

Upregulation of IgE synthesis by staphylococcal toxic shock syndrome toxin-1 in peripheral blood mononuclear cells from patients with atopic dermatitis

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Summary

Background Atopic dermatitis (AD) is a chronic skin disease associated with increased IgE synthesis and colonization with *Staphylococcus aureus* secreting exotoxins, such as Toxic Shock Syndrome Toxin-1 (TSST-1).

Objectives In this study, we were interested in determining the *in vitro* effects of TSST-1 on IgE synthesis in peripheral blood mononuclear cells from patients with AD.

Methods We stimulated peripheral blood mononuclear cells (PBMC) from AD patients with a wide range of TSST-1 concentrations and measured IgE synthesis by enzyme-linked immunosorbent assay (ELISA) after 14 days.

Results We show herein that TSST-1 produced antagonistic effects on IgE synthesis by PBMC from AD patients, depending on the concentration used: IgE synthesis was inhibited at 1000 pg/mL ($P < 0.05$) and enhanced at 0.01 pg/mL ($P < 0.01$) of toxin. TSST-1 was found to induce the production of much higher amounts of interferon-gamma ($\text{IFN}\gamma$) at 1000 pg/mL than at 0.01 pg/mL of toxin ($P = 0.0001$). More importantly, immunoglobulin E (IgE) synthesis was enhanced by TSST-1 at 1 pg/mL in the presence of antibodies blocking $\text{IFN}\gamma$ activity. The other immunoglobulin (Ig) isotypes were also increased after TSST-1 stimulation suggesting that the enhanced IgE synthesis was secondary to a polyclonal B cell activation rather than an isotype switch. TSST-1-stimulated IgE synthesis was T cell-dependent because purified tonsil B cells were only able to synthesize increased amounts of IgE when small numbers of T cells were added to the cultures. Anti-HLA-DR and anti-LFA-1 monoclonal antibodies (MoAb) inhibited TSST-1-enhanced IgE synthesis, suggesting that the bridging of the T cell receptor (TCR) and major histocompatibility complex (MHC) class II on B cells was necessary for activation of B cell differentiation.

Conclusion These data indicate that staphylococcal superantigens are able, at concentrations inducing low amounts of $\text{IFN}\gamma$, to stimulate IgE synthesis by PBMC from AD patients, and suggest that staphylococcal toxins may contribute to elevated IgE synthesis in AD.

Keywords: atopic dermatitis, immunoglobulin E, T cells, toxic shock syndrome, toxin-1 superantigens, *Staphylococcus*

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Introduction

Atopic dermatitis (AD) is a chronic inflammatory skin disorder that frequently occurs in patients with respiratory allergy [1]. More than 80% of patients with AD have elevated serum immunoglobulin E (IgE) levels, and clinical evidence of allergies, i.e. foods and inhalants exacerbate this common skin disease [2,3,4]. In addition, peripheral blood mononuclear cells (PBMC) of AD patients produce increased levels of IgE *in vitro* [5,6]. They also produce higher levels of interleukin-4 (IL-4), but have diminished capacity to synthesize interferon-gamma (IFN γ) in response to stimulation with allergens and mitogens [6–8]. It is well established that greater than 90% of AD patients are heavily colonized with *Staphylococcus aureus*, which produce exotoxins such as toxic shock syndrome toxin-1 (TSST-1) [2,9]. The role of *S. aureus* in the pathogenesis of AD is strongly suggested by the reduction in skin inflammation following treatment with anti-staphylococcal drugs [10,11].

TSST-1, similar to other staphylococcal toxins, is a prototypic superantigen, which causes marked stimulation of monocytes and T cells triggering the production of high levels of cytokines, such as IFN γ [12,13]. TSST-1 has been shown to bind monomorphic determinants on major histocompatibility complex (MHC)-class II molecules in an unrestricted manner [14,15]. The MHC class II plus TSST-1 complex interacts primarily with the V β chain of the TCR to induce the activation and proliferation of a large number of T cells [12,13].

Paradoxically, several investigators have described an inhibition of Ig production by PBMC stimulated with these antigens [11,16,17]. This observation correlates well with the immunological features of toxic shock syndrome (TSS), in which the majority of the cases are related to TSST-1 [16]. In TSS, there is a marked activation of T cells and macrophages (M ϕ) but an inhibition of Ig production [16]. In contrast, chronic staphylococcal colonization or recurrent infections in other diseases, such as AD or hyper-IgE syndrome, are associated with elevated IgE synthesis. Previous studies have demonstrated that neutral pH and other factors optimize TSST-1 production [18]. Thus, *S. aureus* is likely to produce lower levels of TSST-1 on the skin of patients with eczema than vaginal *S. aureus* during menstrual bleeding in TSS. To further examine the potential role of staphylococcal toxins in IgE synthesis, the current study was carried out to test the effect of different TSST-1 concentrations on IgE synthesis.

Materials and methods

Subjects

We obtained blood from eight adult patients with

moderate to severe AD (greater than 20% of skin involvement). Their diagnosis was based on well-defined criteria [19,20]. All patients had a history of AD dating back to early infancy and had flexural eczema. None of the patients had previously used systemic corticosteroids. Control blood was obtained from eight healthy non-atopic adults, who had no personal history of atopic diseases, asthma or skin disease. Tonsils were obtained immediately after tonsillectomy of six non-atopic patients. Informed consent was obtained from all subjects before blood was drawn for these studies.

