

Staphylococcal Toxins Augment Specific IgE Responses by Atopic Patients Exposed to Allergen

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Microbial agents are known to play a significant role in aggravating allergic diseases. Recently described viral and bacterial superantigens represent one important strategy by which infectious agents can stimulate the immune response. In previous work, we reported that the staphylococcal toxin toxic shock toxin-1 (TSST-1), a prototypic superantigen, induces *in vitro* total IgE synthesis after cross-linking T and B cells. This study was carried out to establish a potential link between superantigens and the enhanced IgE response to specific allergens in allergic patients. Peripheral blood mononuclear cells from atopic patients were isolated during and outside the pollen allergen season and stimulated with TSST-1, a prototypic superantigen. Total IgE and interferon- γ production were measured in supernatants of these cultures. Outside the pollen season, TSST-1 significantly increased total IgE production only in the presence of exogenous interleukin-4, whereas during the pollen season IgE production was significantly enhanced without the

need of exogenous interleukin-4. This increase in the absence of exogenous interleukin-4 was associated with significantly lower interferon- γ production by peripheral blood mononuclear cells stimulated by TSST-1 during the pollen season. Moreover, TSST-1 stimulation of peripheral blood mononuclear cells from inhalant allergic patients was followed by an increased production of allergen-specific IgE that was restricted to the allergen to which the patient was allergic and recently exposed. In addition, TSST-1 induced on B cells the expression of B7.2, a molecule that has recently been demonstrated to enhance T helper 2 responses and to be involved in IgE regulation. This study, by demonstrating that superantigens can augment allergen-specific IgE synthesis and B7.2 expression, provides a mechanism by which microbial superantigens may modulate allergic responses. **Key words:** allergy/dermatitis/superantigens/T cells. *J Invest Dermatol* 112:171-176, 1999

Allergic diseases, such as asthma and atopic dermatitis (AD), are characterized by increased production of IgE directed against specific allergens, triggering inflammation in the target organ (Leung, 1993a, 1994). Aside from allergens, it has been shown that microbial antigens, like staphylococcal toxins in AD patients or viral antigens in asthma patients, may influence the course of allergic diseases (McIntosh *et al*, 1973; Leyden *et al*, 1974). Based on these clinical observations, the potential role of infectious antigens in triggering the IgE response in allergic patients was proposed over 20 y ago (McIntosh *et al*, 1973); however, the mechanism by which this occurs is unknown.

AD is a chronic inflammatory skin disorder that frequently occurs in patients with respiratory allergy (Leung *et al*, 1993b). More than 80% of patients with AD have elevated serum IgE, and clinical evidence of allergies, i.e., foods and inhalants, can exacerbate their

skin disease (Burks *et al*, 1988; Norris *et al*, 1988; Leung, 1995). In acute AD skin lesions, T cells secrete a T helper (Th) 2 cytokine profile [interleukin (IL)-4, IL-5, IL-13], which is known to induce IgE production (Van der Heijden *et al*, 1991; Hamid *et al*, 1994). Furthermore, Langerhans cells infiltrating into the AD skin lesions bear IgE on their cell surface (Bruijnzeel-Koomen *et al*, 1986; Leung *et al*, 1987; de Bieber *et al*, 1992). These cells may play an important role in cutaneous allergen presentation to Th 2 cells and contribute to atopic skin inflammation (Mudde *et al*, 1990). AD is an interesting disease model to investigate the events involved in IgE sensitization, because AD is an early manifestation of atopy in children, and nearly 80% of these children develop allergic rhinitis and asthma later in childhood (Leung, 1995). Of note, most AD patients are colonized by *S. aureus* (Hauser *et al*, 1985; Lever *et al*, 1988; Hoeger *et al*, 1992) on their skin and a majority of these strains secrete exotoxins that can trigger skin inflammatory responses (Marrack and Kappler, 1990).

Staphylococcal toxins, such as toxic shock syndrome toxin-1 (TSST-1), are prototypic superantigens leading to a polyclonal expansion of T lymphocytes expressing a specific T cell receptor variable region BV gene and the production of high cytokine levels (Marrack and Kappler, 1990). Superantigens are also able to activate B lymphocytes by bridging the T cell receptor on the T lymphocyte with the class II major histocompatibility molecule on the B lymphocyte (Mourad *et al*, 1989). We have previously shown that

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Abbreviations: AD, atopic dermatitis; Th, T helper; TSST-1, staphylococcal toxic shock syndrome toxin-1.

