

THE STAPHYLOCOCCAL TOXIC SHOCK SYNDROME
TOXIN 1 TRIGGERS B CELL PROLIFERATION AND
DIFFERENTIATION VIA MAJOR HISTOCOMPATIBILITY
COMPLEX-UNRESTRICTED COGNATE
T/B CELL INTERACTION

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Toxic shock syndrome toxin 1 (TSST-1)¹ is a 22-kD exotoxin produced by most strains of *Staphylococcus aureus* isolated from patients with toxic shock syndrome (TSS) (1). TSST-1 is a potent activator of monocytes and T cells. It acts on monocytes to induce the synthesis of IL-1 and TNF (2-4). TSST-1 also triggers T cell activation and proliferation (5), as well as the production of large amounts of various lymphokines such as IL-2 (6) and IFN- γ (7). The massive induction of monokine and lymphokine production by TSST-1 is thought to play an important role in the pathogenesis of TSS.

A significant insight into the mechanism of action of TSST-1, as well as other related staphylococcal exotoxins, came with the observation that these toxins bind directly to MHC class II molecules (8-11). Among the MHC class II molecules, TSST-1 binds equally well with high affinity and saturation kinetics to HLA-DR and -DQ antigens (8). TSST-1 also binds to HLA-DP alleles, albeit with a much lower affinity than is observed for HLA-DR and -DQ alleles (Scholl, P., unpublished data). The MHC class II-bound toxin behaves as a superantigen that interacts with T cells via the TCR β chain (12, 13) to induce MHC-unrestricted T cell activation and proliferation.

The ability of MHC class II-bound TSST-1 to engage T cells raises the possibility that TSST-1 may mimic nominal antigen in initiating cognate interaction between T and B cells resulting in B cell proliferation and Ig production. We demonstrate here that TSST-1 induces both the proliferation of resting human B cells and their differentiation into Ig-secreting lymphocytes. Triggering of B cell proliferation and differentiation by TSST-1 is dependent on the presence of T cells, and proceeds via

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¹ *Abbreviations used in this paper:* AET, 2-aminoethylisothiuronium bromide; TSS, toxic shock syndrome; TSST-1, toxic shock syndrome toxin 1.

MHC-unrestricted cognate T/B cell interaction involving the TCR/CD3 complex and the MHC class II antigen/toxin complex.

Materials and Methods

Reagents and Antibodies. TSST-1 was obtained from Toxin Technology (Madison, WI). PHA-P, PMA, 2-aminoethylisothiuronium bromide (AET), and ionomycin were purchased from Sigma Chemical Co. (St. Louis, MO). Ficoll-Hypaque and Percoll were obtained from Pharmacia Fine Chemicals (Piscataway, NJ). RPMI 1640, AB⁺ serum, L-glutamine, penicillin, and streptomycin were obtained from Whittaker M.A. Bioproducts (Walkersville, MD). FCS was obtained from HyClone Laboratories (Logan, UT). mAb SPVL3, directed against a monomorphic determinant on human HLA-DQ alleles, was kindly provided by Dr. Hergen Spits (DNAX Research Institute, Palo Alto, CA). mAb ST259, directed against a monomorphic determinant on human HLA-DR and -DQ molecules (14), was kindly provided by Dr. E. Yunis (Dana Farber Cancer Institute, Boston, MA). mAbs L243, directed against monomorphic determinants on human HLA-DR molecules, OKT3 (anti-CD3), OKT4 (anti-CD4), OKT8 (anti-CD8), and OKM1 (anti-CD11b/CD18) were all generated as ascites from their respective hybridomas (American Type Culture Collection, Bethesda, MD). mAb 10F12 directed against CD18, the common β chain of the Leu-CAM family of adhesion molecules (CD11a, -b, -c) was a kind gift of Dr. S. J. Burakoff (Dana Farber Cancer Institute). The anti-CD20 mAb Leu-16 and goat anti-mouse Ig-FITC were obtained from Becton Dickinson & Co. (Sunnyvale, CA). [³H]Thymidine and Econofluor were from New England Nuclear (Boston, MA).