Reagents

TSST-1 was prepared by one of the authors (P.M.S.) as previously described [21]. The toxin was homogeneous when 20 μ g was tested by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). A neutralizing rabbit polyclonal antibody to human IFN γ was provided by Genentech (South San Francisco, CA, USA). r-human IL-4 was obtained from Dr Paul Trotta (Schering Research Institute, Bloomfield, NJ, USA). A monoclonal antibody (MoAb) against human CD40 (clone 626.1, IgG1) was a kind gift of Dr Shu Man Fu (University of Virginia, Charlottesville, VA, USA). Anti-HLA-DR (clone L243, IgG2a specific for a non-polymorphic HLA-DR epitope) and against the α chain of the leucocyte function-associated antigen-1 (anti-LFA-1 α , clone G25.2, IgG2a) MoAbs were purchased from Becton Dickinson, Mountain View, CA, USA.

Cell preparation

PBMC were isolated from heparinized venous blood by density gradient centrifugation on Ficoll-Paque (Pharmacia, Uppsala, Sweden), and washed three times in HBSS (Gibco, Grand Island, NY, USA). Tonsillar mononuclear cells were obtained by passing minced tissue through a stainless steel mesh, washing two times in RPMI 1640 (Bio-Whittaker, Walkersville, MD, USA) supplemented with 250 μ g/mL of amphotericin B, 100 U/mL penicillin, 100 μ g/mL streptomycin and 50 μ g/mL gentamycin, and isolated by Ficoll-Paque density gradient centrifugation as for PBMC.

To obtain highly purified B cells, T cells were removed by rosetting once with 2-aminoethylisothiuronium bromide (Sigma Chemical Co., St Louis, MO, USA) treated SRBC (Colorado Serum Co., Denver, CO, USA) as previously described [22]. Monocyte/M ϕ were depleted by plastic adherence in tissue culture flasks twice (Costar Corporation, Cambridge, MA, USA) at 37°C. The remaining non-B cells were removed by two cycles of negative selection with immunobeads as previously

described [23]. Briefly, cells were incubated with MoAb anti-CD3, -CD56 (Becton Dickinson) and -OKM 1 (Ortho Diagnostics, Raritan, NJ, USA) on ice for 30 min, then washed two times in HBSS and incubated with magnetic beads coated with anti-mouse IgG (Dynabeads M-450, Dynal, Oslo, Norway) on ice for 60 min. Coated cells were removed by a magnet (Advanced Magnetism, Inc., Cambridge, MA, USA). Purity of cell populations was assessed by immunofluorescence with anti-CD3, -CD14, -CD56 MoAb and flow cytometry analysis (Becton Dickinson). The resulting B cell population was more than 98% pure and contained less than 1% of T cells and less than 1% CD14+ cells.

Cell cultures

For IFN γ synthesis and lymphocyte proliferation studies, PBMC were suspended in RPMI 1640 (Bio-Whittaker) supplemented with 10% heat inactivated fetal calf serum (FCS) (Hyclone Sterile Systems, Inc., Logan, UT, USA), 20 mM Hepes buffer, 100 U/mL penicillin, 100 μ g/mL streptomycin and 2 mM L-glutamine (Gibco). For Ig synthesis, PBMC or purified tonsil B cells were suspended in Iscove's modified Dulbecco's medium (Gibco) supplemented with 10% heat inactivated FCS (Hyclone Sterile Systems), 0.5% BSA, 50 μ g/mL human transferrin, 5 μ g/mL bovine insulin, 1 μ g/mL oleic, linoleic and palmitic acid (Sigma Chemical Co.) as described previously [24,25]. PBMC or purified B cells were cultured at the concentration of 1×10^6 cells/mL in round-bottomed 96-well plates (Costar Corp.) at 37°C, in a humidified 5% CO $_2$ atmosphere. IL-4 and/or antibodies were added as indicated in each experiment (see Results section). Supernatants were collected after 4 days (cytokine production) or 14 days (Ig production) and stored at -20°C until assayed. Preformed and cell-associated IgE were measured by acid elution at day 0 and at day 14 of culture according to the method of Turner *et al.* [26].

Lymphocyte proliferation assay

To assess lymphocyte proliferation responses, PBMC were cultured at the concentration of 1×10^6 cells/mL using TSST-1 concentrations ranging from 1000 to 0.001 pg/mL. After 4 days, 0.8 μ Ci/well [^{3} H]thymidine (Amersham Corp., Arlington Heights, IL, USA) was added and cells were incubated for 6 additional hours. Cells were harvested onto filter paper disks and radioactivity counted by liquid scintillation (Beckman Instruments, Fullerton, CA, USA). All samples were run in triplicate.

ELISA for Ig and cytokine determination

The IgE assay was carried out as previously described [27]. Ninety-six-well microtitre plates (Dynatech, Chantilly, VI, USA) were coated with 0.1 mL of a 1 : 1 mixture of purified monoclonal anti-human IgE (4-15 and 7-12, a kind gift of Dr A. Saxon, University of California, Los Angeles, CA, USA) diluted in 0.1 M NaHCO $_3$ at pH 9.6, at a concentration of 10 μ g/mL. After overnight incubation at 4°C, the wells were blocked with 0.1% gelatin in 0.1 M NaHCO $_3$ at room temperature for 2 h. Serial dilutions of culture supernatants were incubated in duplicate for 2 h at room temperature and overnight at 4°C, with parallel human IgE standard controls (Pharmacia). The plates were then washed, a 1 : 100 dilution of affinity-purified biotinylated goat antihuman IgE (Vector, Burlingame, CA, USA) was added, and plates were incubated for 90 min at 37°C. After a subsequent wash, wells were incubated with a 1 : 1500 dilution of streptavidin-alkaline phosphatase (Tago, Inc., Burlingame, CA, USA) for 90 min at 37°C. The wells were then developed with 2 mM *p*-nitrophenyl phosphate substrate (Sigma Chemical Co.), and the optical density was read at 405 nm on an Emax microplate reader (Molecular Devices, Menlo Park, CA, USA). This assay did not detect polyclonal IgG and therefore did not detect idiotype determinants, as described previously [27]. The concentrations of IgE in the supernatants were read from an IgE standard curve. The lower limit of sensitivity of this assay was 500 pg/mL.

