The prevalence of antibody responses against *Staphylococcus aureus* antigens in patients with atopic dermatitis: a systematic review and meta-analysis.

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ABSTRACT

Background

Staphylococcus (*S.*) *aureus* plays a role in the pathogenesis of atopic dermatitis (AD), possibly via the expression of various virulence antigens. An altered antibody response towards these antigens might contribute to inflammation.

Objective

To provide an overview of the varying prevalences and odds of antibody responses against *S. aureus* antigens in patients with AD.

Methods

Data were systematically obtained from Embase, MEDLINE, Web of Science, Scopus, Cochrane, PubMed and Google Scholar up to 12 February 2016. We selected all original observational and experimental studies assessing antistaphylococcal antibodies in serum of patients with AD. Prevalences and odds ratios (ORs) of IgE, IgG, IgM, IgA against *S. aureus* in patients with AD vs. healthy controls were pooled using the random-effects model. We calculated I^2 statistics to assess heterogeneity and rated study quality using the Newcastle-Ottawa Scale.

Results

Twenty-six articles (2369 patients) were included, of which 10 were controlled studies. Study quality was fair to poor. Patients with AD had higher prevalences of IgE against staphylococcal enterotoxin (SE)A (OR 8.37, 95% confidence interval 2.93-23.92) and SEB (OR 9.34, 95% confidence interval 3.54-24.93) compared with controls. Prevalences of antistaphylococcal IgE were 33% for SEA, 35% for SEB and 16% for toxic shock syndrome toxin-1. However, study heterogeneity and imprecision should be taken into consideration when interpreting the results. Data on IgG, IgM and IgA, as well as other antigens, are limited.

Conclusions

Patients with AD more often show an IgE antibody response directed against *S. aureus* superantigens than healthy controls, supporting a role for *S. aureus* in AD pathogenesis.

INTRODUCTION

Atopic dermatitis (AD) is a multifactorial disorder that arises from interactions between immune dysregulations, genetic predisposition, skin barrier defects and environmental factors.[1,2] Both the lesional and nonlesional skin and the noses of patients with AD are more likely to be colonized with *Staphylococcus* (*S.*) *aureus* compared with healthy controls.[3] Recent studies have shown that abundance of *S. aureus* is associated with AD severity, suggesting a causal role for *S. aureus* in the pathogenesis of AD.[2,4-9] However, the exact mechanisms by which *S. aureus* aggravates inflammation in AD are not fully understood.[10] *S. aureus* expresses a variety of virulence factors that could contribute to AD inflammation. Based on their biological function these antigens can be divided in four groups: (i) microbial surface components recognizing adhesive matrix molecules (MSCRAMMs) such as clumping factor A (ClfA), which helps *S. aureus* adhere to the host cells, (ii) cell-membrane damaging molecules such as alpha toxin, which can induce keratinocyte cell death, (iii) household enzymes such as lipase, which provides cell nutrition and (iv) immunomodulatory proteins (superantigenic and nonsuperantigenic).[10-13] The latter include the group of staphylococcal superantigens, which have the ability to activate mast cells and T cells directly, resulting in the release of proinflammatory cytokines.[14-16] Expression of these *S. aureus* antigens varies between the different *S. aureus* isolates. However, it has been proven difficult to identify associations between the genetic composition of *S. aureus* strains and AD.[17-22]

Evaluation of the antibody response to these *S. aureus* antigens gives an indication of the antigens that are expressed by the bacterium *in vivo* and will give insight into how the immune system of patients with AD counteracts these antigens. This might help us to understand the role of *S. aureus* in AD pathogenesis as well as the mechanisms by which *S. aureus* causes inflammation. Since 1982 several studies have reported serum antibodies against *S. aureus* in patients with AD.[23-35] However, the prevalences of antistaphylococcal antibodies in these studies vary widely. This is probably due to low sample sizes and different methods used to detect antibodies [e.g. enzyme-linked immunosorbent assay (ELISA) or AlaSTAT]. Moreover, studies often focus on few antigens and/or antibody classes.

The aim of this systematic review was to provide an overview of the pooled prevalence and odds of antibodies (IgE, IgG, IgM and IgA) against *S. aureus* antigens in serum of patients with AD compared with healthy controls. Additionally, we reviewed the relationship between AD severity and anti-*S. aureus* antibodies.

MATERIALS AND METHODS

Study participants and outcomes

All original observational and experimental human studies were included. No restrictions were made on publication date or language. Case reports were excluded. Patients of all ages with AD, irrespectively of disease severity, in which anti-*S. aureus* antibodies were measured. Healthy controls were defined as persons who had neither AD nor an atopic constitution (food allergy, asthma, allergic rhinitis) nor another skin disease. The primary outcome was the proportion of patients with AD with antibodies (IgE, IgG, IgM, IgA) in serum against *S. aureus* antigens compared with healthy controls. The secondary outcome was the relationship between AD severity and antistaphylococcal antibodies.

Search strategy

The systematic electronic search was conducted in Embase, MEDLINE, Web of Science, Scopus, Cochrane, PubMed and Google Scholar up to 12 February 2016 (table S1). A cross-reference check was performed to identify other relevant studies.

Study selection and data extraction

Initially, all studies identified in the systematic search were screened for relevance by title and abstract. Duplicates and studies that did not meet our inclusion criteria were excluded (appendix S1, see Supporting information). The remaining articles were assessed for eligibility by full-text review. Translation of non-English studies was conducted officially. Study selection and data extraction were performed independently by two researchers (F.J.M.vB. and either J.E.E.T. or J.dW). Disagreements were resolved and consensus was reached. If one population was described in different articles, we included the study with the most detailed description of the results. The methodological quality of the articles was scored based on an adapted version of the Newcastle-Ottawa Scale (NOS).[3] Studies could reach a maximum score of nine points for case-control studies and five points for uncontrolled studies. Using a scoring algorithm, the controlled studies were classified as being of poor, fair or good methodological quality, based on their NOS scores (appendix S2, see Supporting information).[36] The overall quality of evidence was discussed according to the principles of the GRADE approach (i.e. limitations in study design or execution, inconsistency of results, indirectness of evidence, imprecision and publication bias).[37]

Statistical analysis

A meta-analysis was performed using a random-effects model in case of at least two available studies. We extracted the prevalences of antistaphylococcal antibodies in patients with AD and controls from the included studies. If required we calculated