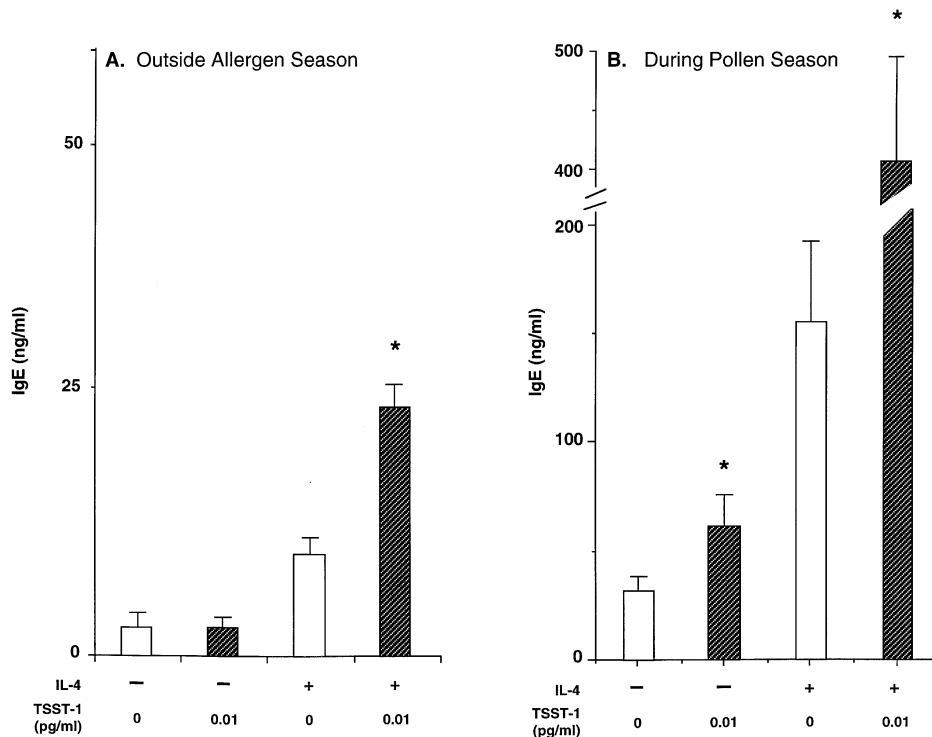


Figure 1. Stimulation of IgE production by TSST-1 during and outside the pollen season. PBMC from eight pollen-allergic subjects isolated outside (A) or during (B) the pollen season were incubated with TSST-1 at a concentration of 0.01 pg per ml in the presence or absence of exogenous recombinant IL-4. IgE production was measured by enzyme-linked immunosorbent assay after 14 d of culture. Mean $\pm$ SEM of eight experiments [medium alone (0, $\square$) versus TSST-1 ($\blacksquare$): * $p < 0.05$] are shown.

in vitro TSST-1 at low concentrations is able to stimulate IgE production by peripheral blood mononuclear cells (PBMC) from AD patients, and that this IgE production was inhibited by interferon (IFN)- γ at higher TSST-1 concentrations (Hofer *et al*, 1995). Increased IgE production was secondary to T cell-dependent polyclonal B cell activation after bridging of T and B cells by TSST-1. In this regard, TSST does not induce purified B cells to synthesize IgE in the absence of T cells. Furthermore, anti-LFA-1 monoclonal antibodies inhibit TSST-1 enhanced IgE synthesis. In contrast to anti-CD40 antibodies, TSST-1 did not induce IgE synthesis by purified B cells, which implies that TSST-1-induced IgE synthesis is T cell dependent. In this study, we show that staphylococcal toxins augment IgE response to specific allergens. These data provide for the first time a mechanism by which infectious agents may affect the course of allergic responses, in particular that staphylococcal infection in AD may augment IgE sensitization to inhalant allergens, and thereby contribute to the development of respiratory allergy.

MATERIALS AND METHODS

Subjects For the studies during and outside the pollen season we investigated eight patients with moderate to severe AD (greater than 20% of skin involvement). Their diagnosis was based on well-defined criteria (Hanifin and Rajka, 1980; Leung *et al*, 1993b). All patients had a history of AD dating back to early infancy and had flexural eczema. These patients had concomitant asthma or allergic rhinitis and were allergic to grass and/or ragweed pollen, as demonstrated by positive immediate skin test reactivity to grass and ragweed, respectively, and a prior history of increased asthmatic or rhinitis symptoms during the allergen season (late spring and/or fall). None of the patients had previously used systemic corticosteroids. Heparinized blood was collected from all pollen sensitive patients at two separate time points: during the pollen season (grass or ragweed, depending on the pollen to which the patient was sensitized) and outside the pollen season (December and January).

