

Deficiency in immunoglobulin G2 antibodies against staphylococcal enterotoxin C1 defines a subgroup of patients with atopic dermatitis

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Summary

Background and aim Atopic dermatitis (AD) is a common chronic inflammatory skin disease often accompanied by cutaneous *Staphylococcus aureus* colonization and, in this regard, especially complicated by the presence of superantigen-producing strains. Because IgG antibodies comprise an important defence mechanism of the adaptive immune system against bacteria, it was investigated whether AD patients have an abnormal pattern or distribution of superantigen-specific IgG subclass antibodies in association with disease severity and activity.

Methods Staphylococcal enterotoxin B (SEB) and staphylococcal enterotoxin C1 (SEC1) specific IgG antibody subclasses were assessed in $n = 89$ adult AD patients with mild to severe disease activity as determined by the SCORAD score and in $n = 28$ healthy age-matched controls. Results were correlated with the current status of bacterial skin colonization and severity score.

Results Thirty-eight per cent of the AD patients showed a selective deficiency in IgG2 antibodies against SEC1 compared with only 14% in the control group. The absence of these antibodies was found in both currently colonized and non-colonized AD patients and was associated with a severe phenotype (SCORAD more than 40 points in two-thirds of the deficient patients). However, these patients had normal production levels of IgG2 antibodies against *pneumococcal capsular polysaccharide* (PCP) and SEB, but higher IgG1 and IgG4 titres against SEC1. Except for elevated total IgG1, total IgG subclass levels were normal in this AD subgroup. Yet, peripheral blood mononuclear cells (PBMCs) derived from these patients clearly produced IL-4 and IL-5 upon SEC1 re-stimulation whereas PBMCs from those providing SEC1-specific IgG2 antibodies failed in the production of these cytokines.

Conclusion A subgroup of AD patients suffers from a selective deficiency to produce anti-SEC1 IgG2 antibodies. This patient group is characterized by a severe AD phenotype.

Keywords adaptive immunity, atopic dermatitis, IgG subclass, immunodeficiency, staphylococcal enterotoxins, superantigens

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Introduction

Atopic dermatitis (AD) is a chronically relapsing inflammatory disease of still unknown aetio-pathogenesis. AD patients show an enhanced susceptibility to cutaneous infections with certain viral, fungal and microbial pathogens [1]. In this regard, the prominent role of *Staphylococcus aureus* skin colonization as a factor contributing to the exacerbation of AD has been well established [2–4]. A link between the degree of colonization with *S. aureus* and the intensity of skin inflammation has been described [5]. *S. aureus* carries many pro-inflammatory components including α -toxin, protein A and less well-characterized pyrogenic exoproteins [6]. Many,

but not all, *S. aureus* strains carry the capacity to produce enterotoxins with superantigenic properties [7–10]. Former studies could demonstrate a close link between superantigen production, disease severity and total serum IgE levels in children [11, 12]. More recently, this association has also been confirmed in adult AD patients [13, 14]. Pathogenetically, AD is determined by abnormal T cell functions [15]. In this respect, it was shown that local application of superantigens triggers recruitment and activation of T cells into the dermis [16, 17].

The underlying pathomechanism that results in increased colonization of and susceptibility to *S. aureus* infection still remains to be fully elucidated. Both the innate and adaptive immune system participate in the recognition and defence of microbial pathogens. Regarding innate defence mechanisms, Ong et al. [18] have previously reported that AD patients carry a deficiency in the production of some defensins. More recently, Nomura et al. [19] have shown a decreased expression of human beta defensin-3 in AD. The adaptive

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immune system participates via specific T and B cell responses. Concerning B cell responses, it is well established that at least a subgroup of AD patients produce IgE antibodies against superantigens [20]. However, it is unknown whether and how IgG antibody responses participate in the pathogenesis of this phenomenon. IgG is the most common immunoglobulin, especially in the extravascular fluids where it neutralizes toxins and combats micro-organisms by fixing complement via the C1 pathway, and facilitates the binding to phagocytic cells by receptors for C3b and Fc γ [21]. Therefore, the objective of this study was to investigate the presence and, in particular, the distribution pattern of IgG (subclass) antibodies with superantigenic specificities in AD patients and to assess their possible involvement in disease.

Methods

Study populations

A total of 89 patients with mild to severe AD (median age 31 years, range 14–78 years) fulfilling the diagnostic criteria of Hanifin and Rajka [22] as well as 28 healthy control subjects, members of the clinical department (median age 27 years, range 17–53 years), were enrolled in this study. Patients' written consent as well as approval of the study by the local ethics committee were obtained. All patients had a steroid-free interval of at least 4 weeks and did not receive systemic or topical antibiotic treatment prior to examination. The body surface area affected by the eczema varied among patients, i.e. some patients had only flexural eczema, while others were in a phase of acute exacerbation (i.e. had acute eczema) when they visited our department. Furthermore, they did not suffer from any clinically relevant bacterial superinfection. The clinical severity of AD was determined according to the SCORAD score, a scoring system that evaluates objective and subjective symptoms of AD (median value 45 points; range 15–90.5 points) [23].