The protocol for IgG assay [27] was identical to that for IgE except that the initial capture antibody was an affinity-purified polyclonal goat anti-human IgG antibody (Tago) and the second antibody was biotinylated goat antihuman IgG (Vector). The IgG standards were obtained from Tago. IFN γ was measured by a commercially available enzyme immunoassay kit (Endogen, Boston, MA, USA) with a lower limit of sensitivity of 5 pg/mL (0.1 U/mL).

Statistical analysis

Data are expressed as the mean $\pm$ SEM. Due to the exponential range of the data, log transformation was performed before the data were analysed by paired Student's *t*-test.

Results

Stimulation of IgE synthesis in AD PBMC by TSST-1

PBMC obtained from eight AD patients and eight non-atopic subjects were stimulated with a range of TSST-1

concentrations from 1000 pg/mL down to 0.001 pg/mL and analysed for spontaneous IgE synthesis. Preliminary studies showed that IgE production by AD PBMC increased progressively with decreasing concentrations of TSST from 1000 down to 0.01 pg/mL (data not shown). We therefore chose three concentrations corresponding to maximal inhibition (1000 pg/mL), similar to medium alone (1 pg/mL) and maximal stimulation (0.01 pg/mL) for our study. As shown in Fig. 1(a), there was significant inhibition of IgE synthesis by PBMC from AD patients at 1000 pg/mL of TSST-1 (690 ± 240 pg/mL, $P < 0.05$) compared with medium alone (2410 ± 710 pg/mL). However, there was significant enhancement of IgE synthesis at 0.01 pg/mL of TSST-1 (4600 ± 2160 pg/mL, $P < 0.01$). In contrast to

AD PBMC, in PBMC from non-atopic subjects (Fig. 2), there was no IgE synthesis detectable after stimulation with TSST-1 at any concentration tested.

Next, the effect of TSST-1 on PBMC stimulated with IL-4 was examined. As shown in Fig. 1(b), the IL-4-induced IgE production by AD PBMC was inhibited maximally at 1000 pg/mL (800 ± 420 pg/mL, $P < 0.05$) compared with IL-4 alone (3890 ± 990 pg/mL). In contrast, there was marked enhancement of IgE at 0.01 pg/mL of TSST-1 (20980 ± 8080 pg/mL, $P < 0.001$). In PBMC of eight non-atopic subjects (Fig. 2), TSST-1 inhibited IgE synthesis at 1000 pg/mL (< 500 pg/mL, $P < 0.05$) compared with IL-4 alone (1970 ± 850 pg/mL), and did not have a significant effect on IgE synthesis at any other concentration tested.

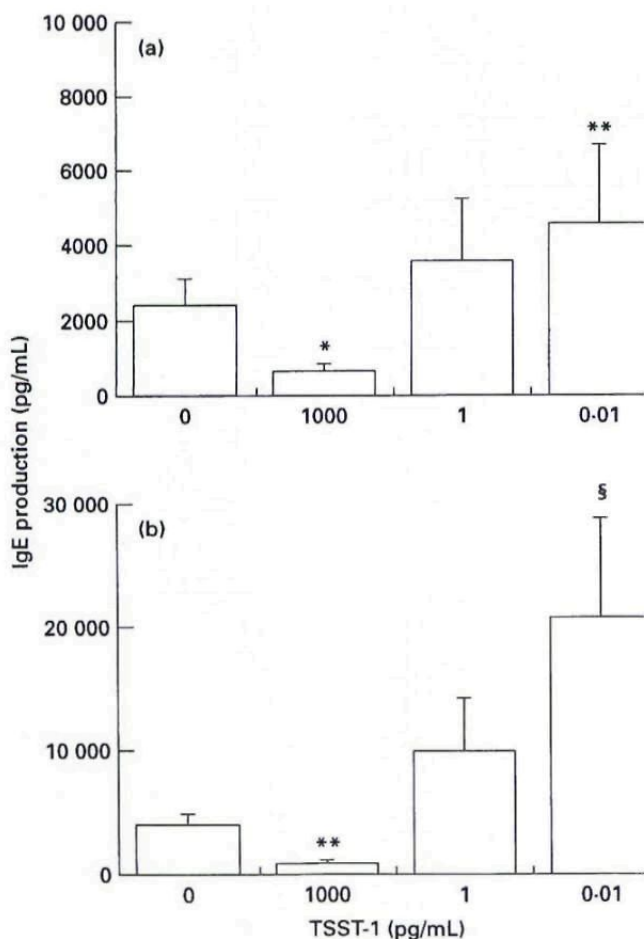


Fig. 1. IgE production by AD PBMC was significantly enhanced after stimulation with low concentrations of TSST-1. PBMC from eight AD patients were incubated in the absence (a) or presence (b) of IL-4 at 400 U/mL with a range of TSST-1 concentrations (medium alone (0) vs TSST-1: * $P < 0.05$, ** $P < 0.01$, § $P = 0.0001$). In AD PBMC, preformed IgE was less than 500 pg/mL.