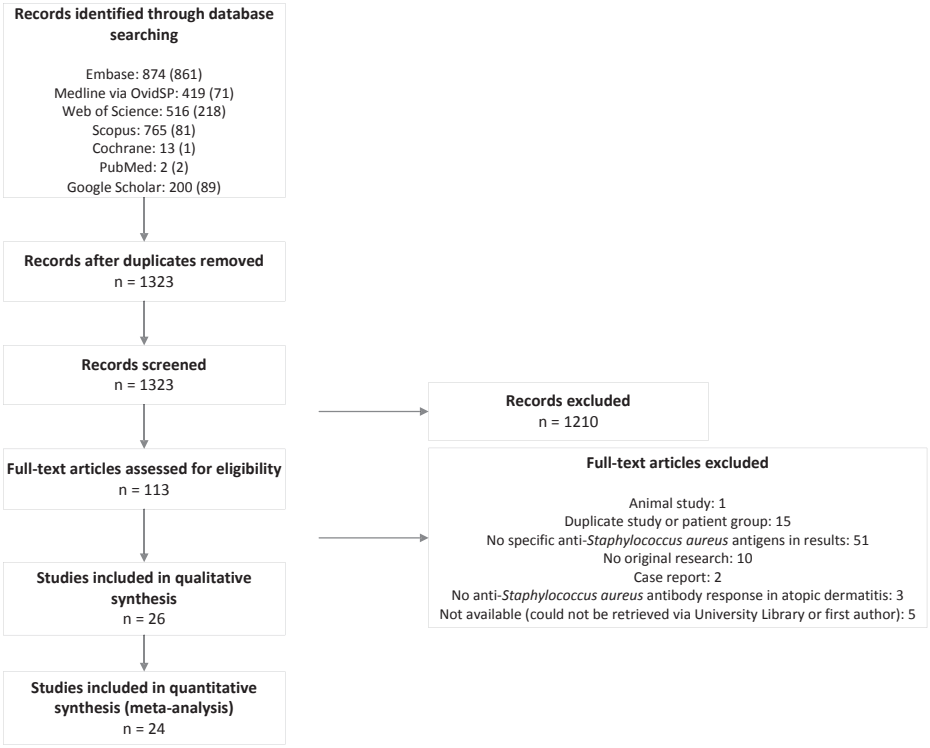
prevalences with the available raw data. The prevalences of antistaphylococcal antibodies were pooled. Furthermore, in controlled studies these prevalences in patients and controls were compared, expressed as ORs with a 95% confidence intervals (CIs). Antibody prevalences were descriptively presented for single studies. When the antibody prevalence in the control group was 0% an OR could not be calculated and a continuity correction factor using the Mantel-Haenszel method was added to both the patient and control groups (based on the unbalanced group ratio).[38,39] Heterogeneity was assessed using the Higgins I^2 test.[40,41] However, I^2-values should be interpreted cautiously in small meta-analyses.[42] In case of substantial (I^2 = 50-90%) or considerable (I^2 = 75-100%) heterogeneity, sources were explored using subgroup analyses for the variables age, method of antibody identification and geographical region of the study centres (≥10 available studies). Possible publication bias was assessed in case of ≥10 studies using funnel plots and Egger's test (P-value <0.05).[43,44] Analyses were performed using Comprehensive Meta-Analysis Version 2.2 (Biostat, Englewood, NJ, U.S.A.). This systematic review was conducted and reported according to the Meta-Analysis of Observational Studies in Epidemiology (MOOSE) guidelines.[45]

RESULTS

Study characteristics and quality

The literature search identified 2789 studies. After removal of duplicates, 1323 studies remained. Screening on title and abstract yielded 113 full-text articles. Finally, 26 articles with a total of 2369 patients were included for further analysis (figure 1).[24,25,27-35,46-60] Twenty-one articles reported the sex of the patients, with a mean percentage of males of 53.4% (range 28.1-81.8).[25,28-30,33-35,46-49,51-60] The mean age was 24.1 years (range 4.4-68.9), reported in 15 articles.[25,28-30,33,34,46-49,51,53-55,58] Thirteen articles scored the AD severity using the SCORing Atopic Dermatitis (SCORAD) with three articles reporting a mean of 33.6 (range 32.2-36.0), corresponding with moderate AD.[30,32-35,46,47,49-52,57,59,61] Nine articles used other scoring criteria for AD severity.[25,28,29,31,48,54-56,58] Most studies were conducted in Europe and Asia. Methods for identification of antistaphylococcal antibodies consist mainly of radioimmunoassay (RIA) tests, ELISA and AlaSTAT, an enzyme immunoassay method for the measurement of allergen-specific IgE (table S2). One study measuring multiple antibodies used both a RIA test for IgE and an ELISA for IgG.[31] NOS scores of the 11 controlled studies were rated as good (n=1), fair (n=5) and poor (n=5).[24,25,27-33,36,53,60] The main reason for downgrading the quality of these studies was incomparability of the patient and control groups. The quality scores of the 15 studies without a control group varied between 2 and 4 points out of 5 (table S2 and appendix S2).[34,35,46-52,54-59]

Figure 1. Flow chart of search strategy and study selection



Prevalence of antibodies against *Staphylococcus aureus*

IgE

Twenty-four studies including 2206 patients reported the prevalence of antistaphylococcal IgE. [24,25,27-31,33-35,46-59] These studies predominantly determine the antibody response against staphylococcal enterotoxin (SE)A, SEB, SEC, SED and toxic shock syndrome toxin (TSST)-1 (19, 23, seven, three and 10 studies, respectively) (table S3).[24,25,27-31,34,35,46-59] Pooled prevalences of antistaphylococcal IgE in patients were 33% for SEA (95% CI 23-45; I^2 94.23)[24,25,27-30,34,35,46-48,50,51,53,54,56-59], 35% for SEB (95% CI 27-43; I^2 91.36)[24,25,27-31,34,35,46-59], 14% for SEC (95% CI 8-22; I^2 78.26)[24,34,46,47,52,56,58], 5% for SED (95% CI 1-16; I^2 70.49)[24,34,47] and 16% for TSST-1 (95% CI 10-25; I^2 85.28)[24,27,34,35,46-48,52,56,58] (table 1 and figure S1a-e). There was a great variation in prevalence between studies (0.8-78.8% for SEA, 1.4-72.9% for SEB, 5.4-40.0% for SEC, 0.0-10.7% for SED and 1.4-53.3% for TSST-1), probably resulting in the substantial to considerable heterogeneity. One study showed a prevalence of 35.8% of fibronectin-binding protein (FBP)-specific IgE, while another study found a prevalence of 48.1% of IgE against lipoteichoic acid (LTA).[27,33] Undetectable to very low prevalences of IgE against the staphylococcal antigens SEE, SEI, SEH, SEK, SEJ, exfoliative toxin (ET)-1 and ETA were found in several single studies (table 2).[24,31,47]

Table 1. IgE against SEA, SEB, TSST-1, SEC and SED in patients with atopic dermatitis

***Staphylococcus aureus* antigen (subgroup)**	**Studies**	**Patients**	**Pooled proportion of patients with detectable antigens (95% CI)**	**Heterogeneity (I^2)**
SEA				
All studies	19	1852	0.33 (0.23-0.45)	94.23
Studies including age <18	6	507	0.31 (0.11-0.63)	96.56
Studies including age ≥18	7	859	0.27 (0.17-0.42)	88.22
Studies including RIA methods*	8	1139	0.19 (0.12-0.29)	86.36
Studies including ELISA method	3	169	0.61 (0.34-0.82)	86.83
Studies including AlaSTAT method	6	461	0.42 (0.28-0.57)	89.04
Studies including Immunoblot method	1	27	0.48 (0.30-0.66)	-
Studies performed in Europe	11	1220	0.24 (0.16-0.34)	87.87
Studies performed in Asia	7	576	0.51 (0.33-0.70)	93.75
Studies performed in USA	1	56	0.32 (0.21-0.45)	-
SEB				
All studies	23	2111	0.35 (0.27-0.43)	91.36
Studies including age <18	8	631	0.25 (0.13-0.43)	92.73
Studies including age ≥18	8	968	0.38 (0.29-0.48)	84.70
Studies including RIA methods*	11	1418	0.25 (0.18-0.34)	86.77
Studies including ELISA method	4	209	0.47 (0.24-0.72)	89.39
Studies including AlaSTAT method	6	461	0.48 (0.33-0.64)	90.42
Studies including Immunoblot method	1	27	0.63 (0.44-0.79)	-
Studies performed in Europe	12	1304	0.28 (0.21-0.36)	84.70
Studies performed in Asia	10	751	0.48 (0.36-0.61)	90.06
Studies performed in USA	1	56	0.18 (0.10-0.30)	-
SEC				
All studies	7	540	0.14 (0.08-0.22)	78.26
SED				
All studies	3	317	0.05 (0.01-0.16)	70.49
TSST-1				
All studies	10	1110	0.16 (0.10-0.25)	85.28
Studies including age <18	5	631	0.13 (0.05-0.28)	85.00
Studies including age ≥18	3	1039	0.12 (0.05-0.27)	84.65
Studies including RIA methods*	6	918	0.12 (0.05-0.25)	90.38
Studies including ELISA method	2	109	0.18 (0.11-0.26)	0.00
Studies including Immunoblot method	1	27	0.41 (0.24-0.60)	-
Studies performed in Europe	7	945	0.15 (0.07-0.28)	90.09
Studies performed in Asia	2	109	0.18 (0.11-0.26)	0.00
Studies performed in USA	1	56	0.21 (0.13-0.34)	-

