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Differential effects of staphylococcal toxic shock syndrome toxin-1 on B cell apoptosis

(immunoglobulins/superantigen/Fas/IFN-γ)

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ABSTRACT **Superantigens, such as toxic shock syndrome toxin 1 (TSST-1), have been implicated in the pathogenesis of several autoimmune and allergic diseases associated with polyclonal B cell activation. In this report, we studied the *in vitro* effects of TSST-1 on B cell activation. We show herein that TSST-1 produced antagonistic effects on Ig synthesis by peripheral blood mononuclear cells (PBMC) from normal subjects, depending on the concentration used; Ig production was inhibited at 1000 pg/ml ($P < 0.01$) and enhanced at 1 and 0.01 pg/ml ($P < 0.01$) of toxin. Cultures of PBMC were then examined for morphologic features and DNA fragmentation characteristic for apoptosis. B cells exhibited a significantly higher ($P < 0.01$) incidence of apoptosis after stimulation with 1000 pg/ml of TSST-1 compared with 1 or 0.01 pg/ml of toxin or medium alone. Abundant expression of Fas, a cell surface protein that mediates apoptosis, was detected on B cells after stimulation with 1000 pg/ml of TSST-1 and was significantly higher on B cells undergoing apoptosis than on live cells ($P = 0.01$). Additionally, increased Fas expression and B cell death occurred at concentrations of TSST-1 inducing the production of high amounts of γ interferon (IFN-γ), and both events could be blocked by neutralizing anti-IFN-γ antibody. These findings suggest that high concentrations of TSST-1 can induce IFN-γ-dependent B cell apoptosis, whereas at low concentrations it stimulates Ig synthesis by PBMC from normal subjects. These findings support the concept that staphylococcal toxins have a role in B cell hyperactivity in autoimmunity and allergy.**

Staphylococcus aureus produces a large family of exotoxins (1), which have been implicated in the pathogenesis of several allergic and autoimmune diseases (2, 3). Staphylococcal toxic shock syndrome toxin 1 (TSST-1) is a prototypic superantigen, which causes marked stimulation of monocytes and T cells, triggering the production of high levels of cytokines such as γ interferon (IFN-γ) (3, 4). TSST-1 has been shown to bind monomorphic determinants on major histocompatibility complex (MHC) class II molecules in an unrestricted manner (5, 6). The MHC class II plus TSST-1 complex interacts primarily with the Vβ chain of the T cell receptor to induce the activation and proliferation of a large number of T cells (3, 4). However, MHC class II molecules are important not only for T cell activation, but also for B cell activation and proliferation (7). Cross-linking MHC class II molecules can induce an increase in cAMP levels, tyrosine kinase activation, and Ca^{2+} mobilization (7–9). TSST-1 has also been found to induce cAMP accumulation in B cells (5). In mice, ligation of MHC class II molecules on resting B cells with anti-class II monoclonal antibody (Ab) lead to apoptosis, via elevated levels of cAMP (10). Also when the interaction occurs with antigen-specific MHC-restricted T cells, the process results in apoptotic death of the B cells (K.N. and J.H.F., unpublished observation).

In human peripheral blood mononuclear cell (PBMC) cultures, staphylococcal toxins have been reported to inhibit B cell function by decreasing Ig production (11, 12). 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 a small number of T cells (4, 5, 11, 12). These observations suggest that a massive activation of T cells may prevent polyclonal B cell activation. Indeed, in staphylococcal toxic shock syndrome (TSS), inhibition of Ig synthesis is observed, whereas in other diseases, such as Kawasaki disease or atopic dermatitis likely associated with lower levels of TSST-1 production, B cell activation occurs (13–15). To determine the mechanism for the apparent antagonistic effects of TSST-1 on B cell activation observed *in vivo*, the current study tested the *in vitro* effects of a wide range of TSST-1 concentrations on B cell activation. Concurrently, the potential mechanism of inhibition of immunoglobulin production induced by TSST-1 was also examined.

MATERIALS AND METHODS

Reagents. Purified TSST-1 was prepared by P.M.S. as described previously (16). The toxin was homogeneous when 20 μg was tested by sodium dodecyl sulfate polyacrylamide gel electrophoresis. A neutralizing rabbit polyclonal Ab to human IFN-γ was provided by Genentech. Anti-Fas Ab (IgG1) was purchased from Panvera (Madison, WI), and IgG isotype controls were from Southern Biotechnology Associates.

