N)	Superantigen [1]	128	92.38 (28.81-328.06)	63	1769.00 (384.00-3827.50)	< 0.001
SEO (Staphylococcal enterotoxin O)	Superantigen [1]	127	34.00 (10.50-140.00)	63	305.25 (90.25-546.50)	< 0.001
SEQ (Staphylococcal enterotoxin Q)	Superantigen [1]	119	34.75 (12.75-130.50)	67	591.50 (47.00-4981.75)	< 0.001
SER (Staphylococcal enterotoxin R)	Superantigen [1]	123	147.75 (59.50-424.50)	49	429.00 (168.38-1488.75)	< 0.001
TSST-1 (Toxic shock syndrome toxin 1)	Superantigen [1]	125	163.75 (53.13-7202.00)	74	2626.00 (67.63-6578.75)	= 0.152
Immune modulators (non-superantigen)						
HlgB (Gamma-hemolysin component B)	Pore forming toxin [1]	128	8217.13 (4881.06-11112.38)	62	12963.00 (9359.13-14555.13)	< 0.001
LukD (Leukotoxin D)	Cell lysis of neutrophils [1]	127	8894.00 (3666.75-12978.50)	62	14705.13 (12837.31-15792.00)	< 0.001
LukE (Leukotoxin E)	Cell lysis of neutrophils [1]	127	10373.75 (5276.75-14596.75)	62	15897.13 (14282.13-16821.13)	< 0.001
LukS (Leukotoxin S)	Cell lysis of neutrophils [1]	126	4648.50 (2034.31-8396.38)	62	9663.00 (5065.19-12566.82)	< 0.001

Table S1. Overview of 55 *S. aureus* antigens with function and MFI values (continued)

***S. aureus* antigen**	**Description**	**N**	**IgG SMA** (median, IQR)	**N**	**IgG DAVOS** (median, IQR)	***P*- value** *
SSL1 (Staphylococcal superantigen like-protein 1)	Immune-modulation. Limits neutrophil chemotaxis[2]	125	3587.50 (491.38-13491.00)	75	12624.13 (6916.50-15991.94)	< 0.001
SSL3 (Staphylococcal superantigen like-protein 3)	TLR signalling inhibition[3]	125	7765.50 (1355.75-12847.38)	75	12696.00 (9673.75-15214.75)	< 0.001
SSL5 (Staphylococcal superantigen like-protein 5)	Prevents neutrophil rolling on activated endothelial cells[3]	125	1983.25 (891.63-4833.00)	75	4066.00 (2792.75-5798.00)	< 0.001
SSL9 (Staphylococcal superantigen like-protein 9)	Complement inhibitor[4]	125	11502.50 (5653.50-14845.38)	76	14879.38 (11952.56-15889.25)	< 0.001
SSL10 (Staphylococcal superantigen like-protein 10)	Phagocytosis inhibition[3]	124	5597.88 (2168.06-10963.44)	76	13037.25 (11159.19-14609.50)	< 0.001
SSL11 (Staphylococcal superantigen like-protein 11)	Chemotaxis inhibition[3]	125	179.50 (37.88-647.75)	75	1239.25 (480.75-2996.50)	< 0.001
SCIN (Staphylococcal complement inhibitor)	Chemotaxis inhibitory, inhibits C3 convertase [1]	123	4420.00 (1442.00-10108.50)	49	14291.00 (11844.75-15457.75)	< 0.001
CHIPS (Chemotaxis inhibitory protein of Staphylococci)	Chemotaxis inhibition[3]	122	5442.13 (621.44-8926.31)	60	11027.63 (7898.25-12846.00)	< 0.001
SAK (Staphylokinase)	Binding/ inactivate complement C3b and IgG bound to surface bacterial cells [5]	118	646.88 (75.94-3691.31)	63	2433.00 (936.25-5507.50)	< 0.001
EsxB (ESAT-6 secretion system extracellular B)	Interferes with host cell apoptotic pathways [6]	125	28.00 (6.25-71.38)	76	53.50 (25.75-97.00)	= 0.001
FlipR (Formyl peptide receptor-like 1 inhibitor)	Inhibition of opsonophagacytosis and killing by neutrophils[5] Chemotaxis inhibition[3]	122	4336.88 (499.81-9194.56)	66	10812.25 (7690.13-13461.31)	< 0.001