SE, staphylococcal enterotoxin; TSST-1, toxic shock syndrome toxin; RIA, radioimmunoassay; ELISA, enzyme-linked immunosorbent assay; CI, confidence interval

* CAP fluorescent enzyme immunoassay, ImmunoCAP, and UniCAP

Table 2. IgE, IgG and IgM against *Staphylococcus aureus* antigens in patients with atopic dermatitis

Antibody and *Staphylococcus aureus* antigen	Studies	Patients	(Pooled) proportion of patients with detectable antigens (95% CI)
IgE			
SEE	1	140	0.01
SEI	1	140	0.01
SEH	1	140	0.00
SEK	1	140	0.00
SEJ	1	140	0.00
ETA	1	26	0.00
FBP	1	95	0.36
LTA	1	27	0.48
IgG			
SEA	1	74	0.77
SEB	2	114	0.64 (0.42-0.81)[a]
TSST-1	1	74	0.77
ETA	1	26	0.35
IgG_2			
SEB	1	77	0.87
SEC_1	1	78	0.62
IgM			
SEB	1	40	0.63

Ig, immunoglobulin; SE, staphylococcal enterotoxin; TSST-1, toxic shock syndrome toxin 1; ET, exfoliative toxin; FBP, fibronectin-binding protein; LTA, lipoteichoic acid; CI, confidence interval; [a] Heterogeneity: $I^2 = 78.8$

IgG, IgM and IgA

Prevalences of IgG against *S. aureus* antigens were determined in four studies.[31,32,55,60] The pooled prevalences of IgG against SEB, reported in two studies (114 patients), was 64% (95% CI 42-81; I^2 78.84) (figure S1f).[55,60] In single studies the IgG prevalences were 77.0% for SEA, 77.0% for TSST-1 and 34.6% for ETA.[31,60] IgG subclass 2 (IgG_2) was found in 87.0% of the patients with AD against SEB and in 61.5% against SEC_1.[32] Only one study determined antistaphylococcal IgM, and detect antibodies against SEB in 62.5% of the patients with AD (table 2).[55] None of the selected articles studied antistaphylococcal IgA.

Odds of antibodies against *S. aureus*

Of the 26 articles, 11 studies compared patients with AD against healthy controls (759 patients vs. 328 controls).[24,25,27-33,53,60] Nine studies reported the prevalence of antistaphylococcal IgE (596 patients vs. 189 controls).[24,25,27-31,33,53] These studies mainly described antibody responses against SEA and SEB (seven and eight articles, respectively).[24,25,27-31,53] Pooled analyses showed that antistaphylococcal IgE was found significantly more

Table 3. IgE against SEA, SEB and TSST-1 in patients with atopic dermatitis versus healthy controls

***Staphylococcus aureus* antigen**	**Studies**	**Patients**	**Controls**	**Pooled OR in patients vs controls (95% CI)**	**Heterogeneity (I^2)**
SEA	7	475	139	8.37 (2.93-23.92)*	0.00
SEB	8	501	172	9.34 (3.54-24.93)*	0.00
TSST-1	2	83	20	23.33 (0.47-1153.93)	0.00

OR, odds ratio; CI, confidence interval. * Significant result

often in the serum of patients compared with controls, with ORs of 8.37 for SEA (95% CI 2.93-23.92; P<0.001; I^2 0.00)[24,25,27-30,53] and 9.34 for SEB (95% CI 3.54-24.93; P<0.001; I^2 0.00)[24,25,27-31,53] (table 3 and figure S2a-b). A pooled OR of IgE against TSST-1, reported in two studies (83 patients vs. 20 controls), was 23.33 (95% CI 0.47-1153.93, P=0.114, I^2 0.00) (table 3 and figure S2c).[24,27] Prevalences of other antigens, including SEC, SED, ETA, ET-1, FBP and LTA, were described in single controlled studies and pooled estimates could not be provided.[24,27,31,33] The prevalences of all these *S. aureus* antigens were equal or increased in patients vs. controls (table 4). As most antibody prevalences in control groups were 0% the ORs could not be calculated. Prevalences of IgG in patients and controls were compared in three studies.[31,32,60] Compared with controls, patients were found to

Table 4. IgE and IgG against *Staphylococcus aureus* antigens in patients with atopic dermatitis versus healthy controls

Antibody and *Staphylococcus aureus* antigen	**Studies**	**Patients**	**Controls**	**Mean proportion of patients with detectable antigens**	**Mean proportion of controls with detectable antigens**
IgE					
SEC	1	56	15	0.05	0.0
SED	1	56	15	0.05	0.0
ETA	1	26	33	0.00	0.0
ET	1	56	15	0.02	0.0
FBP	1	95	17	0.36	0.0
IgG					
SEA	1	74	111	0.77	0.88
SEB	1	74	111	0.73	0.69
TSST-1	1	74	111	0.77	0.85
ETA	1	26	14	0.35	0.14
IgG_2					
SEB	1	77	27	0.87	0.78
SEC_1	1	78	28	0.62	0.86

SE, staphylococcal enterotoxin; TSST-1, toxic shock syndrome toxin 1; ET, exfoliative toxin; FBP, fibronectin-binding protein

have higher IgG prevalences to ETA and SEB and lower prevalences of IgG to SEA and TSST-1.[31,60] In patients, the IgG_2 prevalence to SEC_1 was lower and to SEB higher than in controls.[32] However, most differences in prevalences between patients and controls were small. No studies compared the antistaphylococcal IgM or IgA responses between patients and controls.

Subgroup analysis

Subgroup analyses of the variables age, method of antibody identification and geographical region of the study centres were performed to detect possible sources of heterogeneity. The prevalences of IgE against SEA, SEB and TSST-1 did not significantly differ between children and adults (31% vs. 27%, 25% vs. 38% and 13% vs. 12%, respectively). Studies using the ELISA method showed higher pooled prevalences of IgE against SEA, SEB and TSST-1 than studies using RIA tests (61% vs. 19%, 47% vs. 25% and 18% vs. 12%, respectively). Lastly, studies conducted in Asia showed higher pooled prevalences of IgE to SEA, SEB an TSST-1 compared to studies conducted in Europe (51% vs. 24%, 48% vs. 28% and 18% vs. 15%, respectively) (table 1).

