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Preferential Expression of T-cell Receptor Vβ-chains in Atopic Eczema

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Chronic skin colonization with *Staphylococcus aureus* is a characteristic feature of atopic eczema, and about 60% of *S. aureus* strains isolated from the skin of patients with atopic eczema secrete enterotoxins. T-cell stimulation by staphylococcal enterotoxins is restricted to the Vβ-chain of the T-cell receptor. Therefore, the expression of different Vβ-chains (Vβ3, 5 a,b,c, 6, 8, 12) on peripheral blood T-cells (CD4+) from patients with atopic eczema was measured by flowcytometry before and after stimulation with staphylococcal enterotoxin B. Lymphocytes from healthy donors served as controls. Additionally, the expression of Vβ-chains in normal skin and in lesional skin of patients with atopic eczema was determined by immunofluorescence histology. In atopic eczema, higher numbers of CD4+ T-cells expressed Vβ3, Vβ8 and Vβ12 compared to the control group. No correlation between *S. aureus* enterotoxin B-stimulated Vβ-expression and HLA-haplotypes was found. In lesional skin of patients with atopic eczema most of the infiltrating T-cells were Vβ3+, whereas in normal skin only very few T-cell receptor-expressing cells were detected. To evaluate the significance of these T-cell clones for allergic inflammation, T-cells from patients with atopic eczema and normal donors were stimulated with monoclonal antibodies against Vβ3, 5(c) and 8. Afterwards, the proliferative response of lymphocytes as well as IL-5 and IFNγ synthesis were measured. T-cells from patients with atopic eczema showed a significantly higher proliferation and IL-5 secretion than normal donors after stimulation with monoclonal antibodies against Vβ3 and Vβ8. In contrast, the monoclonal antibodies directed to Vβ5(c) induced a markedly elevated proliferation and IFNγ production of normal lymphocytes compared to patients with atopic eczema. Our results suggest a preferential expression of certain Vβ-subgroups during inflammation in atopic eczema; this may be explained by a selective stimulation of T_H2-cells via *S. aureus*-derived enterotoxins.

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Normal as well as diseased skin of patients with atopic eczema (AE) is severely colonized with *Staphylococcus aureus* (1–3). Patients with either impetiginized AE or without superinfection show a better response to combined treatment with antistaphylococcal antibiotics and topical corticosteroids than to corticosteroids alone (4, 5). Recently, it has been shown that the majority of *S. aureus* strains isolated from the skin of patients with AE produce staphylococcal toxins like enterotoxin B (SEB) or toxic shock syndrome toxin-1 (TSST-1) (6, 7). About 50% of the patients have specific IgE directed to staphyloccocal toxins. Additionally, basophils release histamine on exposure to the relevant exotoxin (6). The activation of T-cells from AE patients by staphylococcal superantigens results in the release of a T_H2-like cytokine pattern (IL-4, IL-5) *in vitro* (8, 9), which via induction of several effector cells may be the cause of increased IgE-synthesis and eosinophilia. SEB belongs to a group of bacterial exotoxins that strongly and specifically stimulate $CD4^+$ and $CD8^+$ T-lymphocytes by cross-linking the T-cell-antigen receptor (TcR) with major histocompatibility complex class-II molecules on accessory or target cells. Interestingly, and in contrast to nominal antigens, these molecules did not require any processing for their presentation to T-cells (10–12). The recognition of "superantigens" by the TcR depends almost entirely on the variable region of the beta chain (Vβ).

Selective changes in the circulating Vβ T-cell receptor repertoire, indicating superantigen stimulation, have been documented for multiple sclerosis (13), rheumatoid arthritis (14), and psoriasis (15). Therefore, it was the purpose of this study to investigate the distribution of Vβ-chains on peripheral blood and skin T-cells from patients with AE.

MATERIALS AND METHODS

Patients and controls

Lymphocytes were obtained from healthy volunteers and from patients with AE. The AE diagnosis was performed according to the criteria of Hanifin & Rajka (16); the following four basic features were present: a chronic or chronically relapsing dermatitis, flexural lichenification, pruritus, and a personal or family history of atopy (asthma, rhinitis, AE). The serum IgE levels were above 500 kU/l. All patients had a skin colonization with *S. aureus*, as determined by isolating bacterial strains from skin smears. The donors did not receive systemic steroid treatment. As controls served normal donors with no personal history of allergic diseases and serum IgE levels below 100 kU/l.