Table S1. Overview of 55 *S. aureus* antigens with function and MFI values (continued)

***S. aureus* antigen**	**Description**	**N**	**IgG SMA** (median, IQR)	**N**	**IgG DAVOS** (median, IQR)	***P*- value** *
FlipR-like (Formyl peptide receptor-like1 inhibitor like)	Chemotaxis inhibition[3]	123	3537.00 (748.00-8240.00)	74	10785.00 (6972.56-13626.94)	< 0.001
Efb (Extracellular fibrinogen-binding protein)	Inhibits complement activation and blocks opsonophagocytosis [3, 5]	119	1551.25 (688.50-3872.00)	67	6621.75 (3033.50-9304.75)	< 0.001
Eap (Extracellular adhesive protein)	Phagocytic killing inhibition[3] Prevent neutrophil attachment to and migration through endothelial cells [5]	128	8878.63 (3729.81-12629.06)	64	11957.38 (8675.69-14846.63)	< 0.001
EapH1 (Extracellular adherence protein homolog 1)	Phagocytic killing inhibition[3]	128	39.75 (0.00-552.38)	64	1853.00 (509.69-4266.69)	< 0.001
EapH2 (Extracellular adherence protein homolog 2)	Phagocytic killing inhibition[3]	117	4953.25 (646.13-13314.88)	61	11998.25 (8251.13-14424.38)	< 0.001
ETA (Exfoliative toxin A)	Serine proteases: hydrolyze desmosomal proteins in the skin [7]	126	68.25 (18.88-332.12)	62	2969.75 (164.75-10600.31)	< 0.001
ETB (Exfoliative toxin B)	Serine proteases: hydrolyze desmosomal proteins in the skin [7]	127	52.00 (20.75-127.75)	62	162.88 (42.38-5042.50)	< 0.001
MSCRAMMs						
ClfA (Clumping factor A)	Adhesin: (fibrinogen) [1]	120	572.75 (145.25-2259.31)	69	3876.50 (1739.38-6289.63)	< 0.001
ClfB (Clumping factor B)	Adhesin: (fibrinogen) [1]	101	417.75 (55.75-1104.75)	24	7453.25 (3470.50-9834.63)	< 0.001
FnbpA (Fibronectin-binding protein A)	Adhesin: (fibronectin) [1]	108	214.25 (90.75-1061.00)	64	3342.25 (854.50-4989.19)	< 0.001
FnbpB (Fibronectin-binding protein B)	Adhesin: (fibronectin) [1]	119	102.00 (48.25-346.50)	68	425.25 (194.75-1090.81)	< 0.001
SdrD (Serine-aspartate repeat protein D)	*S.aureus* adhesion[8]	120	144.88 (63.38-288.94)	68	395.13 (138.63-659.38)	< 0.001
SdrE (Serine-aspartate repeat protein E)	*S.aureus* adhesion[9]	120	177.25 (50.50-1186.38)	64	2414.63 (1144.19-5548.81)	< 0.001
SasG (*S. aureus* surface protein G)	Biofilm formation [10]	120	101.63 (18.38-387.75)	69	620.00 (342.63-1548.00)	< 0.001

Table S1. Overview of 55 *S. aureus* antigens with function and MFI values (continued)