TSST-1-enhanced IgE production in AD PBMC is inhibited by IFN γ

In order to determine the potential mechanism for inhibition of IgE synthesis at the higher TSST-1 concentrations and for the difference in IgE synthesis between AD and non-atopic PBMC at 0.01 pg/mL of TSST-1, we measured PBMC proliferation and IFN γ production in supernatants of cultures stimulated with several concentrations of TSST-1. Table 1 shows the effect of TSST-1

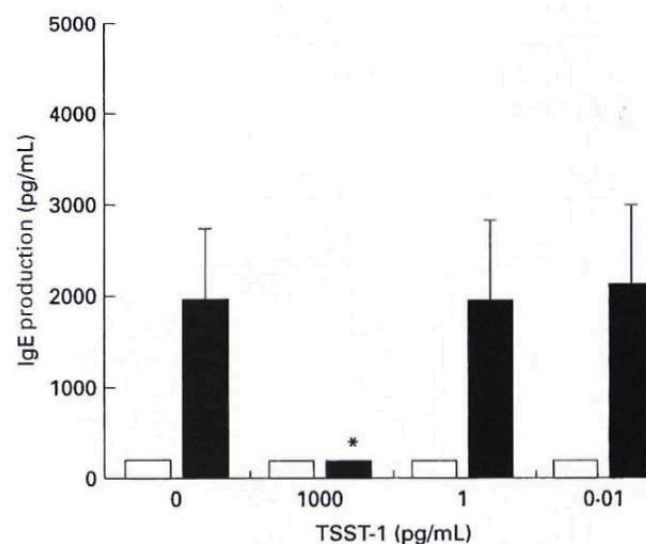


Fig. 2. IgE production by PBMC from non-atopics was not increased after stimulation with various concentrations of TSST-1. PBMC from eight non-atopics were incubated in the absence (□) or presence (■) of IL-4 at 400 U/mL with a range of TSST-1 concentrations (medium (0) vs TSST-1: * $P < 0.05$). In PBMC from non-atopics, preformed IgE was less than 500 pg/mL.

Table 1. TSST-1-induced lymphocyte proliferation and IFN γ production in PBMC of AD patients and normal subjects

	TSST-1 (pg/mL)	Proliferation (cpm)	IFN γ (pg/mL)
AD patients	0	2796 $\pm$ 570	5 $\pm$ 1§
	1000	43 925 $\pm$ 7866	12 839 $\pm$ 6643*
	1	24 819 $\pm$ 6282	295 $\pm$ 73*
	0.01	13 132 $\pm$ 4503	26 $\pm$ 13**
Non-atopic	0	5200 $\pm$ 1110	15 $\pm$ 4
	1000	29 434 $\pm$ 5608	28 898 $\pm$ 1313
	1	24 951 $\pm$ 6290	936 $\pm$ 355
	0.01	13 884 $\pm$ 3811	300 $\pm$ 104

PBMC of seven AD patients and seven normal subjects were cultured for 4 days in the presence of several concentrations of TSST-1. *P*-values compare AD vs non-atopic at each TSST-1 concentration:

P* < 0.01, *P* < 0.005, §*P* < 0.001.

on lymphocyte proliferation and IFN γ production in PBMC of seven AD patients and seven non-atopic subjects. At all concentrations used, TSST-1 showed a mitogenic activity on AD PBMC, but was lowest at the concentration of TSST-1 where enhanced IgE synthesis could be observed (0.01 pg/mL). At this concentration, lymphocyte proliferation was still significantly higher (*P* < 0.005) than for medium alone. Analysis of the relative percentage of different cell types by flow cytometry demonstrated that both B and T cells are equally stimulated at low concentrations of TSST-1, whereas T cells predominantly proliferated after high concentrations of TSST-1 stimulation.

This decrease in lymphocyte activation was paralleled by the IFN production, which was significantly higher in cultures stimulated with 1000 pg/mL (12 839 $\pm$ 642 pg/mL, *P* = 0.0001) and with 1 pg/mL of TSST-1 (295 $\pm$ 73 pg/mL, *P* < 0.0005), than with 0.01 pg/mL of TSST-1 (26 $\pm$ 13 pg/mL), where enhanced IgE synthesis

was observed. TSST-1 induced a similar dose-dependent change in IFN γ production by PBMC from non-atopic subjects. At 0.01 pg/mL of TSST-1, as with all other TSST-1 concentrations tested, non-atopic PBMC synthesized significantly higher levels of IFN γ (300 $\pm$ 104 pg/mL) than AD PBMC (26 $\pm$ 13 pg/mL, *P* < 0.005). This difference in IFN synthesis was not secondary to a lower lymphocyte proliferation, because it was similar in AD and in non-atopic PBMC at each of these concentrations.

These data suggested that IFN γ could play an important role in the inhibition of IgE synthesis by TSST-1. To test this hypothesis, we stimulated PBMC from four AD patients with TSST-1 at 1 pg/mL in the absence and presence of anti-IFN γ antibodies. As shown on Table 2, neutralization of IFN γ activity significantly increased IgE synthesis (43 870 $\pm$ 24 140 pg/mL) when compared with PBMC stimulated with TSST-1 at 1 pg/mL alone (8430 $\pm$ 530 pg/mL, *P* = 0.02).

Table 2. Anti-interferon-gamma increases IgE production stimulated by TSST-1

Treatment	IgE (pg/mL)				Mean $\pm$ SEM
	#1	#2	#3	#4	
None	820	6350	1080	25 920	8540 $\pm$ 5930
TSST-1	1940	1840	<500	29 860	8430 $\pm$ 7150
TSST-1 + anti-IFN γ Ab	66 770	3670	4010	101 020	43 870 $\pm$ 24 140*

PBMC of four AD patients were cultured with or without TSST-1 (1 pg/mL) and anti-IFN γ (1.5 μ g/mL). IgE synthesis was significantly greater in the TSST-1-stimulated cultures neutralized with anti-IFN γ as compared with cultures stimulated with TSST-1 alone (**P* = 0.02).