Cell Preparations. Human tonsils were obtained from children undergoing tonsillectomy. Tonsillar tissues were cut into small fragments with scissors and dispersed into single cell suspensions. Viable cells were separated from dead cells and debris by filtering through glass wool and by centrifugation over Ficoll-Hypaque. T cell-enriched populations were obtained by incubating the unfractionated cells with AET-treated SRBC. Rosette-forming cells were isolated by Ficoll-Hypaque centrifugation and the red cells were lysed with deionized water. They were washed three times with HBSS (Microbiological Associates, Bethesda, MD), resuspended in RPMI 1640 containing 10% AB⁺ serum, and incubated on plastic Petri dishes for 1 h at 37°C to remove adherent cells. The resulting T cell populations contained >95% CD3⁺ cells. To purify B cells, nonrosetting cells were submitted to a second rosetting procedure. The resultant B cells were further depleted of residual T cells and monocytes by treatment with OKT3 and OKM1, respectively, followed by rabbit complement (Pel-Freeze Biologicals, Rogers, AR). High density B cells were then obtained by centrifugation over a discontinuous percoll gradient (30, 45, 50, 60, and 90%) and by collecting the cells at the interface between 50–60% layers. The resulting B cell populations contained >98% CD20⁺ cells and no detectable CD3⁺ or CD11b/CD18⁺ cells. They did not proliferate in response to an optimal concentration of PHA-P (10 μ g/ml), and failed to demonstrate synergistic proliferation in response to treatment with PMA and the anti-CD3 mAb OKT3. They also failed to produce Ig in response to PWM. All of these responses are T cell dependent, and could be detected upon the addition of <1% T cell load to the purified B cell populations under study (data not shown).

The T cell clone G8 is an HLA-DR-5-restricted, tetanus toxoid-specific T cell clone that was generated and maintained as described (15). Clone G8 lymphocytes exhibited no alloreactivity when tested against a panel of 10 unrelated donors.

TSST-1 Binding Assay. The binding of TSST-1 to purified B cells was performed as previously described (8). TSST-1 was iodinated using chloramine T and the specific activity of labeled TSST-1 was in the range of 950 Ci/mmol. B cells were resuspended in PBS containing 1% BSA at 2.5×10^6 cells/ml. ¹²⁵I-labeled TSST-1 was added at 5×10^{-9} M, and the cells were incubated on ice for 30 min in the absence or presence of increasing concentrations of unlabeled TSST-1. The cells were then layered over 200 μ l of a mixture of phthallate oils (1:5 vol/vol dinonyl and dibutyl phthallate oils; Eastman Kodak, Rochester, NY) in 400- μ l polypropylene centrifuge tubes and spun at 8,000 g for 2 min. The tips, containing the cell

pellets, were cut off and counted in a gamma counter. The affinity of TSST-1 binding to B cells and the number of binding sites were determined by Scatchard analysis of the data. To study the inhibition of TSST-1 binding to B cells by mAbs directed against B cell surface antigens, cells were incubated with ^{125}I -labeled TSST-1 and 10^{-8} M in the absence or in the presence of the indicated mAb, used at a concentration of 6.7×10^{-8} M ($10 \mu\text{g/ml}$). Nonspecific binding was determined by adding excess unlabeled TSST-1 (10^{-6} M). The incubations were carried out for 30 min on ice, following which, the cells were processed and the specific binding was determined as described above.

Assays for B Cell Proliferation and Ig Production. For proliferation assays, B cells were cultured in 96-well flat-bottomed plates (Nunc, Roskilde, Denmark) at 10^5 B cells/well suspended in $200 \mu\text{l}$ RPMI 1640 medium supplemented with 10% FCS, 100 U/ml streptomycin, 100 U/ml penicillin, and 2 mM L-glutamine. Cells were cultured for 48 h at 37°C in a 5% CO_2 humidified atmosphere in the absence or presence of the indicated stimuli and/or irradiated autologous T cells or irradiated clone G8 cells, (3,000 rad). Cells were then pulsed with [^3H]thymidine at $1 \mu\text{Ci/well}$ and were further cultured for an additional 16 h. Thereafter, the cells were harvested, and [^3H]thymidine incorporated into cellular DNA was counted.

For Ig production, B cells were cultured in 12×75 -mm tubes at 10^6 B cells/ml of culture medium containing the appropriate stimulus and various numbers of irradiated autologous T cells or irradiated G8 cells (3,000 rad). After 7 d of culture, supernatants were collected, and IgG and IgM synthesis was determined by ELISA, as previously described (15).