Isolation of lymphocytes

Isolation was performed by centrifugation on a Ficoll-sodium metrizoate (Sigma, Munich, Germany) gradient according to Böyum (17). Briefly, heparinized venous blood (20 ml) was layered over Ficoll-sodium metrizoate (density = 1,075 g/ml) and centrifuged at $375 \times g$ for 45 min. Cells at the interface above the Ficoll-metrizoate were removed and washed three times with RPMI-1640. These cells are referred to as "peripheral blood mononuclear cells" (PBMC).

Culture conditions

The basic culture medium was RPMI-1640 supplemented with 2 mM glutamine, 100 µg/ml streptomycin, 100 IU/ml penicillin, 10 mM HEPES and 20 mM sodium hydrogen carbonate. Medium containing 10% fetal calf serum is referred to as RPMI-1640 with 10% FCS. Cell suspensions containing 1×10^6/ml viable cells in RPMI-1640 and 10% FCS were dispensed into each well of 24-well plates. Cultures were incubated at 37°C in a humidified atmosphere of 5% CO_2 in air.

Stimulation experiments

PBMC concentrated 1×10^6 cells/ml were stimulated with 1 µg SEB/ml for 4 days at 37°C. SEB was obtained from Sigma (Munich, Germany). Monoclonal antibodies against Vβ3, 5(c), and 8 (1 µg/l $\times 10^6$ cells)

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for induction of proliferation and cytokine secretion were obtained from Dianova (Hamburg, Germany).

FACS analysis

PBMC (1×10^5 cells/500 µl) from normal ($n=10$) and atopic ($n=10$) donors were incubated with anti-CD4 (distributed from Becton & Dickinson) and anti-Vβ monoclonal antibodies either labelled with phycoerythrin (PE) or with fluorescein isothiocyanate (FITC), respectively. Anti-Vβ3 antibody was obtained from Dianova (Hamburg, Germany), anti-Vβ5(a, b, c), -Vβ6, -Vβ8, and Vβ12 were from T-Cell Diagnostics (purchased from Hermann Biermann GmbH, Bad Nauheim, Germany). PE- or FITC-labelled isotype controls (mouse IgG_1 or IgG_2) were used. Five or 10 µl of each antibody solution was added to either freshly isolated or to harvested cells and incubated for 30 min at 4° C in the dark. After incubation, cells were centrifuged for 5 min at $400 \times g$. The pellet was washed three times with PBS and then fixed in 500 µl 0,1% paraformaldehyde in PBS. FACS analysis was performed on a FACScan (Becton & Dickinson). Analysis of data was conducted with LYSIS II software.

HLA class II-typing

In every donor the HLA-type was determined to control the dependence of Vβ-chain expression on MHC-class II expression. HLA class II-typing from nucleated peripheral blood cells by DNA-analysis was performed as described by Ehrlich et al. (18). Cell membranes were lysed with non-ionic detergents and nuclear proteins were digested by proteinase K. The cellular DNA was bound to minicolumns (DIAGEN, Hilden, Germany), purified from peptides and RNA by washing with ethanol and finally eluted with hot distilled water. HLA-DRB1 alleles (19) were amplified by the polymerase chain reaction (PCR) with locus specific biotinylated primers. Allelic variants could be determined after hybridization of the amplificates to nylon membrane bound allele-specific oligonucleotides (SSO) and detection of the biotin label with streptavidine-peroxidase and a colorimetric reaction of tetramethyl benzidine (20). The HLA-class-II-type of patients with AE were compared with a control group consisting of Caucasian healthy blood and bone marrow donors of the Department of Transfusion Medicine, University Hospital, Hamburg.

The odds ratio was calculated according to Woolf's formula and expressed as a relative risk (RR). The statistical significance of the difference of RR from unity was tested by chi-square analysis with 1 *df*. The level of significance was set to 0.05. To obtain corrected values for *p* by use of the Bonferroni inequality method, *p* was multiplied by the number of alleles compared.