***S. aureus* antigen**	**Description**	**N**	**IgG SMA** (median, IQR)	**N**	**IgG DAVOS** (median, IQR)	***P*- value** *
Membrane damaging molecules						
Alpha toxin	Pore forming toxin [1]	128	1357.88 (348.06-3391.19)	62	4356.75 (2713.56-6074.63)	< 0.001
Hlb (Beta-hemolysin)	Pore forming toxin [1]	127	669.50 (181.00-4042.75)	64	6608.13 (2150.88-11467.31)	< 0.001
Housekeeping function						
IsaA (Immunodominant Staphylococcal antigen A)	Assist in cell wall expansion, turnover, growth, and cell separation [11]	117	5435.25 (2779.50-9894.38)	69	10696.75 (6047.63-14414.50)	< 0.001
LytM (Peptidoglycan hydrolase)	Assist in cell wall expansion, turnover, growth, and cell separation [12]	120	104.00 (40.50-877.13)	68	830.25 (80.81-4097.06)	< 0.001
Alt2 (alanine transaminase 2)	Unknown	128	9086.13 (4358.75-13817.69)	64	15067.00 (13257.38-16258.88)	< 0.001
proAlt	Unknown	128	72.25 (0.00-475.06)	76	3094.75 (404.38-10124.19)	< 0.001
Aly	Unknown	83	216.50 (76.75-506.75)	24	1662.13 (656.13-4573.75)	< 0.001
Lipase	Enzyme: lipase [13] (spreading, nutrition)	124	1815.63 (378.13-5336.38)	75	6898.00 (3489.50-11195.25)	< 0.001
Nuc (Nuclease)	Enzyme: nuclease (nutrition)[14]	126	1580.13 (447.81-6626.31)	63	5543.25 (2052.31-9983.75)	< 0.001
IsdA (Iron-responsive surface determinants A)	Ferritin uptake [1]	117	6535.25 (3178.00-9277.75)	65	8648.25 (4964.63-11689.13)	= 0.001
IsdH (Iron-responsive surface determinants H)	Ferritin uptake [1]	115	373.75 (39.50-1041.50)	53	1686.50 (664.88-2499.88)	< 0.001

N = number of patients of which the MFI values could be included for calculation of medians (see exclusion criteria for measurements in the methods section of the manuscript)
* *P*-value SMA vs. DAVOS, Mann-Whitney U test

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Table S2a. Results linear regression analysis GMA study – association with SA-EASI

Antigen	*Regression coefficient (SE)*	*95% CI*	*P-value*
CHIPS	0.015 (0.052)	-0.088-0.115	0.770
SKIN	0.080 (0.058)	-0.037-0.193	0.171
Alpha toxin	-0.031 (0.053)	-0.135-0.073	0.564
Hlb	-0.032 (0.065)	-0.156-0.106	0.608
Nuc	0.026 (0.065)	-0.118-0.139	0.693
ETA	0.017 (0.062)	-0.102-0.134	0.793
ETB	0.043 (0.060)	-0.076-0.167	0.484
SAK	0.035 (0.059)	-0.095-0.143	0.537
SSL11	0.063 (0.047)	-0.031-0.156	0.181
Lipase	0.021 (0.062)	-0.109-0.140	0.721
Aly	0.077 (0.092)	-0.108-0.244	0.418
Alt2	0.069 (0.055)	-0.045-0.166	0.214
ProAlt	-0.029 (0.064)	-0.152-0.105	0.634
EsxB	-0.062 (0.068)	-0.189-0.081	0.356
SEB	-0.043 (0.061)	-0.164-0.074	0.482
SEC	-0.060 (0.062)	-0.184-0.067	0.322
SED	-0.035 (0.071)	-0.169-0.114	0.633
SEE	0.121 (0.061)	-0.003-0.243	0.051
SEG	0.041 (0.048)	-0.053-0.142	0.405
SEH	0.028 (0.069)	-0.106-0.171	0.683
SEI	0.032 (0.046)	-0.057-0.125	0.473
SEJ	-0.105 (0.071)	-0.248-0.035	0.142
SEM	0.032 (0.045)	-0.053-0.126	0.497
SEN	0.104 (0.057)	-0.009-0.217	0.069
SEO	0.012 (0.049)	-0.079-0.112	0.802
SEQ	-0.041 (0.066)	-0.167-0.089	0.557
SER	0.089 (0.058)	-0.017-0.203	0.152
TSST-1	0.067 (0.067)	-0.071-0.187	0.322
FnbpA	0.007 (0.060)	-0.110-0.124	0.905
FnbpB	-0.059 (0.075)	-0.202-0.097	0.435
SdrD	-0.027 (0.078)	-0.179-0.117	0.723
ClfA	0.006 (0.059)	-0.110-0.115	0.914
ClfB	0.063 (0.077)	-0.095-0.217	0.397
IsdH	0.137 (0.068)	0.013-0.284	0.054
EfB	0.043 (0.047)	-0.042-0.148	0.368
SasG	-0.075 (0.062)	-0.204-0.051	0.062
LytM	-0.056 (0.066)	-0.178-0.078	0.398
EapH1	0.035 (0.066)	-0.090-0.172	0.586
EapH2	0.038 (0.066)	-0.092-0.166	0.568