Isolation of Staphylococcus aureus from skin and detection of Staphylococcus aureus-derived superantigens

Contact samples were obtained from eczematous skin lesions by using sterilized swabs and thereafter plated onto non-selective 5% sheep blood agar as also onto mannitol agar containing 7.5% sodium chloride for better detection of these bacteria. After an incubation period of 24 h at 35 °C, *S. aureus* was identified by standard microbiological methods. For enterotoxin detection, a defined inoculum of *S. aureus* was incubated in tryptone soya broth at 37 °C for 18–24 h with shaking. After spinning the culture at 900g for 20 min, supernatants were tested for the presence of enterotoxins in an agglutination assay by SET-RPLA (Oxoid test kit in order to detect staphylococcal enterotoxins A–D and toxic shock syndrome toxin-1 (TSST-1)) according to the manufacturer's instructions (Oxoid Ltd, Basingstoke, UK).

Determination of total and superantigen-specific immunoglobulin E

Levels of total serum IgE as well as serum-specific IgE to staphylococcal enterotoxins A–D and TSST-1 were measured

by the Pharmacia CAP system (Pharmacia and Upjohn, Uppsala, Sweden) according to the manufacturer's instructions. Results were given in kU/L, with 0.35 kU/L as the lower limit of sensitivity.

Determination of immunoglobulin G and immunoglobulin G subclass-specific antibodies against staphylococcal enterotoxin B and staphylococcal enterotoxin C1

Total IgG against staphylococcal enterotoxin B (SEB) and staphylococcal enterotoxin (SEC1) were measured using a modified ELISA assay as described in Campbell and Kemp [24]. High binding plates were coated with 50 μ L/well of SEB (Sigma-Aldrich, Taufkirchen, Germany) or SEC1 (Toxin Technology, Sarasota, FL, USA) at 2 μ g/mL in carbonate buffer, pH 9.5 and incubated overnight at 4 °C. After washing the plates with phosphate buffered saline (PBS)/Tween and blocking them with 1% gelatine/PBS/Tween for 2 h, sera were added at various concentrations and incubated at room temperature for 90 min. In order to determine the total SEB/SEC1-specific IgG, samples were incubated at serial dilutions from 1:50 to 1:5000 (1% gelatine/PBS/Tween) while a dilution range of 1:1 to 1:5000 (15 series) was necessary for the assessment of SEB/SEC1-specific IgG1 and IgG4. To detect SEB/SEC1-specific IgG2 antibodies, samples were diluted from 1:1 to 1:750. The further incubation step with peroxidase-conjugated secondary antibodies (50 μ L/well for 45 min at room temperature) enabled the binding to serum IgG immunoglobulins. For this purpose, the following antibodies were used: anti-human IgG (Sigma-Aldrich, St Louis, MO, USA); anti-human IgG1 (ICN, Aurora, OH, USA); and anti-human IgG2/4 (Caltag, Burlingame, CA, USA). After final washing, bound antibodies were visualized via addition of 100 μ L/well 3,3',5,5'-tetra-methyl benzidine substrate (Roche Diagnostics, Mannheim, Germany) and the enzyme reaction was stopped by 100 μ L/well of 2 M H₂SO₄. Plates were read in a microplate autoreader (Tecan, Kirchheim, Germany) at 450 nm. Anti-SEB/SEC1 IgG antibodies (Toxin Technology) served as standards. Antibody concentrations in samples were then calculated from the linear portion of the standard curve and expressed in laboratory units (LU).

Determination of total immunoglobulin G subclass antibodies

Serum IgG subclasses IgG1–IgG4 were quantified using a nephelometric assay with the human IgG subclass liquid reagent kit purchased from Binding Site (Birmingham, UK). Anti-IgG3 and IgG4 were coated onto latex particles for higher sensitivities. In order to measure IgG1, IgG3 and IgG4, the samples were diluted 1:5 and 1:100 while for IgG2 1:5 and 1:20 dilutions were applied. Results were given in mg/L. Assay was performed on the Behring BNII analyser (Schwalbach Taurus, Germany).

Determination of immunoglobulin G antibodies against pneumococcal capsular polysaccharide

Serum levels of anti-pneumococcal capsular polysaccharide (PCP) IgG2 were determined by a commercial Enzyme Immunoassay Kit purchased from Binding Site. The samples were diluted 1:100 in a medium containing C-polysaccharide

prior to the addition of the PCP pre-coated wells. Detection of the captured antibodies occurred through a peroxidase labelled rabbit anti-human IgG conjugate with an analytical sensitivity of 1.1 mg/L.