Relationship between AD severity and antibodies against *S. aureus*

Considering the low number of studies reporting a mean SCORAD we could not calculate an overall association between AD severity and antistaphylococcal antibodies. However, several individual studies reported a significant association between superantigen-specific (e.g. SEA, SEB) IgE and AD severity, measured by SCORAD, the criteria of Rajka or the modified Leicester system.[29,32,55,58] This association could not be confirmed in four comparable studies.[25,28,46,58] Sohn *et al*. looked at IgG against SEB and did not find a relationship with AD severity.[55] However, Mrabet-Dahbi *et al*. found that patients with a deficiency of antistaphylococcal IgG_2 to SEC_1 had a more severe AD phenotype.[32] Based on these contradictory studies, no conclusions can be drawn about the association between the antistaphylococcal antibody response and severity of AD.

Publication bias

Funnel plots of the pooled prevalence of IgE against SEA, SEB and TSST-1 showed no asymmetry (figure S3). Egger's tests had intercepts of 0.52 for SEA (95% CI -4.40-5.44, P = 0.826), -0.44 for SEB (95% CI -3.78-2.91, P = 0.789) and -0.82 for TSST-1 (95% CI -4.40-2.76, P = 0.611), confirming no publication bias.

DISCUSSION

This systematic review includes 26 studies and 2352 patients with AD. IgE responses against SEA and SEB in serum were found more often in patients with AD than in healthy controls. IgE, IgG and IgM against a very limited panel of other antigens were reported in single studies. No data are available on antistaphylococcal IgA. Pooled prevalences of antistaphylococcal IgE in patients with AD are 33% for SEA, 35% for SEB and 16% for TSST-1. Substantial to considerable heterogeneity and imprecision (small studies) limit the quality of evidence and should be taken into consideration when interpreting the results. Subgroup analysis were performed to account for differences in outcome measures (indirectness). The quality of evidence was probably not influenced by publication bias.

Subgroup analyses suggest that the antibody prevalence is dependent on the method of antibody identification (ELISA vs. RIA) and the geographic region of the study centres (Asia vs. Europe). This is in accordance with the study of Taylor *et al*. that found ELISA more sensitive than RIA to detect IgG_1 in mouse.[62] It might also explain the higher prevalence of antibodies in Asia compared to Europe, as Asian studies use ELISA techniques more often. Furthermore, an ethnicity-dependent antibody response has been suggested, at least for TSST-1.[63] Because heterogeneity in subgroup analyses remains high, pooled prevalences and odds were probably also influenced by other variables, like AD severity. Unfortunately, we were not able to explore this as only a few studies reported a mean SCORAD. These individual studies showed contradictory results about the association between AD severity and IgE against predominantly superantigens.

The *S. aureus* antigens SEA and SEB belong to the group of immune modulators and act as superantigens. This indicates that they have the ability to stimulate T cells directly, resulting in T-cell proliferation and cytokine release, causing epithelial damage.[14-16] The increased anti-SEA and anti-SEB IgE responses could be the result of increased expression of these antigens by the *S. aureus* bacteria in patients with AD, indicating SEA and SEB as possible bacterial mechanisms to aggravate or even initiate inflammation in AD. However, the studies included in this systematic review predominantly examined the prevalence of antibodies against the superantigens SEA, SEB and TSST-1 and other common antigens such as clumping factor A and lipase were not tested. In addition, SEA, SEB and TSST-1 were present in only 14%, 24% and 14%, respectively, of the *S. aureus* isolates.[64] These data suggest a bias in the assessment of staphylococcal antigens and indicate also a large genetic diversity among the colonizing *S. aureus* strains. Furthermore, the increased IgE responses against these antigens may be the result of immunological cross-reactivity, where the corresponding antigen-coding genes of SEA, SEB and/or TSST-1 are not present in the isolate.[65-67]

This is the first systematic review summarising the available data on the prevalence of antistaphylococcal antibodies in patients with AD and the involved antigens. The broad selection criteria (e.g. all languages, exclusion only of case reports and nonoriginal studies) resulted in collecting the majority of articles about this subject and limiting selection bias. However, there are still some limitations in this study. Firstly, most articles did not report the AD treatment at the time of antibody measurement. The use of antimicrobial therapy might decrease the *S. aureus* load and *S. aureus* antibody titres.[4,68] In addition, the anti-inflammatory effect of systemic glucocorticosteroids could both cause a decrease in serum antibody concentrations and might also reduce *S. aureus*.[6,69,70] Even emollient monotherapy showed a decrease of *S. aureus* on the skin.[71,72] In the studies that did report the treatment at baseline, the therapies consisted mainly of topical corticosteroids or no treatment at all (n=9). Secondly, the cut-off values of antibody identification methods were highly variable, not mentioned or unclear in and between several methods. Through subgroup analysis we tried to correct for this variability partly. Last, mainly antistaphylococcal IgE was assessed, of which the choice for determination was often unsubstantiated or based on results of previous studies. Patients with AD have frequent high IgE responses to environmental antigens, for example *S. aureus*. In addition, IgG is the most common antibody in the extravascular fluid and among others it plays a role in the neutralization of toxins.[73,74]

To further investigate the role of (the immune response against) *S. aureus* in AD pathogenesis, future studies should focus on other antibody subtypes and other *S. aureus* antigens. IgG subclasses should be measured to detect possible biomarkers for AD severity, such as a selective deficiency in IgG_2 against SEC_1 in the study of Mrabet-Dahbi *et al*.[32] Furthermore, assessment of the antibody response against other *S. aureus* antigens, like MSCRAMMs, membrane-damaging molecules, housekeeping antigens and other types of immunomodulatory proteins, might give more insight in whether an increased IgE response is a secondary phenomenon of increased *S. aureus* colonization of AD skin.

CONCLUSION

In conclusion, this systematic review with meta-analysis shows that patients with AD have higher prevalences of IgE against the *S. aureus* antigens SEA and SEB than healthy controls, taking the large heterogeneity into consideration. These antigens, belonging to the group of immune modulators, are known as superantigens and have the ability to cause inflammation and epithelial damage. This supports a role for *S. aureus* in the pathogenesis of AD. IgE, IgG and IgM against a very limited panel of other antigens were studied in single studies. No data are available on antistaphylococcal IgA.

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SUPPORTING INFORMATION

Appendix S1. Inclusion criteria for selecting studies for this systematic review

Types of studies

- All original observational and experimental human studies which assess the presence of antistaphylococcal antibodies in the serum of patients with atopic dermatitis, reported per *Staphylococcus aureus* antigen.
- All study designs, except for case reports.

Participants

- Patients of all ages with atopic dermatitis irrespective of disease severity, and presence of antistaphylococcal antibodies. Atopic dermatitis diagnosed by a medical doctor.

Controls

- Persons who does not have atopic dermatitis neither an atopic constitution (asthma, allergic rhinitis, food allergy) or another skin disease.

Outcome measures

- Primary: Specific antibodies (IgE, IgG, IgM, IgA) against *Staphylococcus aureus* antigens.
- Secondary: The relationship between atopic dermatitis disease severity and specific antibodies (IgM, IgG, IgA, IgE) antibodies against *Staphylococcus aureus* antigens.

Appendix S2. Quality assessment score

Modified Newcastle-Ottawa quality assessment scale for cohort or cross sectional studies.