Cell Isolation and Culture. Informed consent was obtained from all subjects before blood was drawn for these studies. PBMC obtained from normal donors were isolated by density gradient centrifugation on Ficoll-Paque (Pharmacia). PBMC were then washed 3 times in Hanks' balanced salt solution (GIBCO) and resuspended in RPMI 1640 (BioWhitaker) supplemented with 10% heat-inactivated fetal calf serum (HyClone), 20 mM Hepes buffer, antibiotics, and 2 mM L-glutamine (GIBCO). For Ig production, PBMC were cultured at the concentration of 1×10^6 cells/ml in round-bottomed 96-well plates (Costar) at 37°C in the presence of

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Abbreviations: TSST-1, staphylococcal toxic shock syndrome toxin-1; Ab, antibody; FITC, fluorescein isothiocyanate; IFN-γ, γ interferon; MHC, major histocompatibility complex; PBMC, peripheral blood mononuclear cells; TdT, terminal deoxynucleotidyl transferase; Th, T helper cell; TSS, toxic shock syndrome.

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TSST-1 (0.001–1000 pg/ml). Supernatants were collected after 14 days and stored at −20°C until assayed. For flow cytometry analysis, PBMC were cultured at the concentration of 2×10^6 cells/ml in 24-well plates (Costar) at 37°C, in a humidified 5% CO_2 atmosphere. Cells were cultured in the presence of TSST-1 (0.001–1000 pg/ml), anti-CD3 (20 ng/ml), anti-IFN-γ (5 μg/ml) blocking Ab, and/or IgG isotype controls for various time periods (1–6 days) as indicated in each experiment. At the end of the culture, cells were washed and resuspended in staining buffer for immunofluorescence staining (see below).

Enzyme-Linked Immunosorbent Assay for Ig Determination. The IgG assay was carried out as described previously (17). Ninety-six-well microtiter plates (Dynatech) were coated with 0.1 ml of a 1:1 mixture of purified goat anti-human IgG (Tago) diluted in 0.1 M $NaHCO_3$ at pH 9.6 at a concentration of 10 μg/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 IgG standard controls (Sigma). The plates were then washed, a 1:100 dilution of affinity-purified biotinylated goat antihuman IgG (Vector Laboratories) 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) for 90 min at 37°C. The wells were then developed with 2 mM *p*-nitrophenyl phosphate substrate (Sigma), and the optical density was read at 405 nm on an Emax microplate reader (Molecular Devices). The concentrations of IgG in the supernatants were read from an IgG standard curve. The lower limit of sensitivity of this assay was 1 ng/ml.

The protocol for IgA and IgM assay (17) were identical to that for IgG, except that the initial capture Ab was an affinity-purified polyclonal goat anti-human IgA or IgM Ab (Tago), and the second Ab was a biotinylated goat antihuman IgA or IgM (Vector). The IgA and IgM standards were obtained from Tago.

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 μCi of [^{3}H]thymidine (Amersham) was added per well, and cells were incubated for 6 additional hours. Cells were harvested onto filter paper disks, and radioactivity was counted by liquid scintillation (Beckman). All samples were run in triplicate.

Cell Staining and Flow Cytometric Analysis. Five-parameter analysis was performed using a FACScan flow cytometer (Becton Dickinson) with fluorescein isothiocyanate (FITC), phycoerythrin, and peridinin chlorophyll protein used as the three fluorescent parameters. PBMC were washed twice in PBS containing 1% human Ig (Armour Pharmaceutical) and 0.02% NaN_3 and were stained for 30 min at 4°C with anti-CD19 FITC, anti-CD3 peridinin chlorophyll protein (Becton Dickinson), and/or anti-Fas phycoerythrin-conjugated monoclonal Ab (Medical and Biological Laboratories). Cells were washed 3 times in PBS and fixed with 1% formaldehyde. PBMC were analyzed for apoptosis by three methods, as described previously. First, by staining with 5 μl of ethidium bromide (10 μg/ml) just before running the sample on the FACScan (10, 18); second, by forward (a measure of cell size) versus side scatter (a measure of cell granularity) analysis on the FACScan, where two cell populations could be distinguished, one smaller and more granular (apoptotic) and one larger and less granular (nonapoptotic) (19); and third, by the terminal deoxynucleotidyl transferase (TdT) method (20, 21). Briefly, after immunostaining as above with anti-CD19 phycoerythrin-conjugated (Becton Dickinson) cells were fixed during 1 h with 1.5% paraformaldehyde at 4°C and then incubated during 1 h at 37°C with reaction mixture [per sample analyzed: 5 units of TdT enzyme, 0.15 nM FITC dUTP, 20 μM dNTPs, 10 μl 5 × TdT buffer (Boehringer Mannheim), and 50 μl dH_2O]. Cells were washed twice in PBS supplemented with 20 mM EDTA (Sigma). Samples were stored at 4°C in the dark until analyzed.