Determination of T-helper type 2 cytokines in cell culture supernatants

Peripheral blood mononuclear cell (PBMCs) were purified from heparinized blood by density centrifugation on Ficoll Hypache (Nycomed Pharma AS, Oslo, Norway) derived from AD patients either with or without SEC1-specific IgG2 deficiency. After washing, cells were adjusted at 3×10^5 /well in Iscoves medium, supplemented with 4% heat-inactivated AB serum (human's serum), 2 mmol/L glutamine, 50 µg/mL gentamicin, 100 mg/mL penicillin, 100 µg/mL streptomycin and non-essential amino acids. Medium, glutamine as well as antibiotics were purchased from Biochrom KG (Berlin, Germany). Cells were stimulated with 100 pg/mL highly purified SEB or SEC1 (Toxin Technology), 1 µg/mL lipoteichoic acid or 1 µg/mL peptidoglycan (Sigma Aldrich Chemie, Munich, Germany). LPS concentrations of antigen preparations were determined with the Limulus Assay (Biowhittaker, Walkersville, ME, USA) according to the manufacturer's instructions. After 24 h, supernatants were collected and applied for the Cytometric Bead Array (CBA) Kit (BD Biosciences Pharmingen, San Diego, CA, USA) that simultaneously and quantitatively measures multiple cytokines in a single sample. The principle of this test system relied on special bead populations, each with distinct fluorescence intensities that have been coated with capture antibodies specific for various analytes. Subsequently, these beads were mixed with Phycoerythrin (PE)-labelled detection antibodies in the presence of standards or test samples, to form sandwich complexes (e.g. Capture Bead-Ab/ analyte/ PE-Ab complex). After acquisition of sample data using the multi-colour FACScan flow cytometer (Becton-Dickinson, Heidelberg, Germany), results were generated in a graphical and tabular format using the BD CBA Software. The linear portion of the standard curve for each cytokine covered a defined set of concentrations ranging from 20 to 5000 pg/mL.

Statistical analysis

Statistical comparisons between experimental groups were made using the two-tailed Mann-Whitney *U*-test, the two-tailed, unpaired Student's *t*-test as well as the two-tailed χ^2 test. *P* values below 0.05 were considered to be significant.

Results

In accordance with previous reports, a substantial portion of AD patients (61% or 54 out of 89 patients) suffered from skin colonization with *S. aureus*, while all of the 28 healthy control individuals were non-carriers (Fig. 1). Forty-six per cent of staphylococcal strains isolated from the skin of affected patients were toxigenic secreting SEC1 > SEB/SEA > TSST-1 > SED. Patients colonized with staphylococci had a significantly more severe AD phenotype than those who did not harbour these pathogens. The first group had a median

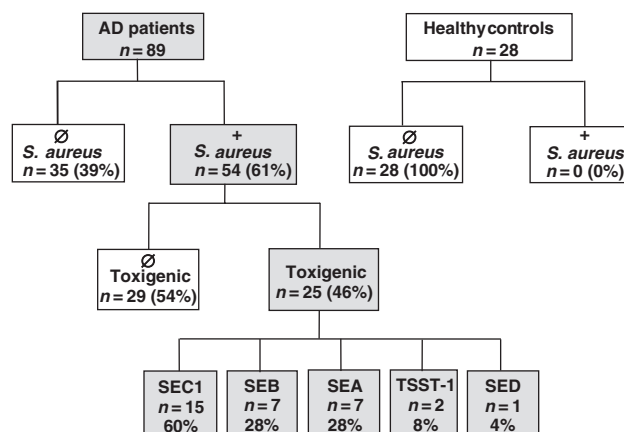


Fig. 1. Prevalence of toxigenic and non-toxicogenic *Staphylococcus aureus* strains on the skin of adults with atopic dermatitis (*n* = 89) and of healthy controls (*n* = 28). In some cases, staphylococci showed multiple toxin production. '+', present; 'Ø', absent.

SCORAD of 60 points compared with the second one with a median value of 40 points (*P* = 0.0025).

Seventy-two per cent of AD patients had antibodies of the IgE isotype against superantigens and almost 30% among them were currently colonized with toxigenic strains. Nearly half of this superantigen-sensitized subgroup developed IgE antibodies against all superantigens tested (SEA, SEB, SEC1, SED and TSST-1), whereas only 5% were sensitized against a single one. Frequencies of IgE antibodies against staphylococcal enterotoxins occurred in the order of SEA > SEB > SED > TSST-1 > SEC1. The presence of superantigen-specific IgE antibodies correlated with disease severity (*P* = 0.0002) as well as with total serum IgE levels (*P* = 0.0001).