In some experiments, B cells were cultured in 24-well flat-bottomed plates (Nunc) at 10^6 cells/ml/well in the presence or absence of TSST-1. 10^6 Irradiated T cells/well, supplemented with 10% irradiated B cells as accessory cells, were cocultured with B cells either mixed together or separated by placing the T cells inside Millicell-HA well inserts (Millipore Corp., Bedford, MA). The two compartments were separated by a $0.4\text{-}\mu\text{m}$ membrane, allowing free diffusion of soluble mediators. The proliferative responses were assessed on day 3, and Ig production on day 7, as described above.

Results

TSST-1 Binds to MHC-Class II Molecules on High Density Tonsillar B Cells. Purified dense tonsillar B cells were incubated with ^{125}I -labeled TSST-1 in the presence of increasing concentrations of unlabeled toxin, and the resulting binding data were subjected to Scatchard analysis. Fig. 1 demonstrates that TSST-1 binds to B cells with high affinity ($K_d = 3.1 \times 10^{-8}$ M) and saturation kinetics (76,000 sites/cell). The binding of ^{125}I -TSST-1 to B cells was significantly inhibited by the anti-HLA-DR mAb L243, and to a lesser degree by the anti-HLA-DQ mAb SPVL3, but not by the anti-HLA-DR and -DQ mAb ST259, or by the anti-CD20 mAb Leu-16. The combination of L243 and SPVL3 mAbs inhibited the binding of TSST-1 to B cells to an extent comparable with that achieved with excess unlabeled toxin (Fig. 2).

TSST-1 Induces B Cell Proliferation and Ig Production in a T Cell-dependent Manner. We next examined the capacity of TSST-1 to trigger B cell proliferation and Ig production. Because TSST-1 is a potent T cell mitogen, the B cells were rigorously purified to rule out any contribution from contaminating T cells to the responses under study. Fig. 3 demonstrates that TSST-1 on its own failed to induce the proliferation of purified B cells. However, in the presence of irradiated T cells, TSST-1 successfully triggered the proliferation of purified B cells. Significant proliferation was observed upon the addition of 5% irradiated T cells, and the maximal response was noted at the highest ratio of T/B cells tested (1:1).

TSST-1 also induced T cell-dependent differentiation of B cells into Ig-secreting

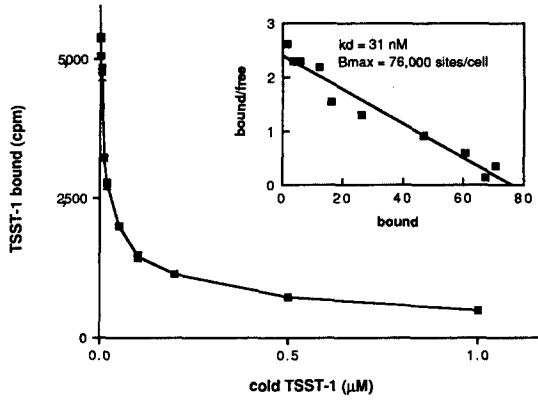


FIGURE 1. Binding of TSST-1 to high density tonsillar B cells. B cells were incubated with ¹²⁵I-TSST at 5 nM, and unlabeled TSST-1 was added at the indicated concentrations. Points represent the mean of triplicate determinations +/- SD. Scatchard plot of the data is shown in the inset.

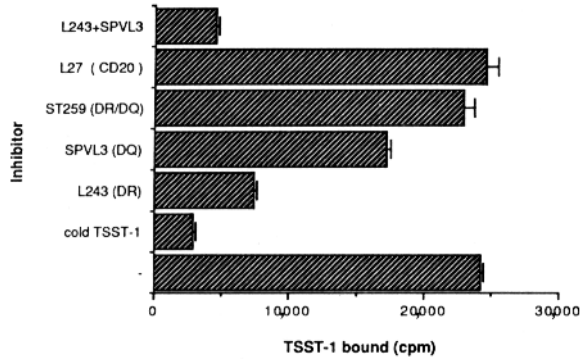


FIGURE 2. Inhibition of binding of TSST-1 to dense tonsillar B cells by MHC class II mAbs. Cells were incubated with ¹²⁵I-TSST at 10 nM. Nonspecific binding was determined in the presence of 10⁻⁶ M unlabeled TSST-1. mAbs were added at a final concentration of 6.7 x 10⁻⁸ M (10 μg/ml). Similar results were obtained in two other experiments.