Methods of cytometer set up and data acquisition were as described previously (22). List mode multiparameter data files (each file with forward scatter, side scatter, and three fluorescent parameters) were analyzed by use of the LYSIS II WINDOWS program (Becton Dickinson). Negative control reagents were used to verify the staining specificity of experimental antibodies.

Statistical Analysis. Data are expressed as the mean ± SE. Due to the exponential range of the data, log transformation was performed before the data were analyzed by paired Student's *t* test on STATVIEW II program.

RESULTS

Dose-Dependent Effect on Ig Production by PBMC After TSST-1 Stimulation. TSST-1 showed a dose-dependent effect on Ig production by PBMC from normal subjects. As shown in Fig. 1*A*, at low TSST-1 concentrations, IgG synthesis, as well as IgA and IgM production, was significantly increased compared with medium alone (1 pg/ml, $P < 0.005$; 0.01 pg/ml, $P < 0.01$). In contrast, when PBMC were stimulated with 1000 pg/ml of TSST-1, the production of all three classes of Ig was inhibited (IgG and IgM, $P < 0.01$; IgA, $P < 0.005$). This inhibition of Ig production was observed at concentrations inducing the highest proliferation as shown by [^{3}H]thymidine uptake (Fig. 1*B*). Thus, concentrations of TSST-1 inducing a higher level of PBMC activation were associated with inhibition of the function of B cells.

Decrease of B Cells in Cultures Stimulated by TSST-1. Since TSST-1 activates and induces proliferation of T cells, we examined the relative number of T and B cells at different concentrations of this superantigen. As shown in Fig. 2*A*, TSST-1 had a dose-dependent effect on the percentage of B cells in PBMC after 6 days of culture. Between 0.001 and 1 pg/ml, TSST-1 did not affect the percentage of B cells, which was similar to the percentage for medium alone. After stimulation with 100 and 1000 pg/ml of TSST-1, the percentage of B cells dropped to 0.3 and 0.2%, respectively. When PBMC were stimulated with anti-CD3 Ab, which induced a higher proliferation index than 1000 pg/ml of TSST-1, the percentage of B cells decreased slightly in the four subjects, but this was a consequence of the increase in absolute T cell number rather than a decrease in absolute B cell number, as it is for high concentrations of TSST-1. Indeed, absolute B cell counts after 6 days of culture with anti-CD3 (83,500/well) and TSST-1 at 1 pg/ml (84,000/well) were similar, but they decreased with 1000 pg/ml of TSST-1 (6800/well).

As shown on Fig. 2*B*, a significant decrease in B cells could be seen even after 24 h of culture following stimulation with 100 ng/ml of TSST-1 and 72 h with 1000 pg/ml. Thus, this almost complete disappearance of B cells in PBMC culture was the consequence of B cell death, which began within 2 or 3 days of stimulation with TSST-1.

TSST-1 Induced B Cell Death by Apoptosis in PBMC. To determine whether B cells are dying by apoptosis after TSST-1 stimulation, we used three different methods. First, we measured ethidium bromide uptake by $CD19^+$ cells, even after 48 h of culture. The number of apoptotic B cells in four experiments was significantly higher with 100 ng/ml (68.0 ± 5.8%) and 1000 pg/ml (62.5 ± 6.6%) TSST-1 than with medium alone (24.1 ± 2.6%: $p < 0.01$). In contrast, cultures stimulated with TSST-1 at 1 pg/ml (22.9 ± 3.8%) showed a similar percentage of apoptosis to medium alone (Fig. 3*A*). The two-dimensional representation of a typical experiment (Fig. 3 *B–D*) shows that a majority of B cells took up ethidium bromide after stimulation with 1000 pg/ml of TSST-1 (76.0%),