TSST-1-enhanced IgE synthesis is due to polyclonal B cell activation

To assess the isotype specificity of the B cell activation by TSST-1, we measured IgG production in supernatants of cultures (Fig. 3). After stimulation with either 1 pg/mL or 0.01 pg/mL of TSST-1, spontaneous IgG production was significantly increased in PBMC from AD patients ($P < 0.01$), as well as in PBMC from non-atopic subjects ($P < 0.005$). IgA and IgM were also measured in culture supernatants of cultures stimulated with TSST-1 at concentrations ranging from 1000 pg/mL to 0.01 pg/mL. At the lower TSST-1 concentrations, we found in AD patients and in non-atopic subjects a significantly increased production of IgA and IgM as well (data not shown).

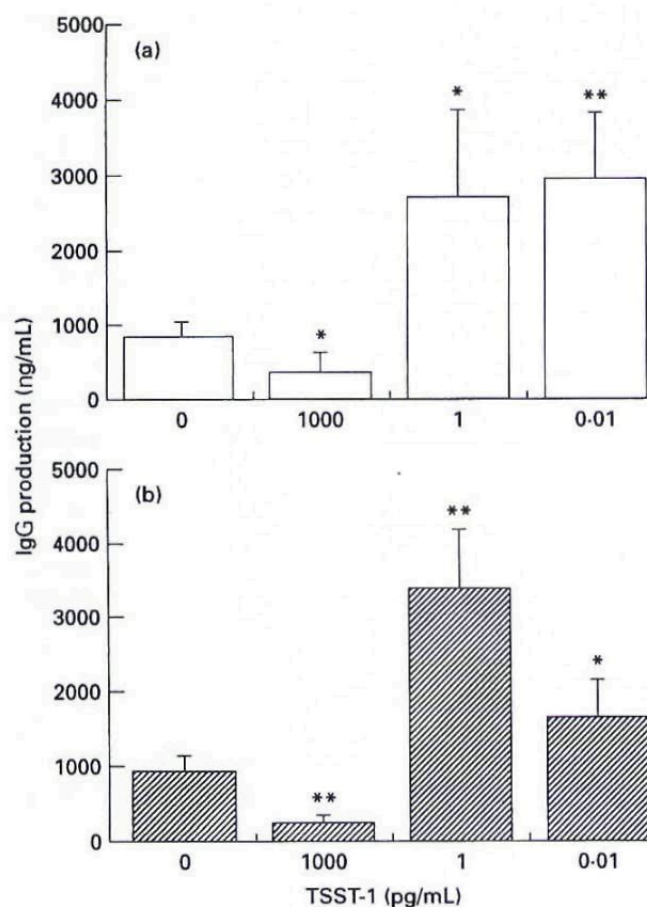


Fig. 3. IgG production was significantly enhanced after stimulation with low doses of TSST-1 in both AD patients and non-atopic subjects. Spontaneous IgG production was measured in culture supernatants of PBMC from (a) eight AD patients and (b) eight non-atopic subjects with a range of TSST-1 concentrations (medium (0) vs TSST-1: * $P = 0.01$, ** $P < 0.005$).

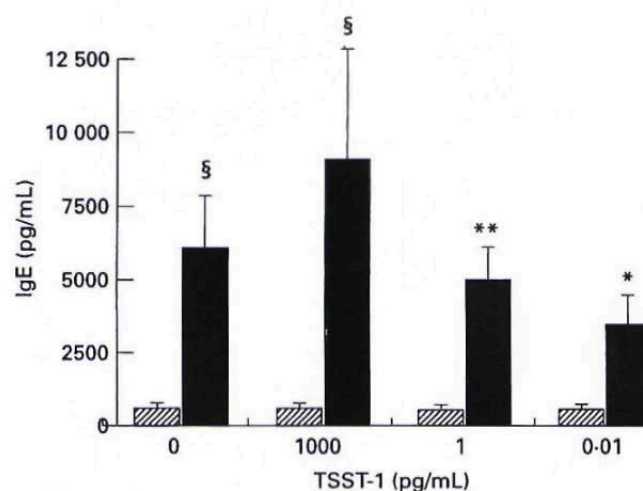


Fig. 4. IgE production from purified tonsil B cells was not induced by TSST-1 at any concentration (▨). IL-4-induced IgE production was measured in cultures of purified B cells from five tonsils with a range of TSST-1 concentrations. Anti-CD40 MoAb (■) significantly increased IgE synthesis of purified B cells in the presence of IL-4 (presence vs absence of anti-CD40: * $P < 0.05$, ** $P < 0.005$, § $P < 0.001$). In tonsil B cells, preformed IgE was less than 500 pg/mL.

In order to determine if TSST-1-enhanced IgE production is secondary to isotype switching, purified tonsil B cells were cultured in the presence of several concentrations of TSST-1. As shown in Fig. 4, IgE synthesis was not stimulated in purified B cells incubated with IL-4 at any concentration of TSST-1. In contrast, when the purified B cells were cultured in the presence of IL-4 and MoAb to CD40, which has been previously demonstrated to induce IgE-isotype switching in purified B cells [28], IgE production was significantly increased in comparison to IL-4 for each concentration of TSST-1 tested (medium + IL-4 + anti-CD40 MoAb: 6220 ± 230 pg/mL, vs medium + IL-4: <500 pg/mL, $P < 0.001$). Incubation of anti-CD40 plus IL-4 stimulated B cells, in the presence of several concentrations of TSST-1, did not inhibit IgE synthesis. This demonstrates that the non responsiveness of purified B cells to TSST-1 was not due to a direct inhibitory effect of TSST-1 on the B cell. Furthermore, in contrast to anti-CD40, TSST-1 does not appear to induce IgE isotype-switching.

TSST-1-enhanced IgE synthesis is T cell-dependent

Next, to examine whether TSST-1-stimulated IgE production is T cell-dependent, we added purified autologous tonsil T cells to B cells with a T : B ratio of 1 : 5. As shown in Table 3, the IL-4-induced IgE production by purified B cells co-cultured with low number of T cells increased after stimulation with TSST-1 at 1 pg/mL.