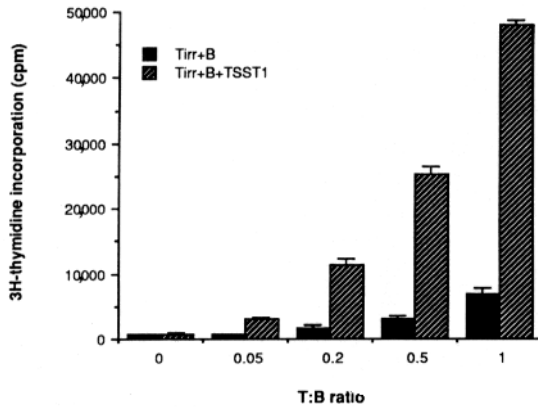


FIGURE 3. Induction of T cell-dependent B cell proliferation by TSST-1. B cells were cultured with the indicated loads of irradiated autologous T cells in the absence or in the presence of TSST-1, used at 1 μg/ml. Results represent means of triplicate cultures +/- SD. Similar results were found in five other experiments.

lymphocytes. Fig. 4 demonstrates that, in the absence of T cells, TSST-1 failed to initiate Ig synthesis by purified B cells. However, in the presence of irradiated autologous T cells, TSST-1 induced resting B cells to synthesize large amounts of IgG and IgM. Ig production induced by TSST-1 was critically dependent on the presence of an optimal number of T cells in culture with B cells. Maximal Ig production

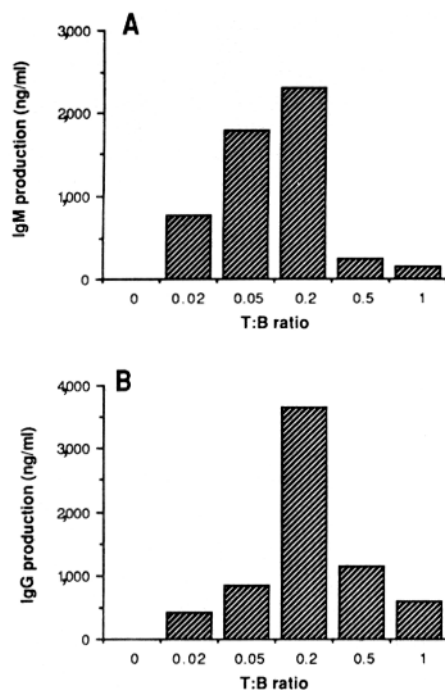


FIGURE 4. TSST-1 induces T cell-dependent IgM (*A*) and IgG (*B*) synthesis in B cells. B cells were cultured with the indicated loads of irradiated autologous T cells in the absence or in the presence of TSST-1, used at 1 $\mu\text{g}/\text{ml}$. The results are expressed as net Ig synthesis in TSST-1-stimulated cultures as compared with control, unstimulated cultures, and represent means of duplicate determinations from duplicate cultures. Similar results were found in five other experiments.

was noted in the presence of 20% irradiated T cells, and progressively decreased upon the addition of higher numbers of T cells (Fig. 4).

TSST-1-triggered B Cell Responses Require Physical T/B Cell Interaction. The T cell requirement for the induction of B cell proliferation by TSST-1 could not be replaced by soluble T cell factors. Addition of various cytokines, including IL-1, IL-2, IL-4, IL-5, IL-6, IFN- γ , and TNF- β , alone or in combination, or of supernatants (diluted 1:2 to 1:20) derived from TSST-1-activated T cells, failed to support TSST-1-triggered B cell proliferation (data not shown). These results suggested that T/B cell contact was required for the induction of B cell proliferation by TSST-1.

To assess whether a short-range or labile mediator could substitute for T/B cell contact, we examined the capacity of TSST-1 to trigger B cell proliferation and Ig production upon separating the T cells from the B cells by means of a well insert with a 0.4- μm membrane that allowed the diffusion of soluble molecules. The T cells in this experiments were placed inside the well insert and were activated by adding TSST-1 and accessory cells in the form of irradiated B cells. Fig. 5 shows that B cells thus separated from T cells failed to proliferate in response to TSST-1. In contrast, the same B cells proliferated to TSST-1 when they were mixed together with the T cells. Similar results were obtained for the induction of IgM and IgG production by TSST-1 (Fig. 6). These results indicate that T/B cell contact was required for TSST-1 to induce B cell proliferation and Ig secretion.