Bootstrapping iter 1000 for all antigens, except SEN

Table S2b. Results linear regression analysis SMA study – association with TARC

Antigen	*Regression coefficient (SE)*	*95% CI*	*P-value*
LukS	0.259 (0.144)	-0.017-0.541	0.064
CHIPS	0.174 (0.196)	-0.207-0.572	0.382
SKIN	0.273 (0.165)	-0.052-0.596	0.099
Alpha toxin	0.075 (0.154)	-0.205-0.402	0.630
Hlb	-0.130 (0.217)	-0.563-0.311	0.559
HlgB	0.207 (0.109)	-0.009-0.424	0.059
Nuc	-0.097 (0.179)	-0.459-0.227	0.600
ETA	-0.106 (0.248)	-0.590-0.412	0.668
ETB	-0.070 (0.216)	-0.522-0.350	0.744
SAK	0.217 (0.196)	-0.190-0.589	0.268
SSL1	0.315 (0.196)	-0.047-0.734	0.113
SSL11	0.105 (0.155)	-0.177-0.411	0.504
FlipR	0.328 (0.190)	-0.031-0.725	0.088
Lipase	0.04 (0.168)	-0.283-0.329	0.981
Aly	0.480 (0.349)	-0.187-1.188	0.179
Alt2	0.237 (0.133)	-0.011-0.514	0.074
ProAlt	-0.051 (0.196)	-0.440-0.333	0.802
EsxB	-0.334 (0.201)	-0.740-0.064	0.096
SEB	0.069 (0.200)	-0.362-0.460	0.720
SEC	-0.106 (0.207)	-0.525-0.277	0.625
SED	-0.379 (0.200)	-0.805-0.020	0.062
SEG	-0.062 (0.203)	-0.452-0.344	0.765
SEH	0.205 (0.221)	-0.237-0.654	0.351
SEI	0.237 (0.186)	-0.100-0.643	0.193
SEJ	-0.233 (0.256)	-0.761-0.258	0.360
SEM	0.274 (0.206)	-0.100-0.703	0.183
SEN	0.131 (0.190)	-0.245-0.507	0.493
SEO	-0.169 (0.192)	-0.533-0.220	0.384
SEQ	0.238 (0.207)	-0.183-0.643	0.259
SER	-0.086 (0.224)	-0.545-0.351	0.707
TSST-1	0.090 (0.210)	-0.331-0.497	0.659
FnbpA	0.160 (0.205)	-0.224-0.581	0.448
FnbpB	-0.265 (0.215)	-0.708-0.154	0.233
SdrD	-0.402 (0.243)	-0.883-0.054	0.106
SdrE-	0.155 (0.244)	-0.633-0.309	0.522
ClfA	0.128 (0.197)	-0.249-0.518	0.507
ClfB	0.215 (0.217)	-0.170-0.678	0.307
IsdH	0.243 (0.199)	-0.132-0.629	0.233
SasG	-0.295 (0.231)	-0.734-0.153	0.203
IsaA	0.128 (0.167)	-0.201-0.456	0.457
LytM	-0.186 (0.242)	-0.684-0.266	0.450
EapH1	0.270 (0.200)	-0.127-0.664	0.179
EapH2	0.365 (0.255)	-0.066-0.950	0.156

Bootstrapping iter 1000 for all antigens, except SEN

Table S2c. Results linear regression analysis DAVOS study – association with SA-EASI