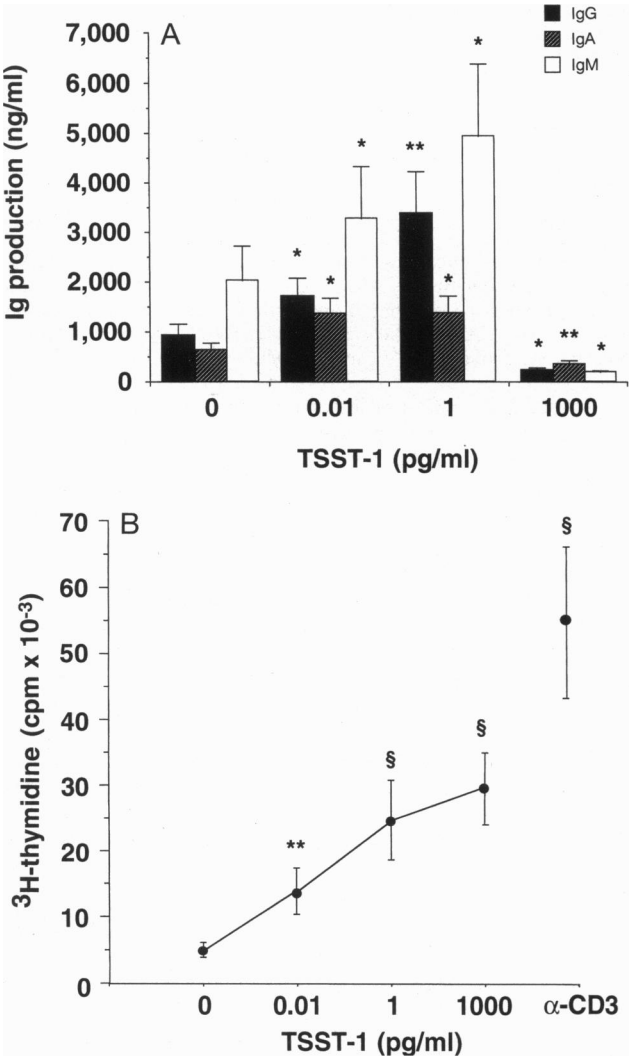

FIG. 1. Concentration-dependent modulation of Ig production by TSST-1. PBMC from eight normal subjects were incubated with a range of TSST-1 concentrations. (*A*) IgG (black bars), IgA (hatched bars), and IgM (white bars) were measured by enzyme-linked immunosorbent assay, and (*B*) proliferation was assessed by [^{3}H]thymidine uptake (medium alone (0) *versus* TSST-1: *, $P < 0.01$; **, $P < 0.005$; §, $P = 0.0005$). Anti-CD3 Ab (20 ng/ml) were used as positive control for T cell proliferation.

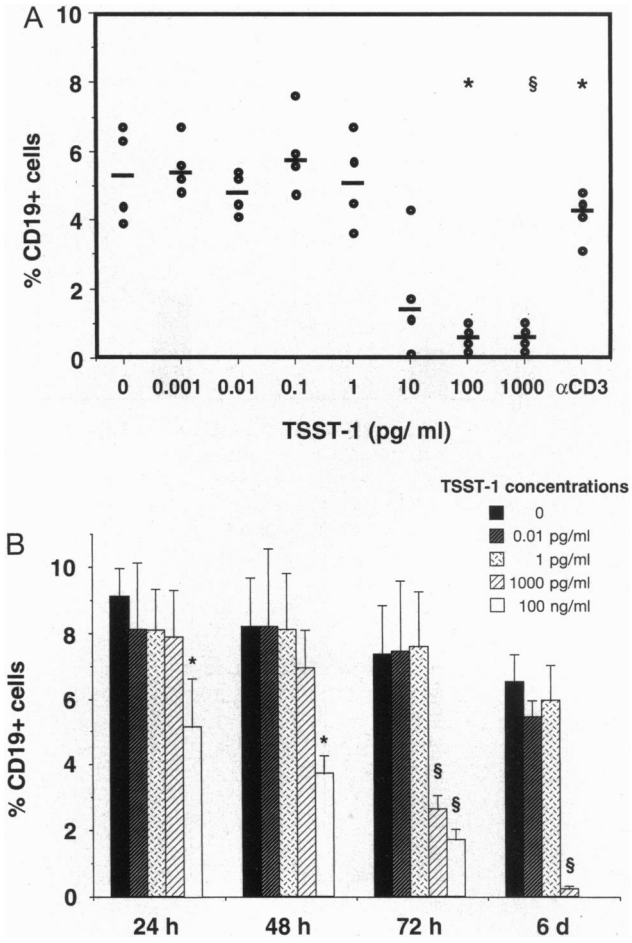

FIG. 2. Effect of TSST-1 on B cells in cultured PBMC. PBMC from four normal subjects were incubated with a range of TSST-1 concentrations or anti-CD3 (20 ng/ml). The percentage of B cells (CD19$^+$ cells) in PBMC culture was measured by immunostaining and FACScan analysis after 6 days (*A*), after 1, 2, 3, or 6 days (*B*) (medium (0) versus TSST-1 or anti-CD3: *, $P < 0.05$, §, $P < 0.005$).