Effect of mAbs against Lymphocyte Surface Antigens on TSST-1-induced B Cell Proliferation and Ig Production. The surface antigens mediating the T/B cell interactions underlying the TSST-1-triggered B cell responses were identified by examining the ability

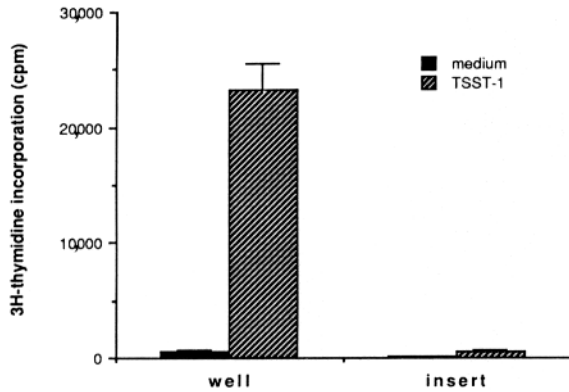


FIGURE 5. TSST-1-triggered B cell proliferation requires intimate T/B cell contact. B cells were cultured with irradiated autologous T cells, used at a 1:1 T/B cell ratio, either mixed together or separated by placing the T cells in a 0.4- μ m well insert. TSST-1 was added at 1 μ g/ml. Results are means of triplicate determinations \pm SD. Similar results were obtained in one other experiment.

of mAbs directed against T and B cell antigens to inhibit these responses (Figs. 7 and 8). Using this approach, the TCR/CD3 complex, as well as MHC class II antigens, were found to be critically important for the progression of TSST-1-triggered B cell responses. The anti-CD3 mAb OKT3 was highly effective in inhibiting both TSST-1-triggered B cell proliferation and Ig production. The anti-HLA-DR mAb L243, which was effective in inhibiting TSST-1 binding to B cells, also inhibited TSST-1-triggered B cell functional proliferation and Ig production. The anti-HLA-DQ mAb SPVL3, which synergized with mAb L243 in inhibiting the binding of TSST-1 to B cells, was found to enhance the inhibition of TSST-1-triggered B cell responses. Three other mAbs to HLA-DR (L227, P4.1, and 2.06), all of which are

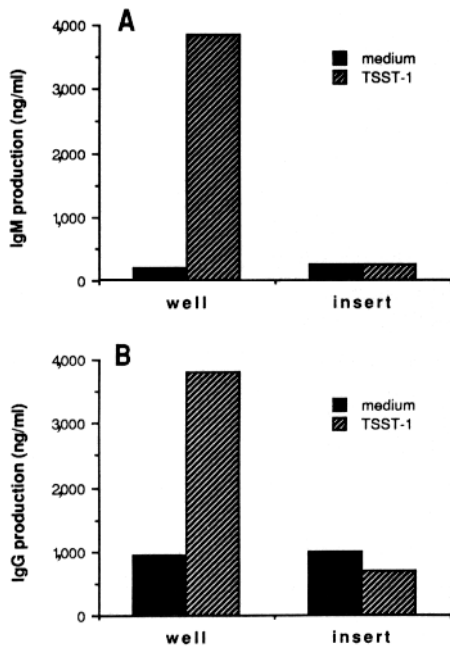


FIGURE 6. TSST-1-triggered Ig production requires intimate T/B cell contact. B cells were cultured with irradiated autologous T cells (at a 0.2 T/B cell ratio) either mixed together or separated by placing the T cells in a 0.4- μ m well insert. TSST-1 was added at 1 μ g/ml. The results represent means of duplicate determinations from duplicate cultures. Similar results were found in one other experiment.