Because IgG2 is a prominent antibody subclass involved in response to Gram-positive bacteria [25], it was examined whether AD patients would produce this antibody subclass against SEB and SEC1, representing the most commonly secreted superantigens in our cohort (Fig. 1). A deficiency in IgG2 antibodies against SEC1 was identified in a considerable portion of AD patients (Fig. 2a). While IgG2 antibodies were missing in 38% of all AD patients, only four healthy subjects showed absence of this antibody subclass (χ^2 test, *P* = 0.02; Table 1). Yet, as shown in Fig. 2b, SEB-specific IgG2 antibodies were absent in ten AD patients and in six healthy controls (χ test, *P* = 0.25; Table 1). Lack of the superantigen-specific IgG2 antibodies was found in AD patients regardless of whether they were currently colonized (with toxigenic or non-toxicogenic *S. aureus* strains, respectively) or non-colonized. However, among all AD patients currently colonized with SEC1-secreting strains (*n* = 15), 1/3 of them were deficient in anti-SEC1 IgG2. In another context, 45% of the subgroup lacking these antibodies even developed IgE sensitization against the latter toxin, pointing towards a previous exposure with this kind of superantigen.

To further analyse whether the subgroup of deficient patients would be generally impaired to produce antibodies against SEC1, IgG1 and IgG4 antibody titres against this toxin were measured as well. As shown in Figs 3a and b the vast majority of healthy controls was positive for SEC1 specific IgG1 and IgG4 antibodies. In addition, almost all patients carried either of these antibodies, indicating that the

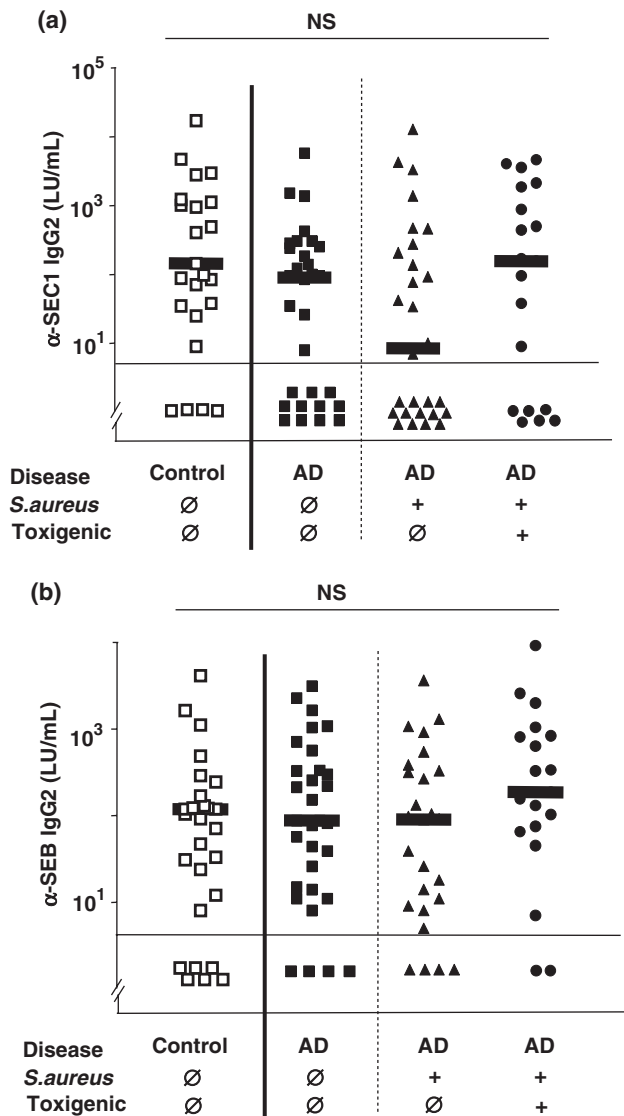


Fig. 2. Amounts of IgG2 antibodies directed against staphylococcal enterotoxin C1 (SEC1) (a) and staphylococcal enterotoxin B (SEB) (b) as determined by ELISA in the sera of controls and atopic dermatitis (AD) patients with different status of staphylococcal skin colonization. Bars represent the median antibody concentration expressed in laboratory units (LU)/mL. NS, not significant; '+', present; 'Ø', absent.

Table 1. Frequency of antibody deficiency among controls and AD patients

IgG2 antibodies against	Subjects		P-value
	Control	AD	
SEC1			
Yes	24	48	0.02*
No	4	30	
Total	28	78	
SEB			
Yes	21	67	0.25
No	6	10	
Total	27	77	

Statistical analysis by two-tailed χ^2 test. * $P < 0.05$.

AD, atopic dermatitis; SEC1, staphylococcal enterotoxin C1; SEB, staphylococcal enterotoxin B.

capacity to produce superantigen-specific IgG1 and IgG4 was not impaired. Median values were higher in the AD group deficient in IgG2 antibodies; however, this did not reach the level of statistical significance. Furthermore, these IgG1 and IgG4 titres did not correlate with each other (data not shown).