For allergen-specific IgE studies, blood was obtained from four cat-allergic patients (two of them were living with their cats and two were not exposed to cat allergen during the study period) and from four pollen-allergic patients (two of them during the pollen season and two during the winter). These patients had respiratory symptoms (asthma and/or rhinitis when exposed to the allergen to which they are sensitized), but none had AD. Control blood was obtained from eight healthy nonatopic subjects, who had no personal history of atopic diseases, asthma or skin disease and

were skin test negative to allergens. Informed consent was obtained from every participant to the study before blood was drawn.

Cell cultures PBMC were isolated from heparinized venous blood by density gradient centrifugation on Ficoll-Paque (Pharmacia, Uppsala, Sweden) and resuspended at 2×10^6 in enhanced Iscove's modified Dulbecco's medium with 10% heat inactivated fetal calf serum, as described previously (Yssel *et al*, 1984). PBMC were incubated in the absence or presence of 400 U recombinant IL-4 (Schering Research Institute, Bloomfield, NJ) per ml with a range of TSST-1 concentrations (1000–0.001 pg per ml). Purified TSST-1 was prepared by P.M.S. as previously described (Blomster-Hautamaa and Schlievert, 1988). After 2 d of culture for cytokine production or 14 d for IgE production, supernatants were collected and stored at -20°C until assayed.

Enzyme-linked immunosorbent assay for immunoglobulin and cytokine determination Total IgE and IgG were measured by a specific enzyme-linked immunosorbent assay, as described previously in Jujo *et al* (1993), read at 405 nm on an Emax microplate reader (Molecular Devices, Menlo Park, CA). The IgE assay did not detect polyclonal IgG and therefore did not detect idiotypic determinants. The concentrations of IgE and IgG in the supernatants were read from a standard curve. The lower limit of sensitivity of this assay was 500 pg per ml. Preformed IgE was measured by acid elution, as described previously (Turner *et al*, 1983) and was less than 500 pg per ml in all experiments. IFN- γ was measured by a commercially available enzyme immunoassay kit (Endogen, Boston, MA) with a lower limit of sensitivity of 5 pg per ml (0.1 U per ml). Specific IgE to timothy, ragweed, and cat allergen were measured by the CAP system. Results were given in kU per liter (Zieg *et al*, 1994). Before running the assay, the supernatants were concentrated 10 times using Centricon centrifugal concentrators (Amicon, Beverly, MA) with a 10,000 MW cut-off. The lower limit of sensitivity of the CAP system was 35 U per liter (0.035 kU per liter).

Cell staining and flow cytometric analysis Four-parameter analysis was performed using a FACScalibur flow cytometer (Becton Dickinson, Mountain View, CA) with fluorescein isothiocyanate and phycoerythrin used as the two fluorescent parameters. PBMC were washed twice in phosphate buffer saline containing 1% human immunoglobulins (Armour Pharmaceutical, Kankakee, IL) and 0.02% NaN_3 , and stained for 30 min at 4°C with anti-CD19 fluorescein isothiocyanate- (Becton Dickinson) and anti-B7.1 (CD80) phycoerythrin- or anti-B7.2 (CD86) phycoerythrin-conjugated monoclonal antibodies (Ancell, Bayport, MN). Cells were washed three times in phosphate buffer saline before analysis. Methods of