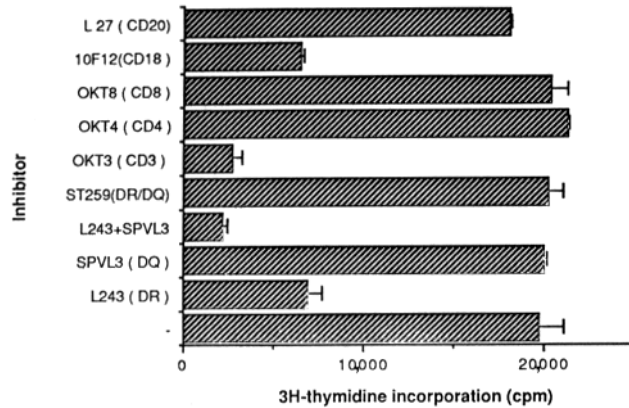


FIGURE 7. Inhibition of TSST-1-triggered B cell proliferation by mAbs directed against T and B cell antigens. B cells were cultured in the presence of irradiated autologous T cells at a T/B cell ratio of 1:1. TSST-1 was added at 1 $\mu\text{g}/\text{ml}$, and the indicated mAbs were added at a final concentration of 10 $\mu\text{g}/\text{ml}$. Results are presented as means of triplicate determinations \pm SD. Similar results were found in two other experiments.

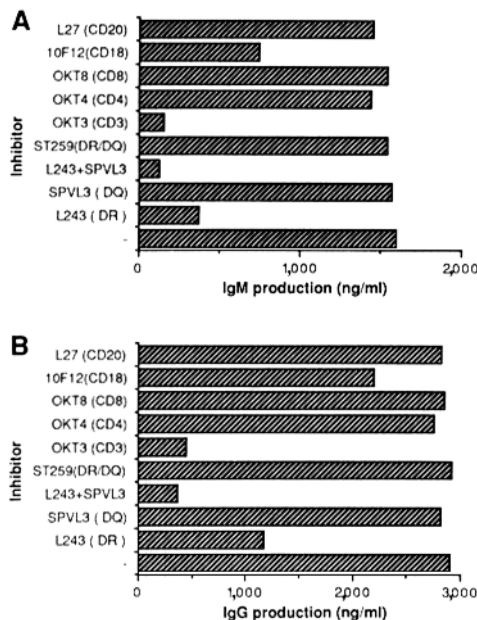


FIGURE 8. Inhibition of TSST-1-triggered Ig production by mAbs directed against T and B cell antigens. B cells were cultured in the presence of irradiated autologous T cells at a T/B cell ratio of 0.2:1. TSST-1 was added at 1 $\mu\text{g}/\text{ml}$, and the indicated mAbs were added at a final concentration of 10 $\mu\text{g}/\text{ml}$. The results are expressed as net Ig synthesis in TSST-1-stimulated cultures as compared with control, unstimulated cultures, and represent means of duplicate determinations from duplicate cultures. Similar results were found in one other experiment.

known to inhibit TSST-1 binding to B cells (8), also inhibited TSST-1-triggered B cell responses (data not shown). In contrast, the anti-HLA-DR and -DQ mAb ST259, which had no effect on the binding of TSST-1 to B cells, failed to inhibit TSST-1-triggered B cell responses. TSST-1-triggered B cell proliferation and Ig production also required the participation of CD11a/CD18 molecules for optimal responses. The anti-CD18 mAb 10F12 significantly inhibited TSST-1-triggered B cell proliferation, and, to a lesser extent, Ig production. The specificity of the inhibitory effects observed with the above-mentioned mAbs was demonstrated by the inability of mAbs directed against other surface antigens, including the T cell antigens CD4 and CD8 and the B cell surface antigen CD20, to inhibit TSST-1-triggered B cell responses.

TSST-1-mediated T/B Cell Interactions Are MHC Unrestricted. We next examined the

TABLE I
TSST-1-mediated T/B Cell Interaction Is MHC Unrestricted

Exp.		[³ H]Thymidine incorporation		Ig production			
				Medium		TSST-1	
		Medium	TSST-1	IgM	IgG	IgM	IgG
		<i>cpm</i>		<i>ng/ml</i>			
1	B cells	188	252	160	190	200	215
	B cells + clone G8	807	47,256	150	200	1,450	3,000
2	B cells	221	179	180	220	200	210
	B cells + clone G8	859	32,541	185	250	2,650	2,800

TSST-1-triggered B cell proliferation and Ig production is supported by clone G8, an allogeneic, nonalloreactive T cell clone. For proliferation studies, B cells were cultured in the presence of irradiated clone G8 lymphocytes at a T/B cell ratio of 1:1. For Ig production, a T/B cell ratio of 0.2:1 was used.