Stars indicate the points allocated if the item criterion is met. A maximum score of 9 can be allocated to each article. Uncontrolled studies can reach a maximum score of 5.

Selection

1. Representativeness of the exposed cohort

a) Truly representative of the general atopic dermatitis population *
b) Somewhat representative of the general atopic dermatitis population *
c) Selected group of atopic dermatitis patients (hospital based, tertiary center, inpatients, outpatients)
d) No description of the derivation of the cohort

2. Selection of the non-exposed cohort

a) Representative of the average community (healthy control, community control) *
b) Selected group of controls (hospital controls, other dermatological condition
c) No description of the derivation of the cohort

3. Ascertainment of atopic dermatitis

a) Diagnosed by dermatologist *
b) Diagnosed by physician other than dermatologist *
c) Diagnosed by clinical assessment*
d) Based on self-report
e) No description of atopic dermatitis case definition

4. Definition of the non-exposed cohort

a) No history of disease (endpoint) *
b) No description of source

Comparability

1. Comparability of exposed and non-exposed cohorts on the basis of design or analysis

a) Patients with AD and healthy controls are matched for age *
b) Patients with AD and healthy controls are matched for any additional factor *
c) No controlling for confounding or matching

Outcome

1. Assessment of outcome: measurement method of antibody response against *Staphylococcus aureus* antigens

a) Determined by ELISA, CAP, RAST, micro-array, AlaSTAT *
b) Not mentioned

2. Treatment during sampling

a) No treatment *
b) Systemic treatment
c) Topical treatment
d) Not mentioned

3. Missing data

a) Same rate for both patients with AD and healthy controls / no missing data *
b) Different rate for both patients with AD and healthy controls, but well described/ missing data, but well described *
c) Different rate for both patients with AD and healthy controls and not explained
d) Not mentioned

Modified Scoring algorithm controlled studies [36]

Quality rating	Points in Selection Domain	Points in Comparability Domain	Points in Outcome domain
Good	≥ 3	≥ 1	≥ 2
Fair	2	0	≥ 2
Poor	0-1	0	0-1

Appendix S3. Supplementary references

22. Leung DY, Harbeck R, Bina P et al. Presence of IgE antibodies to staphylococcal exotoxins on the skin of patients with atopic dermatitis. Evidence for a new group of allergens. J Clin Invest 1993; 92: 1374-80.
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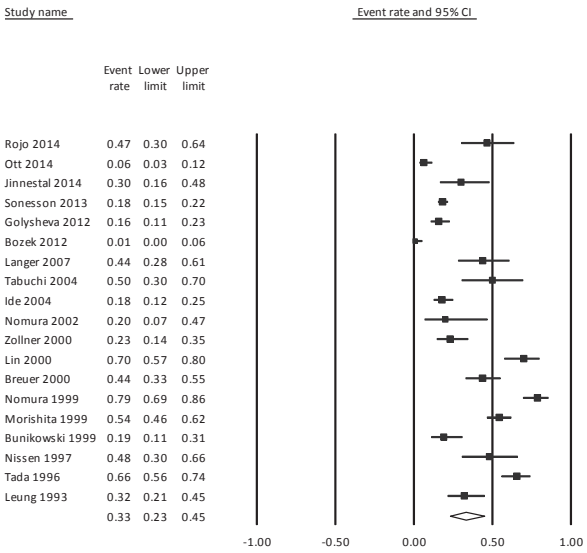
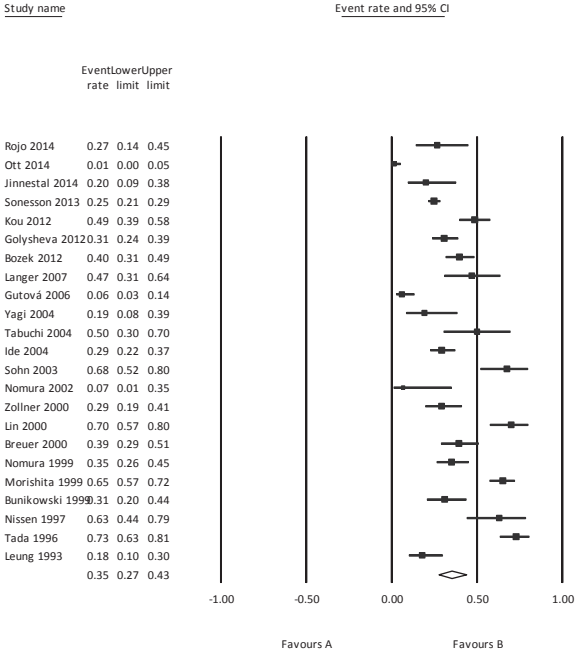
SUPPLEMENTARY FIGURES

Figure S1. Forest plots of prevalence meta-analyses

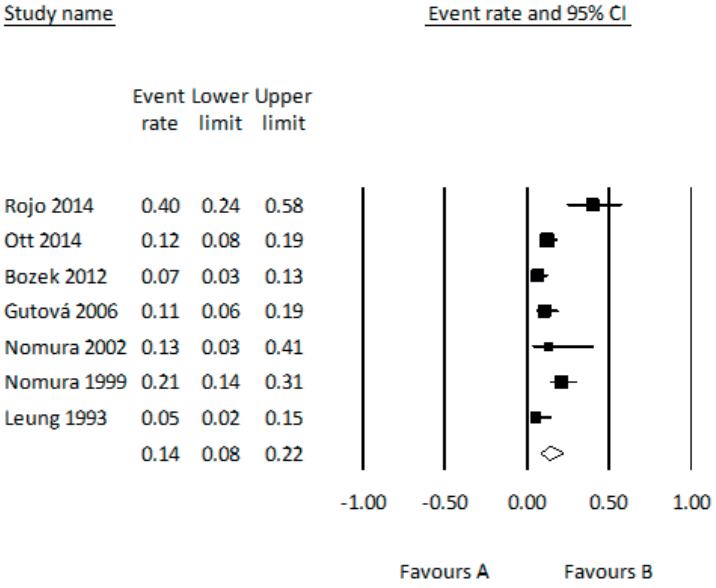
a. IgE against SEA in patients with atopic dermatitis



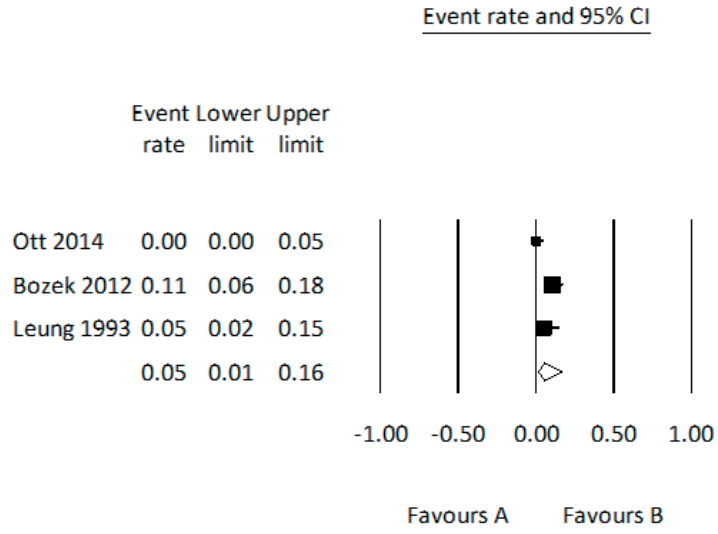
b. IgE against SEB in patients with atopic dermatitis