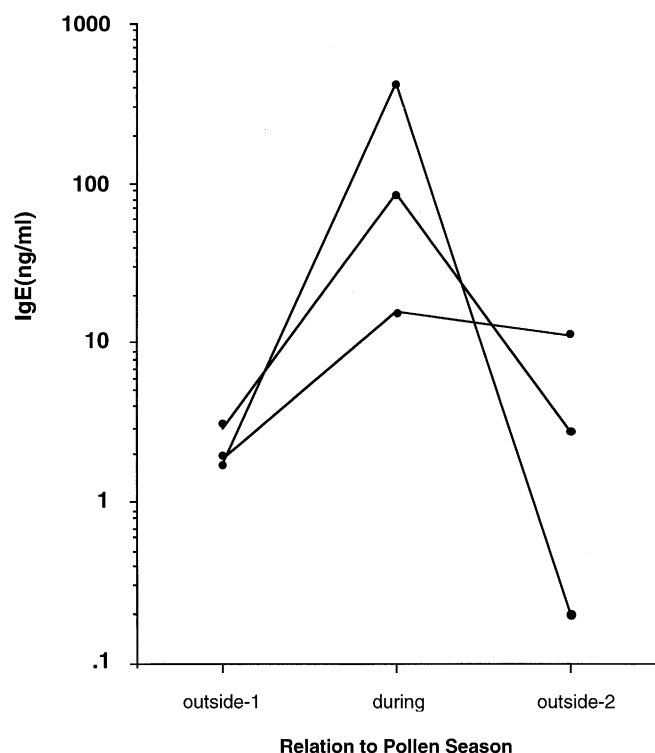


Figure 2. Stimulation of IgE production by TSST-1 in three consecutive seasons. PBMC from three pollen-allergic subjects, isolated outside, during, and outside the pollen season, were stimulated with TSST-1 in the absence of exogenous IL-4 as for Fig 1.

cytometer set up and data acquisition were as described previously (Funderud *et al*, 1987). List mode multiparameter data files (each file with forward scatter, side scatter, and two fluorescent parameters) were analyzed by use of the CellQuest MacIntosh program (Becton Dickinson). Negative control reagents were used to verify the staining specificity of experimental antibodies.

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 analyzed by unpaired or paired Student's *t* test.

RESULTS

TSST-1-enhanced IgE response is primed during the pollen season

Initially, we examined the role of *in vivo* allergen exposure for priming the IgE response to TSST-1 stimulation. PBMC were isolated from eight AD patients with pollen allergy, during and outside the pollen season, and stimulated with a range of TSST-1 concentrations in the presence or in the absence of exogenous IL-4. We chose the TSST-1 concentrations for our experiments based on previous studies, where we showed that higher concentrations inhibited IgE production by inducing B cell apoptosis (Hofer *et al*, 1996). As shown on Fig 1, the spontaneous IgE production in the absence of exogenous IL-4 was only increased significantly during the pollen season (medium alone, 32.0 ± 6.2 ng per ml; TSST-1, 61.5 ± 14.6 ng per ml, $p < 0.05$); however, TSST-1 enhanced IL-4-induced IgE production both outside (medium alone, 9.8 ± 1.5 ng per ml; TSST-1, 24.1 ± 2.2 ng per ml, $p < 0.05$) and during the pollen season (medium alone, 155.7 ± 37.1 ng per ml; TSST-1, 401.8 ± 108.9 ng per ml, $p < 0.05$). Under all conditions, IgE production was significantly higher ($p < 0.05$) during the pollen season compared with outside the pollen season. In three patients studied for three consecutive seasons (outside, during, and outside the pollen season), TSST-1-induced IgE production reproducibly dropped outside the pollen season (Fig 2). As a control, we measured the spontaneous and the TSST-1-induced IgG production, which were not affected by the season

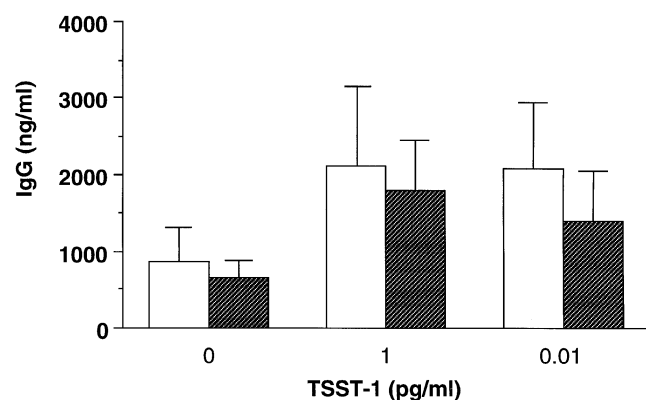


Figure 3. Stimulation of IgG production by TSST-1 during and outside the pollen season. PBMC from eight pollen-allergic subjects, isolated outside (□) or during (▨) the pollen season, were incubated with TSST-1 at concentrations of 0.1 and 0.01 pg per ml in the absence of exogenous IL-4. Mean $\pm$ SEM of all experiments (during versus outside pollen season: all $p > 0.50$).

(Fig 3). Furthermore, the frequency of B cells was similar in resting and TSST-1-stimulated PBMC samples drawn before and during the pollen season: in medium controls, the mean percentage of B cells was 6.1% during pollen season and 5.6% outside pollen season. Following TSST-1 stimulation, the mean percentage of B cells was 7.9% during pollen season and 7.5% outside pollen season. These data highlight the role of allergen exposure in priming for the enhanced IgE production by TSST-1.