Because the deficiency to produce antibodies against superantigens was apparently restricted to the IgG2 subclass, it was investigated whether AD patients could produce IgG2 antibodies against other bacterial antigens. As a surrogate marker for this capacity, the IgG2 titres against PCP were measured. As shown in Fig. 4, both healthy controls and AD patients had similar levels of antibody titres against PCP, regardless of whether AD patients were impaired to produce IgG2 antibodies against SEC1 or not.

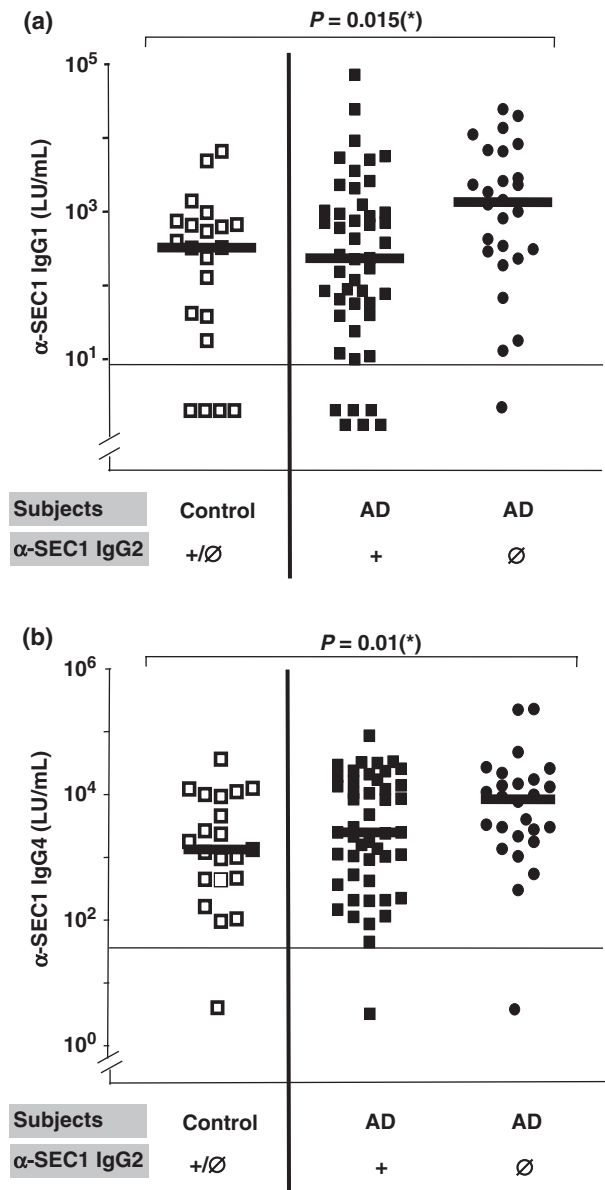


Fig. 3. Levels of staphylococcal enterotoxin C1 (SEC1) specific IgG1 (a) and IgG4 (b) antibodies detected in the sera of controls and atopic dermatitis (AD) patients with and without anti-SEC1 IgG2 deficiency. Bars represent the median antibody concentration in laboratory units (LU)/mL. * $P < 0.05$, by unpaired Mann-Whitney *U*-test. '+', present; 'Ø', absent.

To exclude any major deficiencies in IgG subclass production among AD patients, total IgG1, IgG2, IgG3 and IgG4 levels were measured as well. The data provided in Figs 5a–d indicate that AD patients had normal levels in all IgG subclasses like the healthy controls, except for IgG1 which was significantly elevated in the anti-SEC1 IgG2-deficient

subgroup. These data point towards a selective deficiency among a subgroup of AD patients to produce IgG2 antibodies against bacterial superantigens.

In an attempt to further explore this phenomenon, it was particularly investigated whether PBMCs from these subjects exhibited an abnormal T-helper type 2 (Th2) cytokine profile following stimulation with SEC1. As a result, remarkable levels of IL-4 and IL-5 were measured. In contrast, PBMCs derived from patients without this kind of antibody lack failed in the production of these cytokines upon SEC1 re-stimulation (Figs 6a and b). This differential pattern of IL-4 and IL-5 production was only noted upon stimulation with SEC1, but not with SEB, lipoteichoic acid or peptidoglycan.

Concerning the aspect of a possible relation to disease, more than two-thirds of the AD patients deficient in anti-SEC1 IgG2 had a severity score (SCORAD) of more than 40 points (Table 2). This is in contrast to those patients having SEC1-specific IgG2 antibodies, where almost two-thirds of them ranged below 40 points in the SCORAD score.