MHC restriction of TSST-1-mediated T/B cell interaction. For this purpose, we examined the capacity of the nonalloreactive T cell clone G8 to support TSST-1-mediated B cell activation. Clone G8 proliferated when stimulated with TSST-1 in the presence of autologous or allogeneic monocytes or in the presence of purified B cells obtained from two different tonsils (data not shown). B cells from the same two tonsils were examined for their capacity to proliferate and to secrete Ig in the presence of irradiated clone G8 cells. Table I shows that while neither TSST-1 nor clone G8 could, on their own, induce B cell proliferation or Ig production, vigorous B cell proliferation and Ig production were observed when both clone G8 and TSST-1 were added to B cell cultures. These results indicate that TSST-1-mediated T/B cell interaction is MHC unrestricted.

Discussion

Antigen-driven B cell activation and differentiation is a T cell-dependent process requiring cognate interaction between the TCR/CD3 complex and antigen presented in the context of MHC class II molecules on the surface of B cells (16). This interaction is restricted both by the ability of a B cell to present antigen in the context of the appropriate MHC allele and by the availability in the T cell repertoire of V_H elements able to recognize the antigen MHC class II complex. These restrictions complicate the study of antigen-driven cellular interactions between unprimed T and B cells. In this study, we describe the induction of polyclonal B cell activation and differentiation by the staphylococcal exotoxin superantigen TSST-1. TSST-1 binds with high affinity and with saturation kinetics to MHC class II antigens on high density tonsillar B cells. By itself, TSST-1 did not cause B cell proliferation or Ig production. However, in the presence of irradiated T cells, TSST-1 induced the proliferation of B cells and their differentiation into Ig-secreting lymphocytes. TSST-1-dependent B cell proliferation progressively increased as a function of the load of irradiated cells added. In contrast, TSST-1-induced Ig production was critically dependent on the presence of an optimal load of T cells in culture with the B cells. Optimal Ig production was achieved at 1:5 irradiated T cell/B cell ratio. Higher T cell loads resulted in progressive decline in TSST-1-induced Ig production. This

result may help explain the observation that TSST-1 inhibits spontaneous as well as PWM-induced Ig production in unfractionated PBMC (17). It is possible that inhibitory T/B cell interactions dominate at higher T cell loads. Alternatively, massive activation of a large number of T cells by TSST-1 may result in the attainment of a critical concentration of cytokine(s) inhibitory for TSST-1-triggered Ig production. Experiments are under way to further identify the mechanism(s) underlying this inhibition.

TSST-1-triggered B cell responses were strictly dependent on the presence of T cells in physical contact with B cells. Separation of T and B cells by means of a 0.4- μm membrane that inhibited T/B cell contact while allowing the diffusion of soluble mediators abrogated TSST-1-triggered B cell responses. The requirement for T cells could not be bypassed by the addition of recombinant cytokines in various combinations or by the addition of culture supernatants of TSST-1-activated T cells.

The T/B cell interactions underlying TSST-1-triggered B cell responses were primarily mediated by the MHC class II/toxin complex on B cells and the TCR/CD3 complex on T cells. This was evidenced by the inhibition of TSST-1-induced B cell responses by both an anti-CD3 mAb and by a combination of anti-MHC class II mAbs that interfered with the binding of TSST-1 to B cells. Additional intercellular adhesive interactions, mediated by CD11a/CD18, were also required for optimal TSST-1-triggered B cell proliferation and Ig production. Other T and B cell antigens, including CD4, CD8, and CD20, were not demonstrated to play a significant role in TSST-1-triggered B cell responses. The failure of mAbs directed against CD4 or CD8 to influence TSST-1-triggered B cell responses is in agreement with previous reports showing that TSST-1 induces the activation of both CD4⁺ and CD8⁺ T cell subsets, as well as CD4⁻/CD8⁻ T lymphocytes (18).

Overall, these results support the notion that TSST-1 initiates, upon its binding to MHC class II molecules, a cognate interaction between T and B cells mediated by TCR/CD3 and MHC class II/toxin complex. This view is strengthened by the observation that T cell triggering by MHC class II/toxin complex is preferentially restricted to particular $v\beta$ families, suggesting that upon its binding to MHC class II molecules, TSST-1 engages the $V\beta$ chain on the TCR (12, 13). In this scheme of events, MHC class II molecules would primarily function to bind and present TSST-1 to T cells. However, an additional direct interaction between MHC class II molecules and the TCR cannot be ruled out.