whereas with 1 pg/ml, only fewer B cells (21.9%) were stained by ethidium bromide. Second, we also determined the percentage of apoptosis in PBMC by forward versus side scatter on the FACScan flow cytometer. For B cells, the rate of apoptosis was significantly increased after stimulation with 1000 pg/ml (31.2 ± 6.2%) of TSST-1 as compared with TSST-1 at 1 pg/ml (12.4 ± 3.6%, $P = 0.0005$) or medium alone (17.8 ± 6.1%, $p = 0.01$). These lower values of apoptosis seen by forward versus side scatter compared with the ethidium bromide assay are due to the fact that, in earlier stages of apoptosis, B cells do not show changes in size but are nonetheless able to incorporate ethidium bromide. However for T cells, by forward versus side scatter, the amount of apoptosis was not significantly different between stimulation with TSST-1 (1000 pg/ml, 5.0 ± 0.9%; 1 pg/ml, 4.0 ± 1.0%) and medium alone (4.4 ± 1.1%). Furthermore, after 6 days of culture, T cell apoptosis was similar for medium alone (33.3 ± 7.8%) and after 1000 pg/ml of TSST-1 (34.7 ± 7.3%) and decreased after stimulation with 1 pg/ml of TSST-1 (24.2 ± 6.3%, $P = 0.05$). The only time point where T cells showed a slight increase in percentage apoptosis was after 3 days of culture.

Finally, to confirm that B cell death was apoptotic in nature, we used the TdT method, in which DNA strand breaks are labeled with dUTP FITC-conjugated, and analyzed by flow cytometry. As shown on Table 1, the percentage of B cells positive for uptake of dUTP FITC-conjugated, as well as the mean fluorescence intensity of the green color after gating on B cells, were significantly increased after stimulation with TSST-1 at 1000 pg/ml compared with medium alone ($P < 0.01$) or 1 pg/ml of TSST-1 ($P = 0.02$).

Expression of the Fas Antigen Was Enhanced by TSST-1 Stimulation. Since the Fas antigen is known to be involved in apoptosis (23, 24), we determined its expression on B cells. In five different experiments, Fas antigen expression on B cells was increased after stimulation with 100 ng/ml (58.3 ± 5.2%) and 1000 pg/ml (68.7 ± 3.5%) TSST-1 compared with medium alone (10.7 ± 1.5%, $P < 0.001$), 1 pg/ml (28.1 ± 4.7%, $P < 0.005$), or 0.01 pg/ml (11.3 ± 3.0%, $P < 0.001$) TSST-1. Fig. 4 is an example of a histogram comparing expression of Fas antigen on B cells in medium alone (panel *A*), after 0.01 pg/ml (panel *B*), and 1000 pg/ml (panel *C*) of TSST-1. Panel *B* is similar to panel *A* with a dim and a bright population of Fas-positive B cells. In contrast, stimulation with 1000 pg/ml of TSST-1 primarily induced bright expression of Fas on B cells. Fas expression was significantly higher ($P = 0.01$) on apoptotic than on live B cells, as determined by forward versus