Discussion

Patients with AD are susceptible to chronic *S. aureus* skin colonization, that is associated with increased disease severity. Especially these bacteria have been implied in exacerbation or maintenance of skin inflammation in AD if they secrete superantigens with potent T cell stimulatory activity. This is also associated with the ability to trigger specific IgE antibody

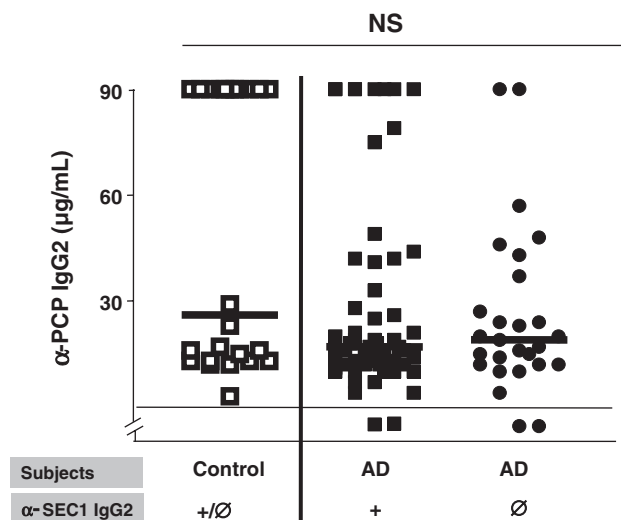


Fig. 4. Levels of pneumococcal capsular polysaccharide (PCP) specific IgG2 antibodies detected in the sera of controls and atopic dermatitis (AD) patients with and without anti-staphylococcal enterotoxin C1 (SEC1) IgG2 deficiency. Bars represent the median antibody concentration given in µg/mL. NS, not significant; '+', present; 'Ø', absent.

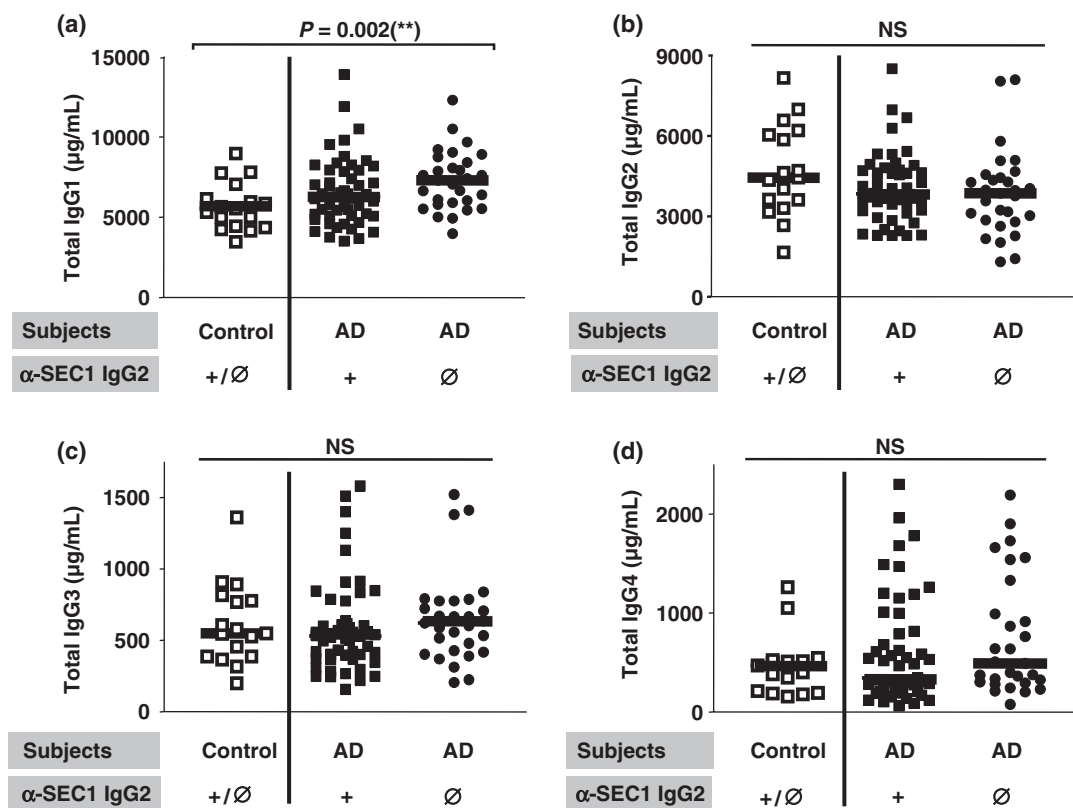


Fig. 5. Total IgG1, IgG2, IgG3 and IgG4 levels among controls and atopic dermatitis (AD) patients with and without anti-staphylococcal enterotoxin C1 (SEC1) IgG2 deficiency. Bars represent the median antibody concentration in µg/mL. NS, not significant; ** $P < 0.01$, by unpaired Mann–Whitney U -test. '+', present; 'Ø', absent.