TSST-1-induced IFN- γ synthesis is decreased during allergen exposure

Allergic patients have in common the predominance of a Th2-type cytokine profile, in particular IL-4 and IL-13, favoring IgE production, in contrast to nonatopic persons, where Th1-type cytokines predominate, such as IFN- γ , which is known to inhibit IgE synthesis (Rousset *et al*, 1991; Hamid *et al*, 1994; Lester *et al*, 1995). We hypothesized that low IFN- γ production contributed to the seasonal difference in TSST-1-stimulated IgE production. PBMC from eight pollen-allergic AD patients and eight nonatopic controls were cultured in medium alone or stimulated for 48 h with a range of TSST-1 concentrations followed by the measurement of IFN- γ and IL-4 in the culture supernatants. As shown in Fig 4, IFN- γ production induced by 10 pg TSST-1 per ml in PBMC from AD patients with pollen allergy was significantly lower during (142 ± 22 pg per ml) than outside the pollen season (2151 ± 499 pg per ml, $p = 0.02$). Results were similar for TSST-1 concentrations higher than 10 pg per ml; however, the spontaneous IFN- γ production was similar regardless of the season. The PBMC from nonatopic controls did not show any significant difference for the TSST-1-induced IFN- γ production during versus outside the pollen season. TSST-1 concentrations lower than 10 pg per ml did not induce sufficient enough IFN- γ to detect significant differences in seasonal production or a significant difference between subject groups studied. IL-4 production was too low to detect in both resting and TSST-1-stimulated cultures. This decreased IFN- γ production during season, as well as the need for exogenous IL-4 for the TSST-1-enhanced IgE production outside the pollen season, resulted in the higher TSST-1-induced IgE production after allergen exposure. When PBMC from allergic patients drawn outside the pollen season were stimulated with a mitogen, phytohemagglutinin, at the concentration of 2 μ g per ml, the IFN- γ production was much lower (96.1 ± 63.3 pg per ml) than after stimulation with TSST-1 (2151 ± 499 pg per ml). For PBMC from nonatopics, the difference was much less (phytohemagglutinin, 1149.6 ± 1042.1 ; TSST-1, 5505.1 ± 1688.0 pg per ml). Furthermore, in cultures done outside the pollen season when IFN- γ was blocked by neutralizing antibodies, TSST-1-stimulated IgE production was significantly enhanced (data not shown). Similarly, we showed previously that neutralizing anti-IFN- γ anti-

Figure 4. Stimulation of IFN- γ production by TSST-1 during and outside the pollen season. PBMC from eight pollen-allergic subjects (A) and eight non-atopic controls (B), isolated outside ($\square$) or during ($\blacksquare$) the pollen season, were incubated with TSST-1 at a concentration of 10 pg per ml. IFN- γ production was measured by enzyme-linked immunosorbent assay after 2 d of culture. Mean $\pm$ SEM of all experiments (during versus outside pollen season: * $p=0.02$).

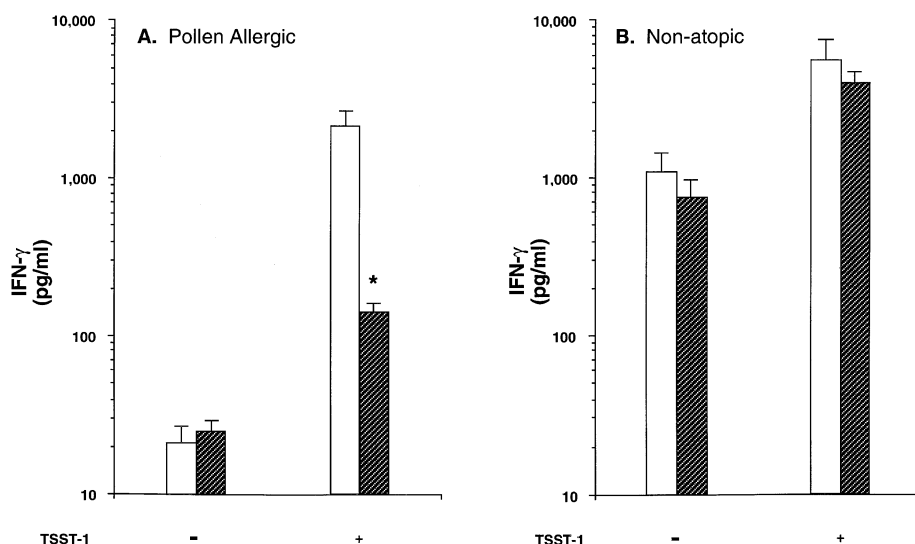


Table I. TSST-1 stimulates specific IgE production in cat and pollen allergic patients^a