The T/B cell interaction initiated upon the binding of TSST-1 to MHC class II molecules is similar in many aspects to nominal antigen-mediated cognate T/B cell interaction. They are both driven by interaction between the TCR/CD3 complex on T cells and the MHC class II molecules on B cells, and they both require the contribution of additional adhesive interactions mediated by CD11a/CD18 (16, 19, 20). However, unlike the case with nominal antigen (19), TSST-1-mediated T/B cell interaction does not require the participation of CD4. This may reflect the high affinity nature of the interaction between the MHC class II/toxin complex and the TCR/CD3 complex as CD4 functions to bolster low affinity but not high affinity antigen-TCR interactions (21). Another distinction between antigen- and TSST-1-triggered T/B cell interactions is that the latter is not restricted to a particular MHC class II allele and can proceed between histoincompatible T and B cells. This was illustrated by the ability of a nonalloreactive T cell clone to support, in the presence of TSST-1,

the proliferation of allogeneic B cells and their differentiation into Ig-producing lymphocytes. The ability of TSST-1 to mediate MHC-unrestricted cognate interaction between large numbers of unprimed T and B cells provides a useful model for the study of antigen-driven, T cell-dependent B cell proliferation and Ig production.

TSST-1-mediated T/B cell interaction initiates a series of events in both T and B cells culminating in B cell activation and Ig production. It has been pointed out that activation of the engaged T cells by means of crosslinking their TCR/CD3 is the primary obligate step in the process of initiating T cell-dependent B cell activation (22–24). In this regard, TSST-1, complexed with MHC class II antigens and arrayed on the surface of B cells, would be expected to effectively crosslink the TCR/CD3 molecules of the engaged T cells. This would result in the reorientation of the microtubule organizing center and the Golgi apparatus of the engaged T cells to face B cells, similar to what is observed for antigen-driven cognate T/B cell interaction (25, 26). These changes would help target the secretory products of the engaged T cells, in particular, lymphokines and growth factors crucial for B cell growth and differentiation, for delivery at the site of T/B cell contact (27).

In addition to triggering T cell activation and cytokine production, TSST-1-mediated T/B cell interaction may also deliver trophic signals to B cells via the engaged MHC class II molecules. The function of MHC class II molecules as signal transducers has been well documented. mAbs to MHC class II antigens modulate the proliferation and Ig production of B cells triggered with a variety of mitogens (28, 29). Engagement of MHC class II molecules by mAbs is associated with the accumulation of the second messenger cAMP and the translocation of protein kinase C from the cytosol to the nuclei of treated B cells (30). Evidence presented elsewhere (Mourad, W., T. Chatila, and R. S. Geha, manuscript submitted for publication) demonstrates that TSST-1 synergizes with antibodies to surface IgM to induce vigorous B cell proliferation. This suggests that TSST-1 transduces a signal through MHC class II antigens that allows the progression of preactivated B cells through the cell cycle. The nature of these signals and the role they play in TSST-1-triggered B cell responses requires further investigation.

Summary

The *Staphylococcus aureus* exotoxin toxic shock syndrome toxin 1 (TSST-1) is a potent activator of T cells and monocytes. We have recently demonstrated that TSST-1 is a superantigen that binds monomorphic determinants on MHC class II molecules. In the present study, we have examined the effect of TSST-1 on the activation and differentiation of high density human tonsillar B cells. TSST-1 bound to tonsillar B cells with high affinity and saturation kinetics. This binding was effectively inhibited by a combination of anti-HLA-DR and anti-HLA-DQ mAbs. Treatment of purified B cells with TSST-1 failed to induce B cell proliferation or Ig production. However, in the presence of irradiated T cells, TSST-1 induced resting B cells to proliferate and differentiate into Ig secretory cells. TSST-1 mimicked nominal antigen in that its induction of B cell responses was strictly dependent on physical contact between T and B cells, and was profoundly inhibited by anti-MHC class II mAbs, anti-CD3 mAbs, and, to a lesser extent, by anti-CD18 mAbs. However, unlike nominal antigen, TSST-1-mediated T/B cell interactions were MHC unrestricted.

These results suggest that TSST-1 induces T cell-dependent B cell proliferation and differentiation by virtue of its ability to mediate MHC-unrestricted cognate T/B cell interaction via the TCR/CD3 complex and MHC class II antigens.

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