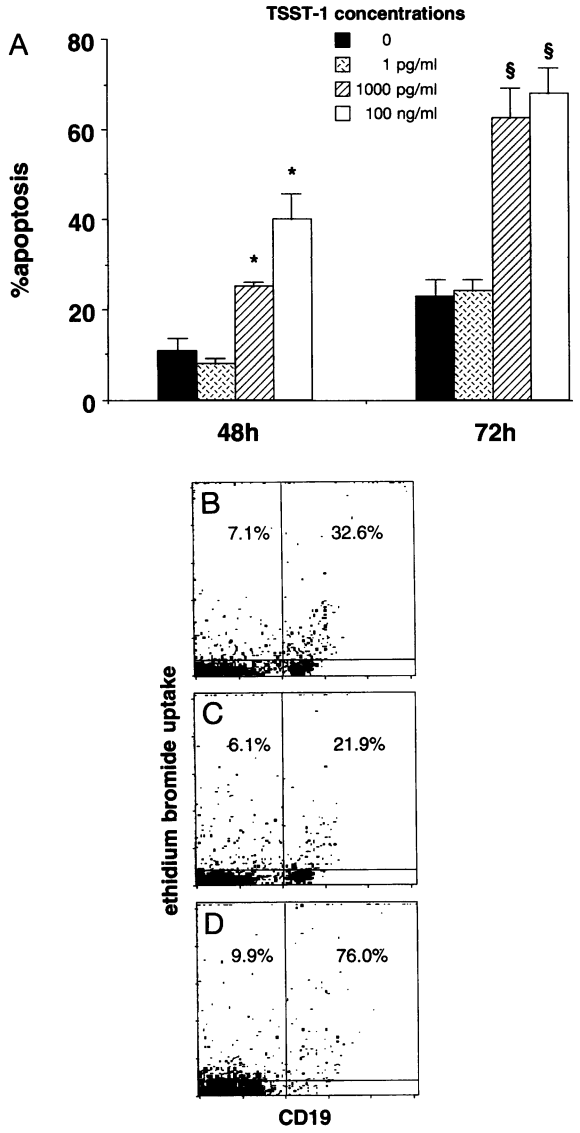

FIG. 3. TSST-1 at high concentrations induced B cell apoptosis. (*A*) PBMC from four normal subjects were stimulated with a range of TSST-1 concentrations, and, after 48 or 72 h, were stained with anti-CD19 FITC-conjugated Ab and ethidium bromide, as described in *Materials and Methods*, and analyzed on the FACScan (medium versus TSST-1: *, $P < 0.05$, §, $P < 0.01$). Two-dimensional representation of ethidium uptake by $CD19^+$ cells after treatment with medium alone (*B*), 1000 pg/ml (*C*), and 1 pg/ml (*D*) of TSST-1 for 72 h. Percentage of apoptosis (upper/upper+lower) in $CD19^-$ (left) and $CD19^+$ (right) cells is indicated for each panel.

side scatter (data not shown). These observations suggest that TSST-1-induced B cell apoptosis was Fas-mediated. To further assess the potential role of Fas in TSST-1-mediated B cell apoptosis, we pretreated PBMC with anti-Fas Ab (10 μg/ml) before culturing them with TSST-1. At the end of the culture, we measured apoptosis by forward versus side scatter and by ethidium bromide uptake. With both methods, we could not see any blocking of TSST-1-induced B cell apoptosis. In contrast, pretreatment with anti-Fas antibodies was followed by an increase in B cell and T cell apoptosis, when compared with cells pretreated with mouse IgG (data not shown). Therefore, we could not directly assess the role of Fas in TSST-1-mediated apoptosis.

Anti-IFN-γ Blocking Ab Rescued B Cells from Death Induced by TSST-1. In a previous study, we demonstrated that TSST-1 at high concentrations induced a much higher production of IFN-γ than at lower concentrations (25). In order to determine the role of this cytokine in B cell apoptosis mediated by TSST-1, we cultured PBMC stimulated with TSST-1 at 1000 pg/ml in the presence of a neutralizing Ab to IFN-γ. Compared with the IgG isotype control, anti-IFN-γ Ab rescued B cells from apoptosis as shown by the higher percentage of B cells after 3 days of culture, and the lower percentage of apoptotic B cells was measured by forward versus side scatter (Table 2). In cultures treated with anti-IFN-γ Ab, B cells had lower Fas expression than with IgG isotype control. Furthermore, when PBMC were stimulated with 1 pg/ml of TSST-1 in the presence of exogenous IFN-γ (10 ng/ml), the percentage of apoptosis in B cells increased from 36.7% (without IFN-γ) to 55.1%, approaching the percentage of apoptosis observed after stimulation with 1000 pg/ml of TSST-1 (58.6%). However, the addition of exogenous IFN-γ to purified B cells cultured with 1 pg/ml of TSST-1 did not increase B cell apoptosis (data not shown). This indicates that B cells need to be activated by TSST-1 in the presence of at least one other population of cells in order to undergo

Table 1. TSST-1-induced B cell apoptosis analyzed by TdT method

	Experiment 1		Experiment 2	
TSST-1 (pg/ml)	%	MFI	%	MFI
0	10.9	200.3	14.0	89.8
0.01	10.9	233.1	13.8	100.3
1	29.7	482.5	30.3	202.7
1000	54.8	755.8	68.1	468.9

PBMC were stimulated with either 1000, 1, or 0.01 pg/ml of TSST-1, and then stained with FITC-dUTP and PE anti-human CD19. Results are expressed as percentage of apoptotic B cells or as mean fluorescence intensity (MFI) of the green color in B cells. Two representative experiments, from a total of five, are shown here.

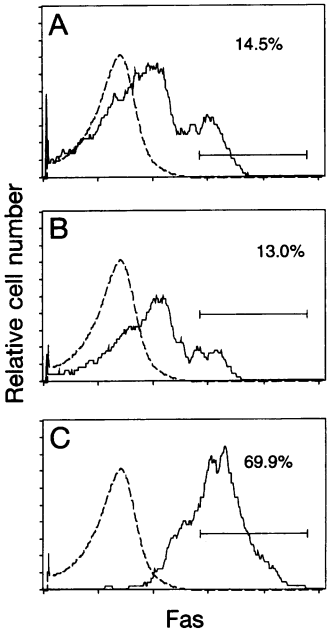

FIG. 4. Expression of the Fas antigen on B cells was increased after stimulation with TSST-1 at 1000 pg/ml compared with 0.01 pg/ml. PBMC were stimulated with a range of TSST-1 concentrations, and, after 48 h, were stained with anti-CD19 FITC and anti-Fas phycoerythrin-conjugated Ab and analyzed on the FACScan. Histogram plots show isotype control (dashed line) and Fas antigen expression on gated B cells (solid line) in medium alone (*A*) and after stimulation with TSST-1 at 0.01 pg/ml (*B*) or 1000 pg/ml (*C*). The percentage of Fas antigen expressing B cells is indicated in each panel. Similar results were found in five different experiments.