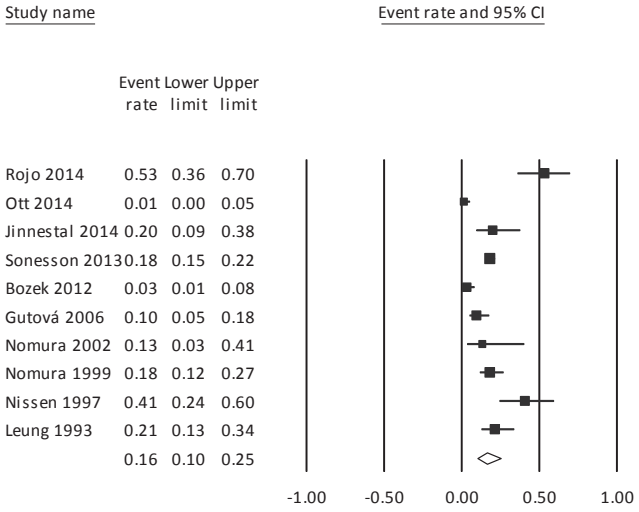
c. IgE against SEC in patients with atopic dermatitis



d. IgE against SED in patients with atopic dermatitis



e. IgE against TSST-1 in patients with atopic dermatitis



f. IgG against SEB in patients with atopic dermatitis

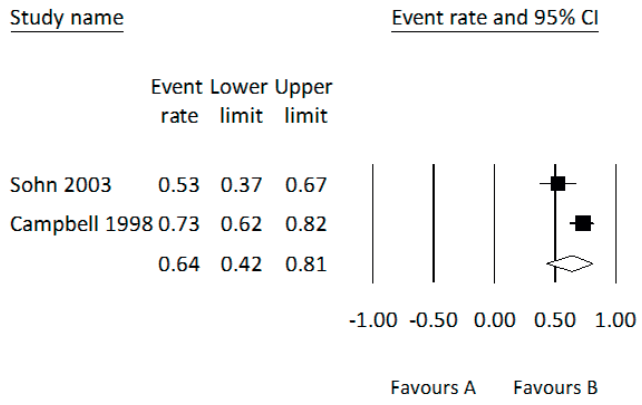
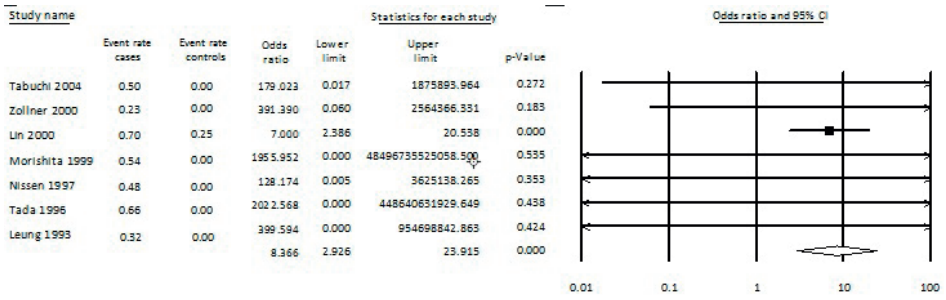
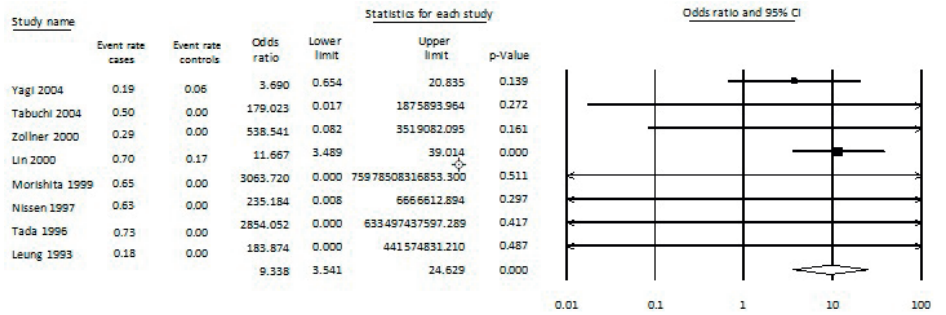


Figure S2. Forest plots of odds meta-analyses

a. IgE against SEA in patients with atopic dermatitis versus healthy controls



b. IgE against SEB in patients with atopic dermatitis versus healthy controls



c. IgE against TSST-1 in patients with atopic dermatitis versus healthy controls

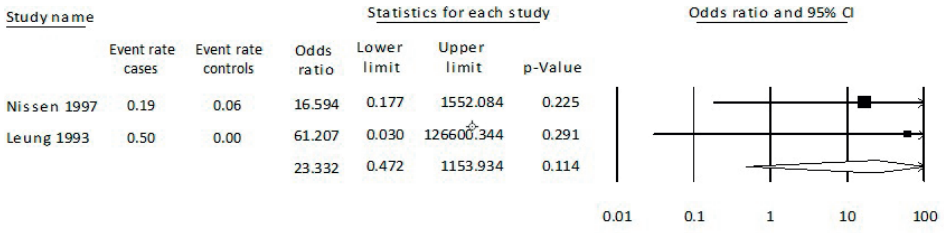
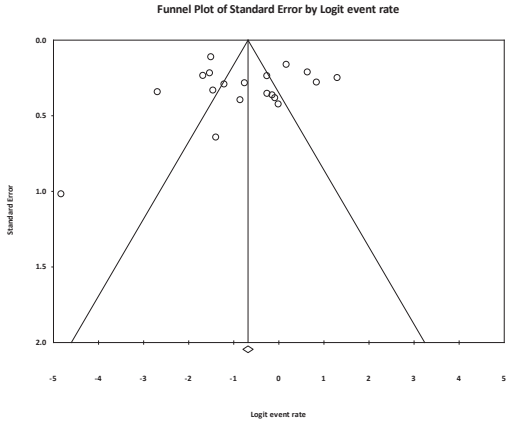
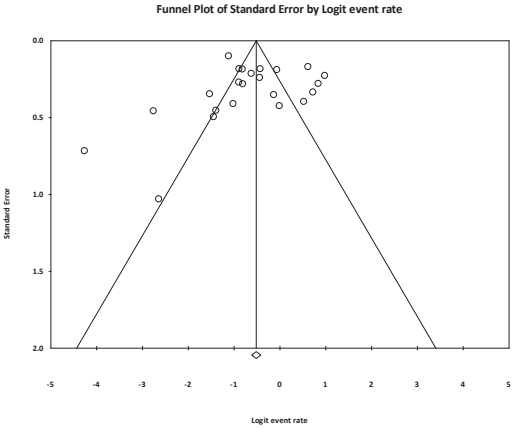