Antigen	*Regression coefficient (SE)*	*95% CI*	*P-value*
LukD	0.058 (0.067)	-0.060-0.197	0.413
LukE	0.107 (0.078)	-0.038-0.216	0.224
LukS	0.103 (0.064)	-0.014-0.233	0.118
CHIPS	-0.009 (0.071)	-0.162-0.109	0.898
SKIN	-0.034 (0.099)	-0.191-0.194	0.762
Alpha toxin	0.074 (0.095)	-0.116-0.277	0.462
Hlb	0.155 (0.058)	0.041-0.262	0.010 *
HlgB	0.022 (0.051)	-0.088-0.120	0.658
Nuc	0.030 (0.063)	-0.099-0.154	0.642
ETA	0.079 (0.059)	-0.049-0.180	0.168
ETB	0.029 (0.067)	-0.119-0.149	0.655
SAK	-0.005 (0.068)	-0.124-0.138	0.938
SSL1	0.080 (0.071)	-0.042-0.242	0.290
SSL3	0.080 (0.094)	-0.052-0.306	0.519
SSL5	0.085 (0.094)	-0.048-0.291	0.473
SSL9	0.095 (0.097)	-0.026-0.323	0.474
SSL10	0.090 (0.093)	-0.029-0.299	0.449
SSL11	0.058 (0.069)	-0.057-0.225	0.384
FlipR	0.085 (0.089)	-0.044-0.293	0.372
FlipRL	0.117 (0.083)	-0.006-0.308	0.299
Lipase	0.111 (0.087)	-0.039-0.298	0.257
Aly	0.149 (0.122)	-0.121-0.367	0.245
Alt2	0.015 (0.065)	-0.109-0.142	0.800
ProAlt	0.010 (0.049)	-0.087-0.111	0.842
EsxB	0.055 (0.064)	-0.066-0.201	0.393
SEA	0.078 (0.066)	-0.046-0.206	0.247
SEB	0.023 (0.072)	-0.122-0.161	0.760
SEC	0.068 (0.067)	-0.082-0.182	0.346
SED	0.074 (0.074)	-0.074-0.224	0.313
SEE	0.119 (0.069)	-0.033-0.248	0.102
SEG	0.165 (0.069)	0.050-0.326	0.019 *
SEH	-0.024 (0.063)	-0.152-0.091	0.703
SEI	0.054 (0.065)	-0.065-0.183	0.409
SEJ	0.067 (0.075)	-0.075-0.223	0.380
SEM	-0.017 (0.072)	-0.0156-0.120	0.819
SEN	0.009 (0.062)	-0.0113-0.133	0.902
SEO	0.098 (0.056)	-0.015-0.209	0.083
SEQ	0.010 (0.063)	-0.113-0.137	0.877
SER	0.023 (0.080)	-0.132-0.179	0.795
TSST-1	0.045 (0.064)	-0.079-0.176	0.479
FnbpA	0.050 (0.067)	-0.099-0.167	0.448
FnbpB	0.151 (0.060)	0.029-0.261	0.015 *
SdrD	-0.045 (0.065)	-0.166-0.091	0.470
SdrE	0.050 (0.064)	-0.086-0.160	0.418
ClfA	0.142 (0.067)	0.023-0.291	0.040 *
ClfB	-0.047 (0.094)	-0.246-0.131	0.618

Table S2d. Results linear regression analysis DAVOS study – association with TARC