	Allergen specific IgE production (U/l)				
	Patient 1	Patient 2	Patient 3	Patient 4	P value ^b
Medium	<0.35	<0.35	<0.35	<0.35	—
TSST-1 (0.01 pg per ml)	68.8	202.5	208.0	47.4	0.04
TSST-1 (0.1 pg per ml)	106.3	387.2	242.0	43.6	0.01
IL-4	54.3	166.3	117.0	38.7	—

^aPBMC from patients without AD, but allergic to cats and living with a cat (patients 1 and 2), or pollen allergic patients in pollen season (patients 3 and 4), were stimulated with or without TSST-1 for 14 d in the presence or in the absence of IL-4 (400 U per ml). At the end of the culture, supernatants were harvested and analyzed for the cat-allergen (patients 1 and 2) or pollen-allergen (patients 3 and 4) specific IgE content by the CAP system.

^bP-values comparing IL-4 versus IL-4 + TSST-1.

bodies significantly increased TSST-1-induced IgE production in nonatopic individuals (Lester *et al*, 1995).

TSST-1 increases the production of allergen-specific IgE in allergic patients Previous studies have demonstrated a significant association between allergen-specific IgE and allergic diseases (Sampson, 1989; Gerriten *et al*, 1990; Nelson *et al*, 1996). In order to explore a potential link between staphylococcal toxin exposure and allergen-specific IgE responses, we examined the effect of TSST-1 on allergen-specific IgE production. PBMC from pollen- and cat-allergic patients were stimulated with TSST-1 in the presence of IL-4 (400 U per ml) and analyzed after 14 d of culture for allergen-specific IgE. As shown in **Table I**, TSST-1 enhanced cat-allergen specific IgE production in PBMC from cat allergic patients living with their cats and symptomatic at the time of blood draw. In contrast, in asymptomatic patients without recent exposure to cat allergen, cat-allergen-specific IgE was below 0.35 U per liter (detection limit of the assay) in all culture condition tested. The increase in allergen-specific IgE was only observed for the allergen to which the patient was sensitized. Similar results for specific IgE production to grass and ragweed pollen were found with PBMC from four pollen allergic patients (the two patients, who were symptomatic and whose blood was drawn during pollen season, are shown in **Table I**). Allergen-specific IgE could not be measured after stimulation with TSST-1 of PBMC from nonatopic controls. With these experiments, we show that TSST-1 is able to stimulate

the production of allergen-specific IgE in PBMC from allergic patients.

TSST-1 upregulates B7.2, but not B7.1, at the B cell surface A costimulatory signal, provided by the interaction between the B7 family of molecules (B7.1 or B7.2) expressed on antigen-presenting cells and CD28, a T cell surface molecule, is necessary in order to activate T cells. Based on work done in mice, B7.2 appears to enhance Th2 cell activation (Gerriten *et al*, 1990). Furthermore, we recently showed that B7.2 is upregulated on the B cell surface from patients exposed to allergens (Hofer *et al*, 1998), and that B7.2⁺ B cells produce significantly higher amounts of IgE than B7.2⁻ B cells (Jirapongsananuruk *et al*, 1998). Therefore, we hypothesized that the B7/CD28 pathway may be differentially affected by the enhancing effect of TSST-1 on allergen-specific IgE production. We examined, by flow cytometry, the expression of both B7 molecules on B cells from four normal and five atopic donors after 2 d stimulation with a range of TSST-1 concentrations. TSST-1 had similar effects on normal and atopic B cells. As shown in **Table II**, TSST-1 significantly upregulated B7.2, but not B7.1, on B cells from nonatopic controls (medium alone, 33.3 $\pm$ 8.7%; TSST-1 at 1 pg per ml, 47.9 $\pm$ 3.6%, $p = 0.05$; TSST-1 at 100 ng per ml, 53.5 $\pm$ 10.0%, $p = 0.05$), and in atopics (medium alone, 59.3 $\pm$ 5.5%; TSST-1 at 1 pg per ml, 67.3 $\pm$ 5.6%, $p = 0.05$; TSST-1 at 100 ng per ml, 79.4 $\pm$ 2.3%, $p = 0.006$).