Table 2. TSST-1-induced B cell death by apoptosis was rescued by anti-IFN-γ Ab

Treatment	Experiment #1		Experiment #2	
	% of B cells (% apoptosis)	Fas on B cells % (MFI)	% of B cells (% apoptosis)	Fas on B cells % (MFI)
Rabbit IgG + TSST-1 (1000 pg/ml)	2.2 (44.2)	60.7 (425.0)	2.7 (28.6)	77.8 (539.4)
Anti-IFN-γ + TSST-1 (1000 pg/ml)	4.5 (32.1)	32.3 (114.2)	5.0 (13.3)	60.6 (342.6)
TSST-1 (1 pg/ml)	6.1 (20.1)	24.4 (77.4)	6.1 (11.5)	50.1 (459.1)

PBMC were stimulated with either 1000 or 1 pg/ml of TSST-1, in the presence or the absence of rabbit anti-human IFN-γ or rabbit IgG. After 3 days, cells were stained with anti-CD19 FITC-conjugated and anti-Fas PE-conjugated, and then analyzed on FACScan. Percentage of apoptosis was determined by forward versus side scatter after gating on the B cell population. Background staining with isotype matched FITC-Ab was slightly higher in apoptotic than in live cells. To calculate the percentage of apoptosis among $CD19^+$ cells, we deducted the number of events observed for the isotype control (<0.1% in live cells, <0.2% in apoptotic cells) from the number of $CD19^+$ cells in both cell populations. Fas expression is shown as the percentage of B cells expressing high intensity of Fas and MFI of Fas after gating on the B cell population.

apoptosis mediated by IFN-γ. Taken together, these results indicate that IFN-γ is necessary but not sufficient to induce B cell apoptosis after stimulation with high concentrations of TSST-1.

DISCUSSION

Our present study demonstrates for the first time that a microbial superantigen can have a concentration-dependent effect on T cell-dependent B cell activation. We found that Ig production by PBMC was dependent on the concentration of superantigen used. At low concentrations of TSST-1, B cells were activated and produced Ig. In contrast, at high concentrations of TSST-1, the B cells did not secrete Ig and actually died by apoptosis, while T cells were stimulated to make IFN-γ. This is consistent with recent data showing that increasing concentrations of superantigens are associated with increased inhibition of B cell responses (26). By several methods, including the TdT technique, we demonstrate that B cell apoptosis is the mechanism for the decrease in Ig production after stimulation at high superantigen concentrations. TSST-1-induced apoptosis was mainly observed in B cells, and the percentage of T cell apoptosis changed only slightly in response to TSST-1 stimulation. Taken together, these data suggest that, at high concentrations of TSST-1, T cells were stimulated to secrete IFN-γ, and interaction with B cells, either directly or indirectly, resulted in B cell death.

Previous reports have shown that activation of the B cell by TSST-1 is T cell-dependent and that physical interaction between T and B cells is required (4, 12). To investigate if TSST-1 induced apoptosis in B cells by a direct effect, we cultured purified tonsil B cells in the presence of a wide range of TSST-1 concentrations. Ig production and the rate of apoptosis were similar at all concentrations tested as compared with medium alone (M.F.H. and D.Y.M.L., unpublished data). Thus, we postulate that T cells constitute a major cellular component involved in superantigen-induced inhibition as well as activation of B cells. Our data suggest that the differential outcome of B cell activation is related to the type and/or the amount of T cell proliferation. Indeed, high concentrations of TSST-1 induced high T cell proliferation rate and B cell apoptosis, whereas after stimulation with low concentrations of TSST-1, T cells proliferated much less and B cell activation was observed.

In a previous study, we have found that TSST-1 induces the production of IFN-γ in a dose-dependent manner (25). Thus, we considered the possibility that increased production of IFN-γ might play a role in the enhanced B cell death after stimulation with high concentrations of TSST-1. In the current study, we were able to partially block B cell death using neutralizing antibodies to IFN-γ. Conversely, exogenous IFN-γ increased the percentage of B cell apoptosis, indicating that this cytokine plays an important role in TSST-1-induced B cell apoptosis. However, the addition of exogenous IFN-γ to TSST-1-stimulated purified B cells did not significantly increase the percentage of apoptosis, indicating that IFN-γ does not act directly on the B cell, but rather possibly acts through an accessory cell.