Skin specimens and immunofluorescence staining

Samples of normal skin ($n=6$) from different body regions were obtained from plastic surgical operations. Biopsies from lesional skin of patients with atopic eczema ($n=6$) were performed after information and after approval of the patients under local anaesthesia. Specimens were stored in liquid nitrogen until used. Serial 5-µm sections were cut on a cryostat at −20°C. For staining the sections were air-dried for 10 min and then incubated for 30 min at 37°C with undiluted FITC-labelled antibodies against Vβ-chains (Vβ3, β5(a, b, c), β6, β8, β12). All investigations in patients and controls were performed after informed consent had been obtained.

Proliferation

PBMC from healthy volunteers ($n=8$) and patients with AE ($n=8$) were harvested after 3 days' stimulation with Vβ3, 5(c) and 8 antibodies and 200 µl of cell supension was transferred to 96-well culture plates in triplicates. Cell proliferation was measured by adding 7.4 KBq/well ^{3}[H] thymidine (Amersham Buchler, Braunschweig, Germany) to PBMC. Triplicates were harvested onto glass fiber filters and radioactivity was counted by liquid scintillation. Data were expressed as counts per minute (cpm).

IL-5 and IFNγ production

The amounts of IL-5 and IFNγ were measured after 4 days of PBMC culture with ELISA-kits purchased from Hermann Biermann GmbH (Bad Nauheim, Germany). Six donors in each group were investigated. The assays were based on the quantitative sandwich enzyme immunoassay technique, as described elsewhere (9).

Analysis of data

All experiments were performed with different donors in the control and in the atopic group, respectively. Data are presented as mean ± standard deviation (SD). The significance was evaluated using nonparametric Wilcoxon signed-ranks test for paired data or the Wilcoxon nonpaired rank-sum for unpaired data and Students *t*-test for independent means. $P<0.05$ was considered significant.

RESULTS

Expression of Vβ-chains on CD4$^+$ T-cells

Unstimulated helper T-cells of patients with AE showed significantly increased expression of Vβ3, Vβ8 and Vβ12, whereas the number of Vβ5(c)$^+$ T-cells was enhanced on either unstimulated or stimulated helper T-lymphocytes from normal donors (Fig. 1A, B). After stimulation with SEB for 4 days the number of Vβ3$^+$, Vβ8$^+$ and Vβ12$^+$ helper T-lymphocytes from patients with AE increased markedly and was still significantly enhanced compared with T-cells from normal donors (Fig. 1B).

Expression of Vβ-chains of lymphocytes in the skin

To investigate whether differences in Vβ-expression of peripheral blood T-cells correspond to the inflammatory T-cell infiltrate in the skin of patients with AE, immunofluorescence staining was performed. The majority of T-cells in lesional skin of 6 patients with AE were Vβ3$^+$ (Table I). In 3 AE patients Vβ8$^+$ T-cells and in 2 AE patients Vβ12$^+$ T-cells among the cellular skin infiltrate were detected. However, only very few T-cells with one of the Vβ-chains we determined were present in normal skin. In one normal donor (N3) a few T-cells expressing Vβ5(b), Vβ5(c) and Vβ6 were observed.

Table I. *Characterization of the Vβ-expression on T-cells in lesional skin of 6 patients with atopic eczema (A1–6) and in skin specimens of healthy donors (N 1-6).*

(+) only single cells are positive; + small infiltrate of positive cells; ++ majority of cells are positive.

	Vβ3	Vβ5(a)	Vβ5(b)	Vβ5(c)	Vβ6	Vβ8	Vβ12	Localization of biopsy
N1	−	−	−	−	−	−	−	Back
N2	−	−	−	−	−	−	−	Back
N3	−	−	(+)	(+)	(+)	−	−	Arm
N4	−	−	−	−	−	−	−	Back
N5	−	−	−	−	−	−	−	Abdomen
N6	−	−	−	−	−	−	−	Arm
A1	++	−	(+)	−	−	−	+	Leg
A2	++	−	(+)	−	−	+	−	Back
A3	++	−	−	−	−	−	−	Back
A4	++	−	−	−	−	+	−	Arm
A5	+	−	−	−	−	+	+	Arm
A6	++	−	−	−	−	−	−	Back