DISCUSSION

This study demonstrates a potential mechanism by which microbial agents could increase IgE production in allergic patients and enhance their sensitization to allergens. First, we showed the importance of *in vivo* allergen exposure for priming total IgE responses to TSST-1 stimulation. *In vivo* allergen priming during the pollen season significantly decreased TSST-1-mediated IFN- γ production and was associated with increased TSST-1-induced IgE production. Of note, in PBMC primed by *in vivo* allergen exposure, TSST-1 increased IgE production even in the absence of exogenous IL-4. Anti-IL-4 neutralizing antibodies did not significantly block TSST-1-induced IgE production. These data suggest that the effect of TSST-1 on IgE synthesis is not only due to the presence of IL-4 and are consistent with the hypothesis of a bridge induced by TSST-1 between Th 2 cells (primed *in vivo*) and B cells leading to enhanced IgE production (**Fig 5**). Our observation suggests that, when T cells are preactivated and shifted toward a Th2-like cytokine profile, infectious agents can contribute to IgE-mediated immune responses.

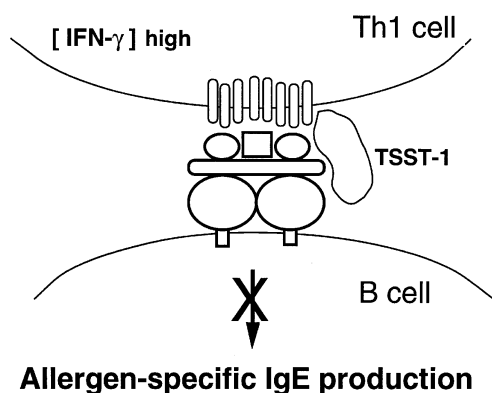
Next, we showed that the increased IgE production after TSST-1 stimulation was associated with enhanced allergen-specific IgE production. It is well established that symptoms following

Table II. B7-2 expression on B cells is upregulated by TSST-1^a

TSST-1 (pg per ml)	%B7.1 ⁺ B cells			B7.2 ⁺ B cells		
	Atopic	Non-atopics	All	Atopic	Non-atopics	All
0	18.0 ± 3.4	23.1 ± 5.1	21.7 ± 3.3	59.3 ± 5.5	33.3 ± 8.7	47.9 ± 6.5
1	14.2 ± 3.2*	21.4 ± 5.1	17.8 ± 3.1*	67.3 ± 5.6*	47.9 ± 3.6*	60.7 ± 5.0*
10 ⁵	14.9 ± 4.5	22.2 ± 5.7	17.0 ± 3.1**	79.4 ± 2.3**	53.5 ± 10.0*	70.9 ± 4.3**

^aPBMC from five atopic patients and four non-atopic controls were stimulated for 48 h with TSST-1. After the culture, cells were harvested, stained with anti-CD19 fluorescein isothiocyanate-conjugated and anti-B7.1 or -B7.2 phycoerythrin-conjugated antibodies, and analyzed by flow cytometry. Results are expressed as mean ± SEM of the nine experiments (medium versus TSST-1: *p < 0.05, **p < 0.005).

A. Outside pollen season



B. During pollen season

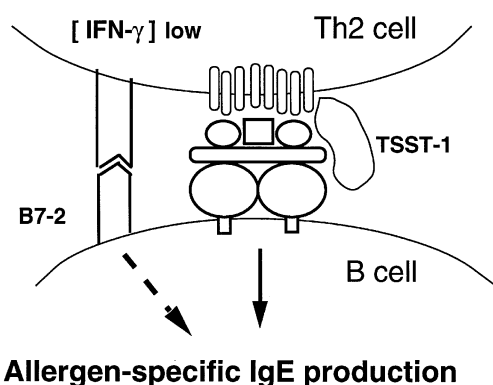


Figure 5. Proposed model of the effects of TSST-1 on allergen-specific IgE responses. After stimulation with TSST-1 outside the pollen season (A), bridging of Th 1 cells with B cells, in the presence of large amounts of IFN- γ will inhibit allergen-specific IgE production. In contrast, after stimulation with TSST-1 during the pollen season (B), very low amounts of IFN- γ are secreted, and bridging TCR on Th 2 cells with major histocompatibility complex class II by TSST-1 induces B cell activation and subsequent allergen-specific IgE production.