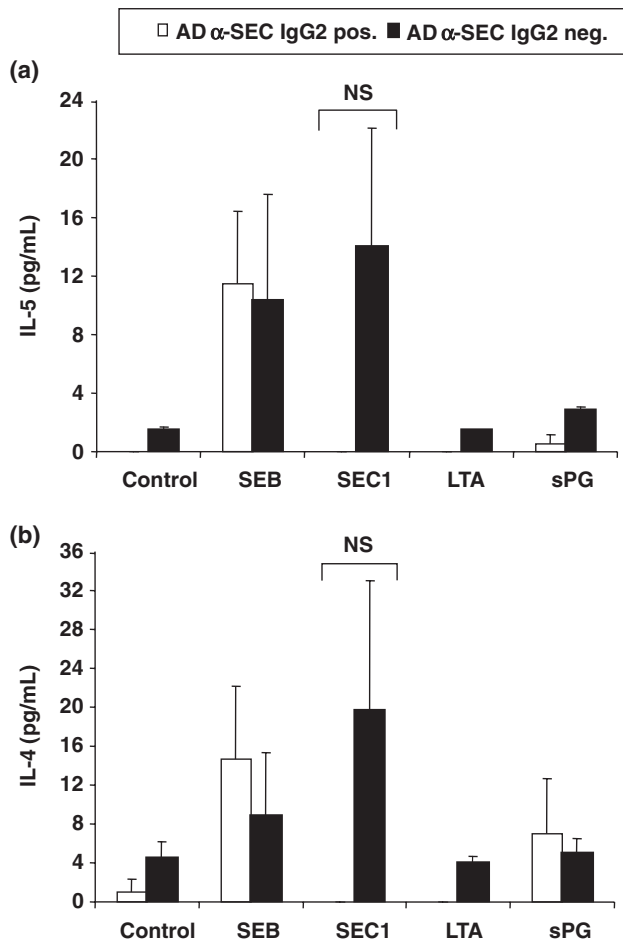


Fig. 6. Amounts of IL-5 (a) and IL-4 (b) were measured in the cell culture supernatants of peripheral blood mononuclear cells from atopic dermatitis (AD) patients with ($n = 4$) and without ($n = 4$) anti-staphylococcal enterotoxin C1 (SEC1) IgG2 deficiency. Briefly, 3×10^5 cells were stimulated for 24 h with highly purified staphylococcal enterotoxin B (SEB) and SEC1 (100 pg/mL) as well as with lipoteichoic acid (LTA) and peptidoglycan (PGN), each at a concentration of 1 μ g/mL. The results are expressed as means $\pm$ SD of each patient subgroup. NS, not significant.

production. The mechanism leading to enhanced staphylococcal colonization in AD might be partly because of an inadequate host defence. In this regard, Ong et al. [18] and Nomura et al. [19] have very recently demonstrated the failure in producing antimicrobial peptides such as human beta defensin-2 and -3, pointing towards a deficiency within the innate immune system. A possible defect in adaptive immunity has not yet been described that could lead to a hindrance in bacterial eradication as well as toxin neutralization. Cambell and Kemp postulated a reduced specific antibody response as a possible reason for superantigen-mediated disease. They found that atopic children were able to produce superantigen-specific IgG antibodies (SEA, SEB and TSST-1) and that these amounts were not decreased in comparison with healthy subjects [24]; however, they did not discriminate between different IgG subclasses.

IgG2 antibodies play a key role in immunity particularly against infections with encapsulated bacteria. This defence does not only depend upon complement activation, because IgG2 has a poor complement-activating property. Probably

Table 2. Antibody deficiency and disease severity

IgG2 antibodies against	SCORAD		P-value
	< 40 points	> 40 points	
SEC1			
Yes	28	20	0.01*
No	9	21	
SEB			
Yes	38	28	0.60
No	3	6	
PCP			
Yes	30	47	0.66
No	2	2	

Statistical analysis by two-tailed χ^2 test. In a few cases SCORAD data are missing. * $P < 0.05$.

SEC1, staphylococcal enterotoxin C1; SEB, staphylococcal enterotoxin B; PCP, pneumococcal capsular polysaccharide.

even more important is the fact that phagocytosis by neutrophilic granulocytes is the major underlying mechanism initiated by the IgG2 binding to Fc γ receptor IIa on the cell membrane of polymorphonuclear cells [26].