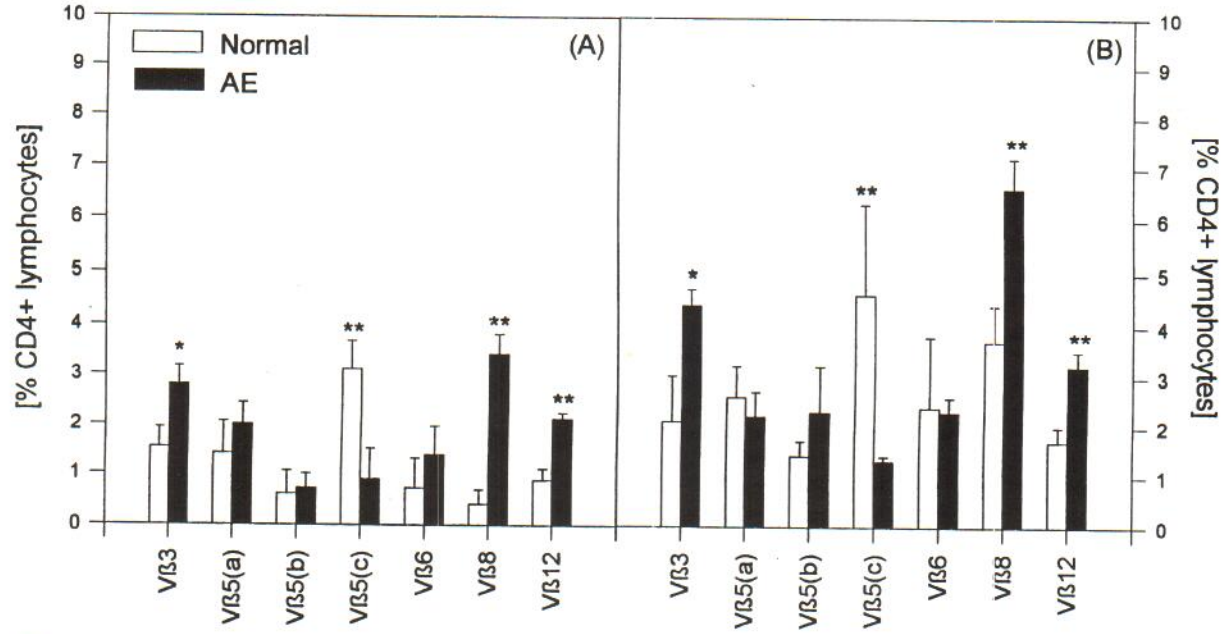

Fig. 1. The distribution of Vβ-chains (3, 5(a,b,c) 6, 8, 12) on $CD4^+$ ('helper') T-cells ($n=10$ in each group) is shown prior to stimulation (A) and after incubation (B) with 1 µg SEB/ 10^6 cells. $*P<0.05$ and $**P<0.01$ are significant compared with cells from normal donors.

HLA class II-typing

To exclude dependence of Vβ-chain expression on HLA-class-II-type, HLA-alleles of each blood donor were determined. No significant correlations to HLA-alleles were found between AE patients and normal controls. Testing the distributions of polymorphic alleles for HLA-locus did not reveal significant differences (data not shown).

Stimulation of PBMC with anti-Vβ antibodies

^{3}[H]thymidine uptake. Stimulation of PBMC with antibodies against Vβ3 and Vβ8 induced a highly significant proliferation ($p<0.01$) in patients with AE but not in healthy volunteers. In contrast, an antibody directed against the Vβ5(c)-chain of the TcR only induced a markedly increased proliferation ($p<0.01$) of normal PBMC (Fig. 2).

IFNγ and IL-5 secretion. Stimulation with moAb against Vβ5(c) induced only in the majority of normal donors a measurable secretion of IFNγ (390.6±476.3 ng/ml). In contrast, antibodies against Vβ3 and Vβ8 stimulated IL-5 secretion (Vβ3: 38.6±49.9 pg/ml; Vβ8: 15.5±12.9 pg/ml) in most of the patients with AE but not in non-atopic donors (Fig. 3).