allergen exposure are caused by allergen-specific IgE bound to high-affinity receptors for IgE (Fc ϵ R1) on mast cells (Ishisaka, 1976), and which on cross-linking with allergens triggers release of histamine and other inflammatory mediators (Segal *et al*, 1977). This reaction requires very small amounts of allergen-specific IgE produced by a small number of IgE-secreting B cells (MacKenzie and Dosch, 1989). Moreover, IgE-switched specific B cell clones remain activated long after allergen exposure and are committed to the continuous production of allergen-specific IgE. This study suggests that TSST-1 stimulates allergen-specific IgE-secreting B cells, which can prolong and amplify IgE responses. We have previously shown that TSST-1 cross-links T and B cells to induce

polyclonal B cell activation. For this reason, we believe that TSST-1 is not acting like a simple antigen, but stimulates T and B cells specific for other antigens, like pollen- or cat-allergens. This suggests that TSST-1 has an adjuvant effect on IgE synthesis.

Bacterial superantigens are known to bridge the T cell receptor on the T lymphocyte with class II major histocompatibility molecule on the antigen-presenting cell (Mourad *et al*, 1989). As we have shown previously, total IgE production is enhanced by TSST-1 after the superantigen has bridged T and B cells (Hofer *et al*, 1995). In order to be activated after antigen presentation, T cells need, in addition to the signal given through the T cell receptor, a costimulatory signal. The B7 family of molecules expressed on antigen-presenting cells such as B cells, deliver this costimulatory signal after ligation of the CD28 T cell surface molecule. Based on work done in mice, two members of the B7 family, B7.1 and B7.2, appear to influence the type of Th cell activation differently (Kuchroo *et al*, 1995). Glimcher *et al* regulated that antibodies to B7.2 could nullify Th 2 differentiation in a Th 2-dominated mouse (Kuchroo *et al*, 1995).

Recently, we demonstrated that B cells from asthmatic patients, exposed to allergen, showed increased B7.2 expression, and that B7.2 was upregulated on B cells by IL-4 (Hofer *et al*, 1998). The functional significance of B7.2 induction by IL-4 in allergic diseases is supported by recent studies demonstrating its direct involvement in the induction of IgE synthesis (Jeannin *et al*, 1997), and by recent data showing that B7.2⁺ B cells produce more IgE than B7.2⁻ B cells. Furthermore, the role of B7.2 in IgE synthesis was confirmed by the decrease of IgE production in cultures incubated with anti-B7.2 antibodies (Jirapongsananuruk *et al*, 1998). Based on the previous studies cited above, we conclude that TSST-1 enhances the IgE production by a mechanism involving B7.2 upregulation on B cells. In this study, we showed that B7.2, but not B7.1, is upregulated on B cells after TSST-1 stimulation. This increased B7.2 expression on B cells is likely to favor allergen presentation and as a consequence stimulate the synthesis of allergen-specific IgE.

In a previous study, we have shown that more than half of AD patients are colonized with *S. aureus* secreting toxins, such as TSST-1 or staphylococcal enterotoxins. Furthermore, the serum of these patients contained specific IgE against these toxins (Leung, 1995). This study extends these observations to include a role for bacterial toxins in the amplification of allergen-specific IgE production by the following postulated pathway: during exposure to allergens, IFN- γ production is decreased leading to a predominance of Th 2-like cytokines; TSST-1 bridges Th 2 cells and B cells inducing B cell activation; TSST-1 upregulates B7.2 expression on B cells and enhances IgE synthesis; IgE binds to CD23 allowing nonspecific B cells to be potent antigen-presenting cells via the costimulatory molecule B7.2. As a result, Th 2-like cells may expand and induce more B cells to switch to IgE, with a subsequent overproduction of IL-4 and allergen-specific IgE. This hypothesis may explain how, in certain allergic patients, sensitivity only to one allergen evolves to multiallergenicity. Thus in AD, TSST-1 may contribute to sensitization to inhalant allergens, which promotes the subsequent development of respiratory symptoms, as it is frequently seen in the evolution of these patients. Our observation indicates a role for *S. aureus* colonization in allergic sensitization of AD patients.

In summary, this study demonstrates that TSST-1 is able to augment IgE response against allergens. This adjuvant effect of TSST-1 on IgE production is greatest when patients are undergoing *in vivo* exposure to allergens, suggesting that exposure to superantigens during the allergen season cross-links IL-4-producing Th 2 cells to B cells, which lead to enhanced IgE synthesis. These data will provide new insights into potential mechanisms by which infectious agents alter the course of allergic diseases.

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