Table 3. TSST-1-induced IgE production by purified tonsil B cells is T cell-dependent

TSST-1 (pg/mL)	IgE (pg/mL)			
	Exp. #1		Exp. #2	
	B (+IL-4)	B + T (+IL-4)	B (+IL-4)	B + T (+IL-4)
0	<500	2080	<500	610
1000	<500	1520	<500	1370
1	<500	5320	<500	5370

Purified tonsil B cells (1×10^6 cells/mL) were stimulated with either 1000 or 1 pg/mL of TSST-1 in the presence of IL-4, with or without purified T cells (2×10^5 cells/mL).

To determine whether TSST-1-enhanced IgE synthesis required direct T-B cell contact we measured IL-4-induced IgE synthesis in supernatants of PBMC stimulated by TSST-1 in the presence of anti-HLA-DR MoAb L243, which is known to inhibit the binding of TSST-1 to B cells [13], and anti-LFA-1 α MoAb, which blocks LFA-1:ICAM interaction and therefore the adhesion between T cells and B cells. As shown in Table 4, the stimulation of IgE synthesis by AD PBMC in the presence of low concentrations of TSST-1 was inhibited by these two MoAbs.

Discussion

It is well established that AD is associated with increased IgE synthesis. Since such patients are colonized with *S. aureus* secreting exotoxins with superantigenic activity, we were interested in determining the effect of TSST-1, over a wide range of concentrations, on IgE synthesis. In this study, we demonstrate for the first time that, *in vitro*, TSST-1 can enhance IgE synthesis from AD PBMC. This could be observed at the concentration of 0.01 pg/

mL of TSST-1, whereas at 1000 pg/mL IgE synthesis was inhibited. The latter is consistent with a previous report on the effect of superantigens on PBMC in AD patients which demonstrated the inhibition of IgE synthesis using SEB at 1 μ g/mL [11].

Several studies have previously shown that staphylococcal exotoxins in the ng and μ g range inhibit IgG and IgM production by PBMC and that polyclonal B cell activation could be achieved only when B cells were cultured in the presence of inactivated T cells (irradiated or mitomycin C treated) or superantigen-reactive T cell clones [13,14,16,17]. These studies suggested that the manner in which T cells could inhibit Ig production is secondary to the massive activation of T cells by TSST-1. The consequence would be either inhibitory T/B cell interactions at higher T cell loads and/or the attainment of a critical concentration of cytokine(s) which inhibit Ig synthesis [13,17].

In our current study, AD PBMC produced much less IFN γ after stimulation with 0.01 pg/mL, than after 1 or 1000 pg/mL of TSST-1. Several reports have demonstrated that an increase in IFN γ production *in vitro*

Table 4. IgE production in AD PBMC stimulated by TSST-1 is blocked by MoAb anti-HLA-DR and anti-LFA-1 α

Treatment	IgE (pg/mL)			
	Exp. #1		Exp. #2	
	IL-4	IL-4 + TSST-1	IL-4	IL-4 + TSST-1
None	1330	5590	1940	3270
Anti-HLA-DR	<500	<500	880	530
Anti-LFA-1	<500	<500	<500	<500

PBMC of two AD patients were cultured with or without TSST-1 (0.01 pg/mL) and in the presence of IL-4 and MoAb anti-HLA-DR (2.5 μ g/mL) or anti-LFA-1 α (1.25 μ g/mL).

correlates with the inhibition of IgE synthesis by PBMC [29]. Concentrations of IFN γ as low as 250 pg/mL have been found to inhibit IgE synthesis by PBMC significantly and 5000 pg/mL to give a maximal inhibition [7,30]. In contrast, IFN γ concentrations higher than 5000 pg/mL were necessary to block IgG synthesis [31]. This may explain differences in the concentration of TSST-1 able to enhance IgE and IgG synthesis. In our current study, AD PBMC stimulated with low concentrations of TSST-1, where IFN γ production was lower than 250 pg/mL, was associated with an enhancement of IgE synthesis by TSST-1. Higher concentrations of this superantigen induced a higher production of IFN γ and did not increase IgE synthesis. The role of IFN γ in the inhibition of TSST-1-stimulated IgE synthesis was further supported in our study by the enhancement of IgE synthesis after antibody neutralization of IFN γ activity.

In contrast to the other Ig isotypes which were increased in PBMC from both AD and non-atopic subjects after stimulation with low doses of TSST-1, only AD patients, but not normal, PBMC showed an increased IgE synthesis. TSST-1, at all concentrations tested, stimulated a significantly lower IFN γ production in AD than in non-atopic PBMC. This difference was not secondary to a decreased T cell activation, because lymphocyte proliferation was similar in AD and in non-atopic PBMC after stimulation with TSST-1. Thus, the higher concentration of IFN after stimulation with 0.01 pg/mL of TSST-1 in non-atopic, compared with AD PBMC, may explain why IgE synthesis is not increased in non-atopic PBMC. Moreover, when purified tonsil T and B cells were cultured together at a much lower ratio (1:5) than in PBMC (10:1), an enhancement of IgE synthesis could be observed at all TSST-1 concentrations tested. The lower number of IFN γ producing T cells could explain this difference in IgE production between purified tonsil T and B cells and PBMC in non-atopic subjects.