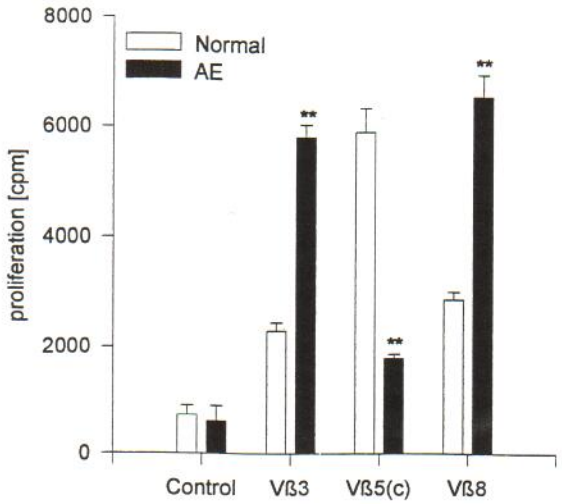

Fig. 2. Proliferation of healthy controls ($n=8$; □) and atopic PBMC ($n=8$; ■) after a 4-day incubation period with anti-Vβ3, 5(c), 8-chain antibodies. As control served the spontaneous ^{3}[H]thymidine uptake of PBMC. $**P<0.01$ is significant compared with cells from normal donors.

DISCUSSION

The analysis of the Vβ-repertoire reported indicates the selective stimulation of helper-T-cells expressing specific TcR Vβ-chains (−3, −8, −12) in AE systematically and in lesional skin. Stimulation of these T-cell clones with moAbs against Vβ3 and Vβ8 resulted in an increased secretion of IL-5, indicating a functional significance of these T-cell clones in AE. If staphylococcal enterotoxins are relevant to inflammation in AE, one would expect changes in the TcR Vβ-chain repertoire. Indeed, addition of SEB induced a marked expansion of $Vβ3^+$, $Vβ8^+$, and $Vβ12^+$ helper T-cells in patients with AE. The fact that in lesional skin of AE patients most T-cells of the lymphohistiocytic infiltrate were $Vβ3^+$ and some were either $Vβ8^+$ or $Vβ12^+$ supports the assumption that the enhanced presence of T-cell clones expressing these Vβ-chains both in blood and skin in AE patients may be related to the skin inflammation. Moreover, the selective expansion of certain Vβ-populations may be a characteristic of superantigen stimulation through the skin. This suggestion is supported by the fact that stimulation with SEB induced the expansion of Vβ3 and Vβ12, which are specific for this toxin (12).