Antigen	*Regression coefficient (SE)*	*95% CI*	*P-value*
LukD	0.022 (0.349)	-0.785-0.576	0.929
LukE	0.148 (0.538)	-0.573-0.871	0.665
LukS	0.400 (0.239)	-0.117-0.840	0.091
CHIPS	0.014 (0.379)	-0.810-0.704	0.965
SKIN	0.065 (0.335)	-0.592-0.755	0.865
Alpha toxin	0.593 (0.341)	-0.043-1.303	0.092
Hlb	0.437 (0.333)	-0.270-1.073	0.192
HlgB	0.054 (0.204)	-0.374-0.448	0.801
Nuc	0.335 (0.292)	-0.240-0.957	0.256
ETA	-0.233 (0.393)	-1.078-0.519	0.528
ETB	0.067 (0.381)	-0.755-0.754	0.850
SAK	0.695 (0.302)	0.084-1.285	0.027 *
SSL1	0.110 (0.392)	-0.882-0.658	0.795
SSL3	0.413 (0.374)	-0.061-1.310	0.435
SSL5	0.300 (0.358)	-0.257-1.071	0.495
SSL9	0.315 (0.358)	-0.207-1.197	0.480
SSL10	0.243 (0.355)	-0.273-1.071	0.557
SSL11	0.515 (0.347)	-0.109-1.212	0.155
FlipR	0.108 (0.395)	-0.654-0.888	0.818
FlipRL	0.375 (0.343)	-0.206-1.149	0.380
Lipase	0.661 (0.351)	0.094-1.454	0.058
Aly	0.485 (0.709)	-0.889-1.879	0.485
Alt2	0.405 (0.324)	-0.258-1.043	0.228
ProAlt	0.035 (0.315)	-0.598-0.646	0.906
EsxB	0.257 (0.297)	-0.363-0.858	0.363
SEA	0.154 (0.354)	-0.557-0.861	0.655
SEB	0.326 (0.297)	-0.203-0.923	0.286
SEC	0.167 (0.272)	-0.312-0.751	0.535
SED	-0.146 (0.267)	-0.685-0.360	0.576
SEE	-0.001 (0.341)	-0.724-0.605	0.998
SEG	0.138 (0.335)	-0.511-0.832	0.673
SEH	0.284 (0.338)	-0.417-0.892	0.402
SEI	0.375 (0.288)	-0.161-1.001	0.176
SEJ	0.215 (0.320)	-0.355-0.886	0.521
SEM	0.110 (0.356)	-0.617-0.749	0.756
SEN	0.123 (0.391)	-0.609-0.926	0.760
SEO	0.324 (0.364)	-0.427-1.085	0.353
SEQ	0.298 (0.336)	-0.372-0.944	0.385
SER	0.270 (0.441)	-0.664-1.128	0.536
TSST-1	0.074 (0.323)	-0.541-0.706	0.809
FnbpA	0.040 (0.340)	-0.659-0.723	0.910
FnbpB	0.522 (0.305)	-0.059-1.122	0.092
SdrD	0.387 (0.320)	-0.257-0.988	0.237
SdrE	0.000 (0.303)	-0.564-0.596	0.998
ClfA	0.355 (0.263)	-0.180-0.910	0.181
ClfB	-0.346 (0.506)	-0.1.328-0.627	0.508

Table S2c. Results linear regression analysis DAVOS study – association with SA-EASI *(continued)*

Antigen	*Regression coefficient (SE)*	*95% CI*	*P-value*
IsdH	0.113 (0.076)	-0.028-0.260	0.138
EfB	-0.023 (0.062)	-0.139-0.101	0.707
IsdA	0.077 (0.082)	-0.040-0.270	0.428
SasG	0.065 (0.066)	-0.064-0.196	0.331
IsaA	-0.045 (0.073)	-0.184-0.105	0.513
LytM	0.026 (0.063)	-0.101-0.151	0.643
Eap	-0.006 (0.069)	-0.133-0.140	0.936
EapH1	0.073 (0.072)	-0.061-0.219	0.322
EapH2	0.026 (0.069)	-0.087-0.182	0.711

* = significant P-value, Bootstrapping iter 1000 for all antigens

Table S2d. Results linear regression analysis DAVOS study – association with TARC *(continued)*

Antigen	*Regression coefficient (SE)*	*95% CI*	*P-value*
IsdH	0.358 (0.329)	-0.240-1.065	0.279
EfB	-0.074 (0.384)	-0.843-0.692	0.858
IsdA	0.416 (0.271)	-0.008-1.031	0.223
SasG	0.215 (0.239)	-0.225-0.751	0.383
IsaA	0.074 (0.284)	-0.487-0.654	0.769
LytM	-0.164 (0.385)	-0.863-0.611	0.683
Eap	-0.229 (0.307)	-0.800-0.412	0.464
EapH1	0.625 (0.336)	-0.027-1.287	0.078
EapH2	0.079 (0.221)	-0.343-0.498	0.741

* = significant P-value, Bootstrapping iter 1000 for all antigens