The high prevalence of skin colonization with SEC1-producing *staphylococci* was a surprising observation in our study. This strongly resembled the phenotype of DS-Nh mice with AD-like dermatitis as described by Yoshioka et al. [27]. Based on these results, they suggested a possible role for SEC in the pathogenesis of AD. In this study, we identified a subgroup of AD patients lacking SEC1-specific IgG2 antibodies, a deficiency that affected healthy individuals just marginally. This kind of antibody lack occurred in both currently colonized and non-colonized patients that might reflect the well-known fluctuation in staphylococcal colonization over time. The impairment to produce IgG antibodies against SEC1 was selectively found within the IgG2, but not in the IgG1 or IgG4 subclass. The higher median values for anti-SEC1 IgG1 and IgG4 among AD patients deficient in IgG2 antibodies may point towards a compensatory effect. However, these antibodies show a lower affinity and thus are expected to be functionally inferior [28]. It is noteworthy that, IgG2 synthesis of SEB and another common Gram-positive bacterial antigen (PCP) were normal as compared with the residual IgG subclasses.

The human immunoglobulin heavy-chain constant region (IGHC) contains nine functional genes (μ - δ - γ 3- γ 1- ψ ϵ - α 1- ψ γ - γ 2- γ 4- ϵ - α 2) organized in two γ - γ - ϵ - α blocks [29]. The genes encoding for IgG2 and IgG4 are both located on the downstream complex of the IGHG and are independently regulated. A defect in the ability to switch from one IgG subclass to another is unlikely as an explanation for our observation, because patients deficient in anti-SEC1 IgG2 would then also lack the corresponding IgG4 subclass.

Different cytokines preferentially mediate isotype switching by making the switch recombination sites accessible to the corresponding recombinase. IFN- γ has been shown to cooperate with IL-6 to induce IgG2 production in human B cells and is, therefore, regarded as an important switching factor for IgG2 [30]. Although it is hard to imagine that a cytokine shift towards Th2 may account for impaired IgG2 formation solely restricted to a single antigen specificity, our data on

preferential IL-4 and IL-5 production by PBMCs derived from patients deficient in anti-SEC1 IgG2 upon re-stimulation with SEC1 but not with other bacterial products may encourage speculation of the existence of predominantly SEC1-specific Th2 cells among these subjects. The toxin itself may additionally contribute to the course of antibody deficiency as SEC1 exclusively stimulates TCRBV8S2-bearing T cells [27] that are known to comprise for the most part natural killer T cells as a major source of IL-4 [31]. So, it is conceivable that these conditions may establish a unique Th2 micro-milieu thereby hindering from antigen-specific IgG2 synthesis. Yet, in turn, the latter concept requires the presence of the special toxin. Although only one-third of all patients currently colonized with SEC1-secreting strains were deficient in anti-SEC1 IgG2, the presence of antibodies directed against this toxin provides indirect information about the exposure with SEC1-secreting *staphylococci* over a longer period assuming that only following these conditions specific antibodies against this superantigen could be induced. In this respect, almost all patients had SEC1-specific IgG1 and IgG4 antibodies and 45% of them even developed IgE sensitization against this particular toxin, suggesting that all of them underwent a period of SEC1 exposure at one time or the other. Especially high levels of the corresponding IgG4 antibodies, a subclass that is often noted to be elevated after chronic exposure to the relevant antigen [32], indicate a previous exposure with this kind of bacteria. Moreover, because only the response to superantigens was impaired but not those to other bacterial antigens, this observation also points towards a special functional property of SEC1. Indeed, this toxin displays differences in protein structure compared with other superantigens as characterized by zinc ligation and zinc-mediated dimerization [33]. This makes it likely that antigenic determinants precisely localized in this dimerization region are not accessible to the corresponding B cell receptor (BCR). Therefore, patients expressing such kind of BCR would fail to recognize SEC1. A selective deficiency in anti-pneumococcal antibody production despite normal IgG2 subclass levels has been described in children suffering from recurrent respiratory infections as well [34]. However, future studies are definitively required to fully understand this phenomenon.

Further characterization of the anti-SEC1 IgG2-deficient subgroup revealed a correlation between increased disease severity and antibody deficiency. More than two-thirds of the affected patients had a severity score (SCORAD) of more than 40 points, while almost two-thirds of the patients carrying SEC1-specific IgG2 antibodies ranged below 40 points in the SCORAD score. Although the precise functional role of these antibodies in AD pathogenesis is still unclear, our findings provide strong evidence that the lack of anti-SEC1 IgG2 is closely associated with disease severity but is not linked to the risk of *S. aureus* colonization or even colonization with *S. aureus*-secreting SEC1. However, our additionally performed T cell cytokine experiments indicate that this particular patient group is strongly related to a different immunological responsiveness towards SEC1. In this respect, the antibody-deficient group shows a much more pronounced Th2 response to this toxin. Because the Th2 disbalance represents a well-known precipitating factor in the immuno-pathogenesis of AD, it might be speculated that this

is the reason why the subgroup of deficient patients has a more severe and more pronounced course of the disease.

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