Since the relative number of Vβ genes is limited, many

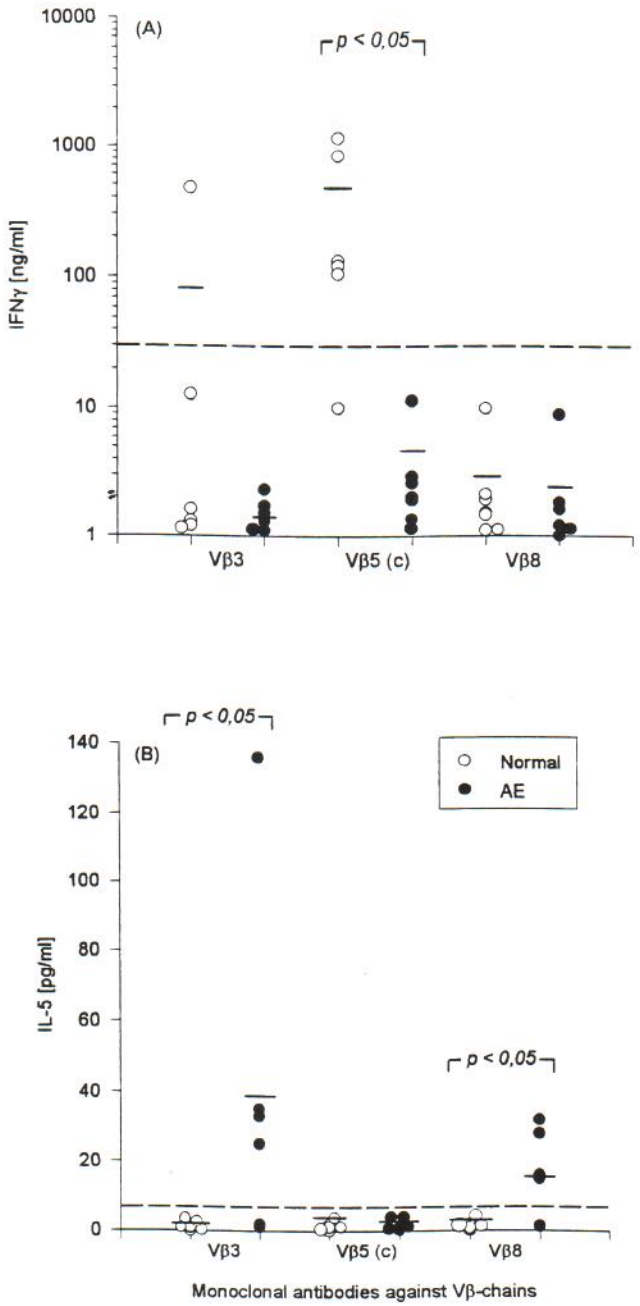

Fig. 3. IFNγ (A) and IL-5 (B) secretion of PBMC from 6 healthy donors (○) and 6 patients with AE (●) after stimulation with moAb against Vβ3, Vβ5(c), and Vβ8. Detection limits of the IFNγ ELISA (12 ng/ml) and of the IL-5 ELISA (7.8 pg/ml) are signed by parallel lines (–).

T-cells within an individual will bear a particular Vβ-element, and a given superantigen is therefore capable of interacting with a large fraction of the T-cell repertoire (21). Depending on the frequency of the responding Vβ-populations, 5–30% of the entire T-cell repertoire could be stimulated by a superantigen, whereas the responding frequency to a conventional antigen is usually much less than 1 in 1,000 (2). The massive amount of T-cell stimulation caused by superantigens could have immediate adverse consequences for the host, primarily through the release of large amounts of cytokines (21). However, none of the staphylococcal superantigens produced on the skin surface of AE patients (e.g. SEA, C, D, E, and TSST-1) (6) is known to stimulate human Vβ8 positive T-cells so far (10, 12).

It has been demonstrated that $V\beta8^+$ T-cells from mice sensitized to ovalbumin preferentially induce B-cells to produce IgE, whereas T-cells expressing other Vβ-chains do not (23). One may speculate that during strong polyclonal stimulation with staphylococcal enterotoxin $V\beta8^+$ T-cells expand independently of specific SE–Vβ-interaction. This is supported by the fact that also in normal donors $V\beta8^+$ cells occur more frequently after SEB stimulation, as compared with the control.

T_H2-cells are characterized by IL-4 and IL-5 synthesis (24). Recently, it has been shown that SEB induces a strong IL-4 and IL-5 synthesis as well as a reduced IFNγ secretion in AE (8, 9). This observation indicates that preferential expression of Vβ-chains in AE corresponds to T_H2-effector cell activation by superantigens. Furthermore, induction of proliferation and IL-5 synthesis by moAbs against Vβ3 and Vβ8 in patients with AE as well as IFNγ secretion by anti-Vβ5(c) supports the hypothesis of a functional association between Vβ-chain expression and T-cell subtype. Thus, Vβ-mediated strong specific stimulation of T_H2-cells by skin penetrating *S. aureus* enterotoxins presented by MHC-II-molecules and/or specific IgE on epidermal Langerhans' cells may pathophysiologically be relevant in AE. Local production of IL-4 and IL-5 would then stimulate IgE synthesis and eosinophilia. SEB specific lysis of MHC-II bearing target cells starts 6–12 h after SEB administration and reaches a maximum after 2 days. It is entirely due to $CD8^+$ T-cells (25, 26). In contrast to the observation that presentation of staphylococcal enterotoxins to T-cells depends on HLA alleles (27), no correlations with MHC haplotypes were observed in our experiments. Therefore, SEB-mediated effects in AE, including preferential Vβ- expression, should not depend on the MHC haplotype.

Since oligoclonal expansion of Vβ-chains has been observed in other inflammatory diseases (e.g. multiple sclerosis, rheumatoid arthritis, psoriasis), one can also assume that in AE disease related infiltrates contain distinct Vβ-chains, although only a limited number of Vβ-chains were investigated (28).

Furthermore, new strategies for the treatment of this increasingly common skin disease may include ones that interfere either with the production of toxins by *S. aureus* or with the immunological mechanisms induced by staphylococcal toxins in AE.

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