To study the cellular requirements for TSST-1-enhanced IgE synthesis, we examined the effect of TSST-1 on purified B cells stimulated with IL-4. Our results indicate that, in contrast to anti-CD40, TSST-1 was not able to induce purified B cells to synthesize IgE in the presence of IL-4. However, the addition of T cells restored the ability of TSST-1 to stimulate an enhancement of IgE synthesis. Thus, unlike anti-CD40, TSST-1 does not act as a second signal in order to induce IgE switching, but needs T-B cell interaction. Furthermore, enhanced IgE production by AD PBMC after TSST-1 stimulation is not due to an isotype switching, but rather the result of polyclonal B-cell activation. This was supported by the increase of the other Ig isotypes after low concentration TSST-1 stimulation.

Our data are consistent with previous reports showing that TSST-1 binds to MHC class II molecules [14,15,32] and mediates the bridging of MHC class II molecules on B cells and TCR/CD3 complex on T cells [13]. LFA-1, an adhesion molecule, has been shown to be necessary for the maintenance of a close association between T helper cells and small resting B lymphocytes [33] and for optimal Ig production [13]. The necessity of a contact between T and B cells in our experiments was illustrated by the fact that antibodies against HLA-DR and LFA-1 α were able to block IgE synthesis by AD PBMC stimulated with TSST-1 at low concentration (0.01 pg/mL). Taken together, these data indicate that TSST-1-stimulated IgE synthesis is T cell dependent and needs the bridging of the TCR and the MHC class II in order to activate B cells.

Recently, it has been shown that staphylococcal protein A can activate B cells by direct binding to the Fab V_H region of Ig and can be considered as a B cell superantigen [34]. Since TSST-1 does not activate Ig synthesis in purified B cells but instead requires T cells, it is unlikely that TSST-1 activates B cells as an Ig superantigen. This concept is further supported by our observation that TSST-1-induced IgE synthesis is blocked by anti-HLA-DR antibodies.

In summary, our results show for the first time that a superantigen, i.e. staphylococcal TSST-1, is able to enhance *in vitro* IgE synthesis by PBMC from AD donors. The concentrations of TSST-1 needed to enhance IgE were much lower than those required for maximal lymphocyte activation. These data suggest that different pathways of lymphocyte activation may be involved after stimulation with high vs low concentrations of TSST-1. In the nanogram range, TSST-1 induces massive T lymphocyte proliferation associated with marked IFN γ production, and, as a consequence, an inhibition of Ig production in both AD and non-atopic PBMC. In contrast, at lower concentrations, TSST-1 stimulates T cells to a lesser extent and promotes a polyclonal B cell activation.

These data are consistent with antibody responsiveness to TSST-1 in TSS patients compared with the general population. Greater than 85% of TSS patients fail to develop an antibody response to TSST-1 and other staphylococcal products upon recovery from the illness [35,36]. This is considered to be the result of exposure to large doses of toxin sufficient to cause serious illness and consequent massive T-cell proliferation with high level IFN γ production. In contrast, there is a steady acquisition of TSST-1 neutralizing antibodies in the general population such that, by age 21, 75% of individuals have neutralizing antibodies [37]. This indicates that low level exposure to TSST-1, in toxin amounts insufficient to

cause TSS, allows production of Ig. Thus, stimulation of the humoral immune response by low concentrations of exotoxins may contribute to the polyclonal B cell activation in Kawasaki syndrome [38] and increase IgE synthesis in AD and in hyper-IgE syndrome. The stimulation of IgE synthesis in PBMC from AD patients by TSST-1 reinforces the importance of chronic colonization or infection with *S. aureus* in the pathogenesis of diseases associated with elevated IgE synthesis.