Vigorous T cell proliferation is required for the induction of the T helper (Th) 1 phenotype, including production of IFN-γ (27). In contrast, the presence of a minimal $CD4^+$ proliferation is needed for the activation of Th2 lymphocytes, which secrete cytokines enhancing B cell responses (27). Th1, but not Th2 cells, express Fas-ligand (28) and are known to induce cell death by triggering a Fas-dependent pathway in potential targets (28, 29). In this study, we showed that TSST-1 at 1000 pg/ml induced a much higher expression of the Fas antigen on B cells than at 1 pg/ml or with medium alone and that the expression of Fas was significantly higher on apoptotic than on live cells. Furthermore, exogenous IFN-γ increased Fas expression on B cells, and Ab to IFN-γ blocked the increase in Fas expression on B cells induced by TSST-1, suggesting that IFN-γ induced B cell death by a Fas-dependent mechanism. The increase of Fas expression on B cells undergoing apoptosis and the inability of TSST-1 to induce B cell apoptosis in the absence of T cells suggest the possibility that TSST-1-induced B cell apoptosis may be Fas/Fas-ligand-mediated. Based on our observations, we propose the model in Fig. 5 for the dose-dependent TSST-1 effect on B cells. At high concentrations, TSST-1 induces an increased proliferation of T cells with the Th1 phenotype, production of large amounts of IFN-γ, and increased Fas expression on B cells. Signals delivered through T cell receptor/MHC class II molecule and/or Fas/Fas-ligand interactions then induce B cell apoptosis. Indeed, previous data (10) suggest that, depending on the activation state of the B cell, a signal delivered through the MHC class II molecule may contribute to the apoptotic process. In contrast, low concentrations of TSST-1 induce minimal IFN-γ production and low expression of Fas on B cells. Under these conditions, T–B cell interaction mediated by TSST-1 induces B cell activation and Ig production.

Our data, which demonstrate dose-dependent effects of TSST-1 on B cell activation, are consistent with reports of differential Ab responsiveness to TSST-1 in TSS patients compared with the general population. In TSS, where patients are exposed to large concentrations of toxin sufficient to cause serious illness and consequently massive T cell proliferation with high level IFN-γ production, greater than 85% of patients fail to develop an Ab response to TSST-1 and other staphylococcal products upon recovery from the illness (13, 30). In contrast, in the general population, by age 21, 75% of individuals have neutralizing antibodies against TSST-1 (31). This indicates that low level exposure to TSST-1, in amounts insufficient to cause TSS, allows the production of a specific immune response, just as for conventional antigens. Likewise, increased Ig synthesis in autoimmune and allergic diseases is probably related to stimulation of the humoral immune system by low concentrations of exotoxins, as it has been suggested for

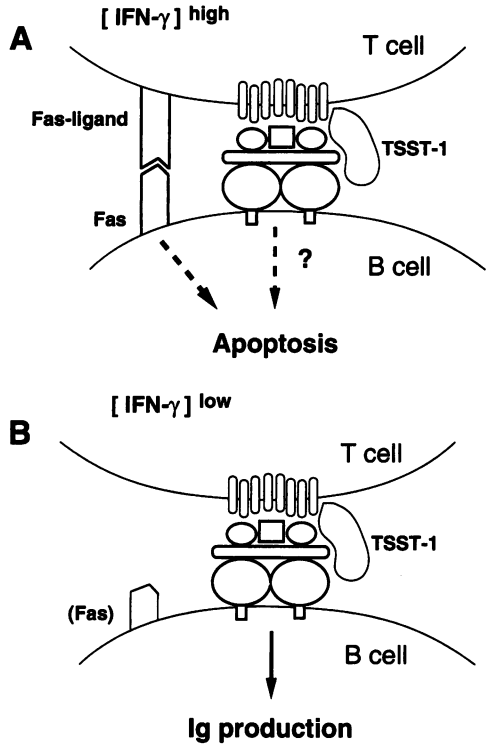

FIG. 5. Our model of the differential effects of TSST-1 on B cell responses. After stimulation with high concentrations of TSST-1 (*A*), large amounts of IFN-γ are produced, and Fas expression on B cells is increased. Signals delivered through T cell receptor/MHC class II molecule and/or Fas/Fas-ligand interactions then induces B cell apoptosis. In contrast, after stimulation with low concentrations of TSST-1 (*B*), very low amounts of IFN-γ are secreted, and Fas expression is low. Bridging T cell receptor and MHC class II by TSST-1 induces B cell activation and subsequent Ig production.

Kawasaki syndrome, a vasculitis associated with polyclonal B cell activation (14). Our data showing stimulation of B cells by TSST-1 reinforces the importance of superantigens in the pathogenesis of diseases associated with abnormal Ig synthesis.

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