Figure S3. Funnel plots for publication bias

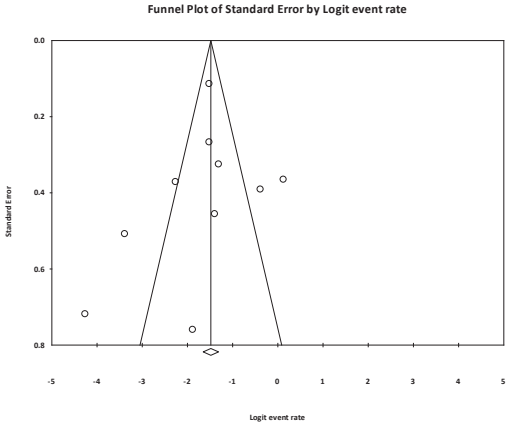
a. IgE against SEA in patients with atopic dermatitis



b. IgE against SEB in patients with atopic dermatitis



c. IgE against TSST-1 in patients with atopic dermatitis



SUPPLEMENTARY TABLES

Table S1. Electronic search

Database	Search
Embase	(eczema/exp OR 'atopic dermatitis'/exp OR (eczem* OR ((atopic OR constitutional*) NEAR/3 (dermatit* OR neurodermatit*))):ab,ti) AND ('Staphylococcus aureus'/de OR ('Staphylococcus aureus' OR 's aureus' OR 'Staph aureus'):ab,ti) AND (antibody/exp OR immunoglobulin/exp OR antigen/exp OR microbiome/de OR 'skin flora'/de OR (antibod* OR immunoglobulin* OR IGA OR IGE OR IGG OR IGM OR IGAs OR IGEs OR IGGs OR IGMs OR antigen* OR superantigen* OR microbiome* OR 'skin flora'):ab,ti)
Medline (OvidSP)	(eczema/ OR "Dermatitis, Atopic"/ OR (eczem* OR ((atopic OR constitutional*) ADJ3 (dermatit* OR neurodermatit*))).ab,ti.) AND ("Staphylococcus aureus"/ OR ("Staphylococcus aureus" OR "s aureus" OR "Staph aureus").ab,ti.) AND (exp immunoglobulin/ OR exp antigens/ OR microbiota/ OR (antibod* OR immunoglobulin* OR IGA OR IGE OR IGG OR IGM OR IGAs OR IGEs OR IGGs OR IGMs OR antigen* OR superantigen* OR microbiome* OR "skin flora").ab,ti.)
Pubmed	(eczema[mh] OR (eczem*[tiab] OR ((atopic OR constitutional*[tiab]) AND (dermatit*[tiab] OR neurodermatit*[tiab])))) AND (Staphylococcus aureus[mh] OR (Staphylococcus aureus*[tiab] OR s aureus*[tiab] OR Staph aureus*[tiab])) AND (immunoglobulin[mh] OR antigens[mh] OR microbiota[mh] OR (antibod*[tiab] OR immunoglobulin*[tiab] OR IGA[tiab] OR IGE[tiab] OR IGG[tiab] OR IGM[tiab] OR IGAs[tiab] OR IGEs[tiab] OR IGGs[tiab] OR IGMs[tiab] OR antigen*[tiab] OR superantigen*[tiab] OR microbiome*[tiab] OR skin flora*[tiab])) AND publisher[sb]
Web of Science	TS=(((eczem* OR ((atopic OR constitutional*) NEAR/3 (dermatit* OR neurodermatit*))))) AND (("Staphylococcus aureus" OR "s aureus" OR "Staph aureus")) AND ((antibod* OR immunoglobulin* OR IGA OR IGE OR IGG OR IGM OR IGAs OR IGEs OR IGGs OR IGMs OR antigen* OR superantigen* OR microbiome* OR "skin flora")))
Scopus	TITLE-ABS-KEY(((eczem* OR ((atopic OR constitutional*) W/3 (dermatit* OR neurodermatit*)))) AND (("Staphylococcus aureus" OR "s aureus" OR "Staph aureus")) AND ((antibod* OR immunoglobulin* OR IGA OR IGE OR IGG OR IGM OR IGAs OR IGEs OR IGGs OR IGMs OR antigen* OR superantigen* OR microbiome* OR "skin flora")))
Google scholar	Eczema|"atopic dermatitis""Staphylococcus|s|Staph aureus" antibody|antibodies|immuno globulin|antigen|antigens|microbiome|"skin flora"|superantigen|superantigens
Cochrane Library	((eczem* OR ((atopic OR constitutional*) NEAR/3 (dermatit* OR neurodermatit*))):ab,ti) AND (('Staphylococcus aureus' OR 's aureus' OR 'Staph aureus'):ab,ti) AND ((antibod* OR immunoglobulin* OR IGA OR IGE OR IGG OR IGM OR IGAs OR IGEs OR IGGs OR IGMs OR antigen* OR superantigen* OR microbiome* OR 'skin flora'):ab,ti)

Table S2. Study characteristics per study
NOTE: Full reference details are provided in Appendix S3 (see Supporting Information)

	Country	Patients				
		N	% Male	Mean age (y)	Treatment at baseline	Mean AD severity score
Rojo 2014[43]	Spain	32/ 30[b]	65.6	23	-	SCORAD -
Ott 2014[44]	Germany	140	60.7	6.2	-	SCORAD 36
Jinnestål 2014[33]	Sweden	30	30.0	32.5 (med)	Topical treatment, no UV treatment	SCORAD -
Sonesson 2013[45]	Sweden	513	32.6	26.6	-	Rajka & Langeland 5.7
Kou 2012[46]	Japan	121/109[b]	57.0	35.7	Topical corticosteroid, 24 patients used cyclosporine	SCORAD 42 (med)
Golysheva[a] 2012[47]	Russia	133	-	Range 1-55	-	SCORAD -
Bozek 2012[32]	Poland	121	63.6	68.9	-	SCORAD 32.2
Reginald 2011[31]	Austria & Germany	95	47.4	34.4	-	SCORAD -
Langer 2007[48]	Germany	32	28.1	31.5	-	SCORAD 33.4
Gutová 2006[49]	Czech Republic	84	50.0	Range 4 mo – 9 y	-	SCORAD -
Mrabet-Dahbi 2005[30]	Germany	89	-	31 (med)	No corticosteroid and systemic or topical AB 4 weeks prior to the study	SCORAD 45 (med)

Controls			Antibody	Cut-off detection method	NOS	Patients	Controls
N	% Male	Mean age (y)				Prevalence positive anti-*S. aureus* antibodies	Prevalence positive anti-*S. aureus* antibodies
			IgE	UniCAP 10 kU/L	2	SEA: 0.47 SEB: 0.27 SEC: 0.40 TSST-1: 0.53	
			IgE	FEIA -	3	SEA: 0.06 SEB: 0.01 SEC: 0.12 SED: 0.00 SEE: 0.01 SEI: 0.01 SEH: 0.00 SEK: 0.00 SEJ: 0.00 TSST-1: 0.01	
10[c]	30.0	41 (med)	IgE	ImmunoCAP 0.35 kU/L	4	SEA: 0.30 SEB: 0.20 TSST-1: 0.20	
			IgE	ImmunoCAP 0.35 kU/L	3	SEA: 0.18 SEB: 0.25 TSST-1: 0.15	
50[c]	54.0	31.0	IgE	UniCAP 0.70 UA/mL	3	SEB: 0.49	
			IgE	ImmunoCAP -	2	SEA: 0.16 SEB: 0.31	
106[c]	-	68.1	IgE	CAP assay 0.35 kU/L	2	SEA: 0.01 SEB: 0.40 SEC: 0.07 SED: 0.11 TSST-1: 0.03	
17	29.4	36.2	IgE	ELISA -	3	FBP: 0.36	FBP: 0.00
9	-	-	IgE	CAP FEIA 0.35 kU/L	2	SEA: 0.44 SEB: 0.47	
10[c]	-	-	IgE	CAP (SEC, TSST-1) - Immulite 2000 (SEB) -	2	SEB: 0.06 SEC: 0.11 TSST-1: 0.10	
28	-	27 (med)	IgG_2	CAP (IgE) 0.35 kU/L ELISA (IgG_2) -	6	SEB: 0.87 (IgG_2) SEC_1: 0.62 (IgG_2)	SEB: 0.78 (IgG_2) SEC_1: 0.86 (IgG_2)