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References

- Wittig HJ, Belloit J, DeFillippi I. Age-related serum immunoglobulin E levels in healthy subjects and in patients with allergic disease. *J Allergy Clin Immunol* 1980; 66:305–13.
- Leung DYM, 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.
- Norris PG, Schofield O, RDRC. A study of the role of house dust mite in topic dermatitis. *Br J Dermatol* 1988; 118:435–40.
- Burks AW, Mallory SB, Williams LW, Shirrell MA. Atopic dermatitis: clinical relevance of food hypersensitivity reactions. *J Pediatr* 1988; 113:447–51.
- Vollenweider S, Saurat J-H, Röcken M, Hauser C. Evidence suggesting involvement of interleukin-4 (IL-4) production in spontaneous in vitro IgE synthesis in patients with atopic dermatitis. *J Allergy Clin Immunol* 1991; 87:1088–95.
- Jujo K, Renz H, Abe J, Gelfand EW, Leung DYM. Decreased gamma interferon and increased interleukin-4 production promote IgE synthesis in atopic dermatitis. *J Allergy Clin Immunol* 1992; 90:323–30.
- Rousset F, Robert J, Andary M et al. Shifts in interleukin-4 and interferon- γ production by T cells of patients with elevated serum IgE levels and the modulatory effects of these lymphokines on spontaneous IgE synthesis. *J Allergy Clin Immunol* 1991; 87:58–69.
- Reinhold U, Wehrmann W, Kukel S, Kreysel H-W. Recombinant interferon- γ in severe atopic dermatitis. *Lancet* 1990; 335 (letter):1282.
- Leyden JE, Marples RR, Kligman AM. Staphylococcus aureus in the lesions of atopic dermatitis. *Br J Dermatol* 1974; 90:525–30.
- Lever R, Hadley K, Downey D, Mackie R. Staphylococcal colonization in atopic dermatitis and the effect of topical mupirocin therapy. *Br J Dermatol* 1988; 119:189–98.
- Neuber K, König W. Effects of staphylococcus aureus cell wall products (teichoic acid, peptidoglycan) and enterotoxin B in immunoglobulin (IgE, IgA, IgG) synthesis and CD23 expression in patients with atopic dermatitis. *Immunology* 1992; 75:23–8.
- Marrack P, Kappler J. The staphylococcal enterotoxins and their relatives. *Science* 1990; 248:705–11.
- Mourad W, Scholl P, Diaz A, Geha RS, Chatila T. The staphylococcal toxic shock syndrome toxin-1 triggers B cell proliferation and differentiation via major histocompatibility complex-unrestricted cognate T/B cell interaction. *J Exp Med* 1989; 170:2011–22.
- Fuleihan R, Mourad W, Geha RS, Chatila T. Engagement of MHC-class II molecules by staphylococcal exotoxins delivers a comitogenic signal to human B cells. *J Immunol* 1991; 146:1661–6.
- Scholl P, Diez A, Mourad W et al. Toxic shock syndrome toxin 1 binds to major histocompatibility complex class II molecules. *Proc Natl Acad Sci USA* 1989; 86:4210–4.
- Poindexter NJ, Schlievert PM. Suppression of immunoglobulin-secreting cells from human peripheral blood by toxic shock syndrome toxin-1. *J Infect Dis* 1986; 153:772–9.
- Moseley AB, Huston DP. Mechanism of Staphylococcus aureus exotoxin A inhibition of Ig production by human B cells. *J Immunol* 1991; 146:826–32.
- Schlievert PM, Blomster DA. Production of staphylococcal pyrogenic exotoxin type C: influence of physical and chemical factors. *J Infect Dis* 1983; 147:236–42.
- Hanifin JM, Rajka G. Diagnostic features of atopic dermatitis. *Acta Dermatol Venereol (Stockh)* 1980; 92:44–7.
- Leung DYM, Rhodes AR, Geha RS, Schneider L, Ring J. Atopic dermatitis. In: Fitzpatrick TB, Eisen AZ, Wolff K, Freeberg IM, Austen KF. *Dermatology in General Medicine*. New York: McGraw-Hill, 1993: 1543–64.
- Blomster-Hautamaa DA, Schlievert PM. Preparation of toxic syndrome toxin-1. *Meth Enzymol* 1988; 165:37–43.
- Madsen M, Johnson HE. A methodological study of E-rosette formation using AET-treated sheep erythrocytes. *J Immunol Methods* 1979; 27:61–74.
- Funderud S, Nustad K, Lea T et al. Fractionation of lymphocytes by immunomagnetic beads. In: Klaus GGB (ed). *Lymphocytes: A Practical Approach*. New York: Oxford University Press, 1987: 55–61.
- Claassen JL, Levine AD, Buckley RH. Recombinant human IL-4 induces IgE and IgG synthesis by normal and atopic donor mononuclear cells. *J Immunol* 1990; 144:123–30.
- Yssel H, deVries JE, Koken M, Blitterswijk WV, Spits H. Serum-free medium for generation and propagation of functional human cytotoxic and helper T cell clones. *J Immunology Methods* 1984; 72:219–27.
- Turner KJ, Holt PG, Holt BJ, Cameron KJ. In vitro synthesis of IgE by human peripheral blood leucocytes. III. Release of pre-formed antibody. *Clin Exp Immunol* 1983; 51:387–94.

- 27 Jujo K, Renz H, Abe J, Trumble A et al. Pokeweed mitogen induced IgE synthesis in the presence of a blocking antibody to the interferon- γ receptor. *J Allergy Clin Immunol* 1993; 91:1201–16.
- 28 Zhang K, Clark EA, Saxon A. CD40 stimulation provides an IFN- γ -independent and IL-4 dependent differentiation signal directly to human B cells for IgE production. *J Immunol* 1991; 146:1836–42.
- 29 Delespesse G, Saifati M, Meusser C. IgE synthesis. *Curr Opin Immunol* 1990; 170:1751–6.
- 30 Punnonen J, Punnonen K, Jansen CT, Kalimo K. Interferon-alpha, interferon-gamma, interleukin-2, and arachidonic acid metabolites modulate IL-4-induced IgE synthesis similarly in healthy persons and in atopic dermatitis patients. *Allergy* 1993; 48:189–95.
- 31 Matsumoto T. Ongoing IgE synthesis by atopic B cells is enhanced by interleukin-3 and suppressed directly by interferon-gamma in vitro. *Int Arch Allergy Appl Immunol* 1991; 85:48–52.
- 32 Fischer H, Dohlsten M, Lindvall M, Sjögren HO, Carlsson R. Binding of staphylococcal enterotoxin A to HLA-DR on B cell lines. *J Immunol* 1989; 142:3151–7.
- 33 Fischer A, Durandy A, Sterkers G, Griscelli C. Role of the LFA-1 molecule in cellular interactions required for antibody production in humans. *J Immunol* 1986; 136:3198–203.
- 34 Sasano M, Burton DR, Silverman GJ. Molecular selection of human antibodies with an unconventional bacterial B cell antigen. *J Immunol* 1993; 151:5822–39.
- 35 Davis JP, Osterholm MT, Helms CM et al. Tri-state toxic-shock syndrome study: evaluation of case definition and prevention of recurrence. *Ann Int Med* 1982; 96:903–5.
- 36 Bohach GA, Fast DJ, Nelson RD, Schlievert PM. Staphylococcal and streptococcal pyrogenic toxins involved in toxic shock syndrome and related illnesses. *Crit Rev Microbiol* 1990; 17:251–72.
- 37 Vergeront JM, Stolz SJ, Crass BA, Nelson DB, Davis JP, Bergdoll MS. Prevalence of serum antibody to staphylococcal enterotoxin F among Wisconsin residents: implications for toxic-shock syndrome. *J Infect Dis* 1983; 148:692–8.
- 38 Leung DYM, Meissner HC, Fulton DR, Murray DL, Kotzin BL, Schlievert PM. Toxic shock syndrome toxin-secreting *Staphylococcus aureus* in Kawasaki syndrome. *Lancet* 1993; 342:1385–8.

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