NOTE: Full reference details are provided in Appendix S3 (see Supporting Information) (continued)

	Country	Patients				
		N	% Male	Mean age (y)	Treatment at baseline	Mean AD severity score
Yagi 2004[29]	Japan	105/26[b]	-	-	-	Modified Leicester system -
Tabuchi 2004[50]	Japan	22	81.8	27.5	-	-
Ide 2004[51]	Japan	140	65.0	4.4	According to guidelines	1999 Japanese Therapeutic Guidelines for Atopic Dermatitis -
Sohn 2003[52]	South-Korea	40	45.0	5.2	-	Criteria of Rajka -
Nomura 2002[53]	Japan	15	73.3	6 mo (med)	Systematic washing of skin using soap and topical corticosteroid, antihistamines and some patients used systemtic AB	Modified Leicester system -
Zollner 2000[28]	Germany	65	41.5	41	No AB or systemic immunosuppressives 4 weeks prior to the study	SCORAD -
Lin 2000[27]	Taiwan	60	66.7	7.2	-	Criteria of Rajka -
Breuer 2000[54]	Germany	71	40.8	32 (med)	No treatment	SCORAD -
Nomura 1999[55]	Japan	94	59.6	7.8	-	Modified Leicester system 35.4
Morishita 1999[26]	Japan	149	44.3	21.4	-	Criteria of Rajka -
Bunikowski 1999[56]	Germany	58	65.5	30 mo (med)	No topical or systemic antimicrobial drugs 2 weeks prior to the study	SCORAD -
Campbell 1998[57]	Australia	74	59.5	-	-	-

Controls			Antibody	Cut-off detection method	NOS	Patients	Controls
N	% Male	Mean age (y)				Prevalence positive anti-*S. aureus* antibodies	Prevalence positive anti-*S. aureus* antibodies
33	-	-	IgE, IgG	UniCAP (IgE) 0.35 kU/L ELISA (IgG) -	3	SEB: 0.19 (IgE) ETA: 0.00 (IgE) ETA: 0.35 (IgG)	SEB: 0.06 (IgE) ETA: 0.00 (IgE) ETA: 0.15 (IgG)
8	50.0	31.9	IgE	AlaSTAT 0.10 IU/mL	3	SEA: 0.50 SEB: 0.50	SEA: 0.00 SEB: 0.00
			IgE	ImmunoCAP 0.7 U/mL	2	SEA: 0.18 SEB: 0.29	
40[c]	65.5	6.6	IgE, IgG, IgM	ELISA 12.11 U/mL (IgE) 26.11 U/mL (IgG) 19.83 U/mL (IgM)	3	SEB: 0.68 (IgE) SEB: 0.53 (IgG) SEB: 0.63 (IgM)	
			IgE	ELISA 1.07 U (SEA) 12.6 U (SEB) 7.4 U (SEC) 8.3 U (TSST-1)	2	SEA: 0.20 SEB: 0.07 SEC: 0.13 TSST-1: 0.13	
65	-	-	IgE	AlaSTAT 0.7 U/mL	5	SEA: 0.23 SEB: 0.29	SEA: 0.00 SEB: 0.00
24	41.7	8.4	IgE	ELISA 0.16 kU/L (SEA) 0.7 kU/L (SEB)	7	SEA: 0.70 SEB: 0.70	SEA: 0.25 SEB: 0.17
			IgE	AlaSTAT 0.35 kU/L	4	SEA: 0.44 SEB: 0.39	
			IgE	ELISA 1.07 U (SEA) 12.4 U (SEB) 7.8 U (SEC) 8.3 U (TSST-1)	3	SEA: 0.79 SEB: 0.35 SEC: 0.21 TSST-1: 0.18	
11	27.3	26.9	IgE	AlaSTAT 0.35 IU/mL	5	SEA: 0.54 SEB: 0.65	SEA: 0.00 SEB: 0.00
22[c]	-	73 mo (med)	IgE	AlaSTAT 0.7 kU/L	3	SEA: 0.19 SEB: 0.31	
111	-	-	IgG	ELISA 2.1 LU (SEA) 2.4 LU (SEB) 8.7 LU (TSST-1)	3	SEA: 0.77 SEB: 0.73 TSST-1: 0.77	SEA: 0.88 SEB: 0.69 TSST-1: 0.85

NOTE: Full reference details are provided in Appendix S3 (see Supporting Information) (continued)

	Country	Patients				
		N	% Male	Mean age (y)	Treatment at baseline	Mean AD severity score
Nissen 1997[25]	Denmark	34/ 27[b]	-	31 (med)	-	-
Tada 1996[23]	Japan	96	42.7	20.2	-	Criteria of Rajka -
Leung 1993[22]	USA	56	-	-	-	-

[a] Only abstract available

[b] Number of patients included in study (characteristics refer to this number) / number of patients included in the outcome

[c] Control group included in the study but the outcome was not reported

Abbreviations: N, number of patients or controls; y, year; mo, months; AD, atopic dermatitis; NOS, Newcastle-Ottawa Scale; *S. aureus*, *Staphylococcus aureus*, med, median; AB, antibiotics; UV, ultraviolet, SCORAD, SCORing Atopic Dermatitis; Ig, immunoglobulin; FEIA, fluorescent enzyme immunoassay; SE, staphylococcal enterotoxin; TSST-1, toxic shock syndrome toxin 1; FBP, fibronectin-binding protein; ET, exfoliative toxin; LTA, lipoteichoic acid

Controls			Antibody	Cut-off detection method	NOS	Patients	Controls
N	% Male	Mean age (y)				Prevalence positive anti-*S. aureus* antibodies	Prevalence positive anti-*S. aureus* antibodies
5	-	-	IgE	Immunoblot -	2	SEA: 0.48 SEB: 0.63 TSST-1: 0.41 LTA: 0.48	SEA: 0.00 SEB: 0.00 TSST-1: 0.00 LTA: 0.00
11	27.7	29.4	IgE	AlaSTAT 0.35 IU/L	5	SEA: 0.66 SEB: 0.73	SEA: 0.00 SEB: 0.00
15	-	-	IgE	ELISA -	9	SEA: 0.32 SEB: 0.18 SEC: 0.05 SED: 0.05 TSST-1: 0.21 ET: 0.02	SEA: 0.00 SEB: 0.00 SEC: 0.00 SED: 0.00 TSST-1: 0.00 ET: 0.00

Table S3. Studies reporting IgE antibodies against *Staphylococcus aureus* antigens in patients with atopic dermatitis

Staphylococcus aureus antigen	Number of studies	Number of controlled studies
SEA	19	7
SEB	23	8
SEC	7	1
SED	3	1
SEE	1	
SEI	1	
SEH	1	
SEK	1	
SEJ	1	
TSST-1	10	2
ETA	1	
FBP	1	
LTA	1	
ET1	1	1

Abbreviations: SE, staphylococcal enterotoxin; TSST-1, toxic shock syndrome toxin 1; ET, exfoliative toxin; FBP, fibronectin-binding protein; LTA, lipoteichoic acid