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Separation of Function Between the Domains of Toxic Shock Syndrome Toxin-1

Jennifer L. Wahlsten2 and S. Ramakrishnan3

Toxic shock syndrome toxin-1 (TSST1) is a pyrogenic toxin superantigen produced by certain strains of Staphylococcus aureus. Structurally, TSST1 is composed of two domains: residues determined by crystallography to directly interact with MHC II molecules reside within the N-terminal domain, while TSST1 residues critical for superantigenicity are within the C-terminal domain. In this study, we expressed the individual N- and C-terminal domains of TSST1 in Escherichia coli and studied their biologic activities. The TSST1 N-terminal domain (TSST(1–87)) did not induce proliferation of human PBLs or release of TNF-β, but did induce TNF-α release. However, TSST1 elicited proliferation and release of both TNF isoforms were induced by a molar excess of TSST(1–87). The TSST1 C-terminal domain (TSST(88–194)) did not bind MHC II molecules, yet it elicited production of TNF-α and TNF-β, and induced TCR Vβ-specific proliferation similarly to intact TSST1. When covalently cross-linked to tumor cells, TSST(88–194) elicited a local in vivo antitumor response indistinguishable from TSST1. Although intact TSST1 causes lethal shock in vivo, the individual domains of this molecule may have therapeutic potential: the N-terminal domain to antagonize lymphocyte activation and TNF release during acute TSST1-precipitated toxic shock syndrome, and the C-terminal domain to stimulate antitumor responses without MHC II binding.


Materials and Methods

Materials

A TSST1 subclone was provided by Dr. P. M. Schlievert (University of Minnesota, Minneapolis, MN). All enzymes for molecular cloning were synthesized by Integrated DNA Technologies (Coralville, IA). The human rIL-2 was a gift from Cetus/Perkin-Elmer (Emeryville, CA). FITC-labeled goat anti-mouse polyvalent Ig, and phycoerythrin-labeled mouse anti-human CD3 (clone UCHT-1) were purchased from Sigma Chemical Co. (St. Louis, MO). Immunoassay kits to quantify TNF-α and TNF-β release were obtained from R&D Systems (Minneapolis, MN). Human rIL-2 was a gift from Cetus/Perkin-Elmer (Emeryville, CA). Raji MHC II-negative mutant R22.2.5 was obtained from Dr. J. M. Boss (Emory University, Atlanta, GA), and permission for its use was given by Dr. R. Accolla (17) (Advanced Technology Center, Genova, Italy).

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Lewis lung carcinoma cells (LLC) were purchased from American Type Culture Collection (Rockville, MD). The heterobifunctional cross-linker, Sulfo-Lo-LC-4-succinimidolysoxybenzyl-α-ethyl-α-(2-pyrididithio)-toluene (SMPT), was obtained from Pierce (Rockford, IL). Female C57BL/6 mice, 8 to 10 wk old, were purchased from Charles River Laboratories (Wilmington, MA), and housed within University of Minnesota specific pathogen-free animal facilities.

Cloning, expression, and purification of H-TSST1 constructs

The TSST1 constructs used in this study are depicted schematically in Figure 1. All sequences were PCR amplified from MNT subclone 6101 (19) using primers designed to incorporate 3′ Ndel and 5′ HindIII restriction sites. The primers used to amplify the mature coding region of TSST1 have been described (20). The downstream primer for TSST1 (1–87) was 5′-gggaagcttttagccacttatttggaa-3′. The upstream primer for TSST1 (88–194), also including a 5′ end cysteine codon, was 5′-ccccatatgtgcctgcgtcgtgcag-3′. Specific radiolabeling at the N terminus of native TSST1 and TSST1 (88–194) was facilitated by addition of a consensus phosphorylation sequence (21) (Kemptide, K-). The upstream primers used to amplify K-TSST1 and K-TSST1 (88–194), each having the Kemptide sequence and a cysteine codon, were as follows: K-TSST1, 5′-ccccatatgtgcctgcgtcgtgcag-3′; K-TSST1 (88–194), 5′-ccccatatgtgcctgcgtcgtgcaggttcagcctggggtaataaa-3′. Because native TSST1 has no cysteine residues, disulfide linkage between TSST1 and cell surface proteins was facilitated through incorporating a cysteine onto the 3′ or 5′ end of the TSST1 coding region. The primers were as follows: upstream for cysts-TSST1, 5′-ccccatatgtgcctgcgtcgtgtaaatttc-3′; downstream for TSST1-cys, 5′-ccggatccatggatcagaatgacc-3′.

All amplified sequences were subcloned into the prokaryotic expression vector, pET 17bH (22), sequenced, and expressed in Escherichia coli host strain BL21 (DE3), according to a previously described protocol (20, 23). Transformed bacteria were grown in M9ZB medium (24) to an A600 of 0.8 to 1 before inducing protein production with isopropyl β-D-thiogalactopyranoside. All proteins expressed from pET 17bH will have 10 sequential end-cysteine codon, was 5′-ccccatatgtgcctgcgtcgtgcag-3′; K-TSST1 (88–194), 5′-ccccatatgtgcctgcgtcgtgcaggttcagcctggggtaataaa-3′. Because native TSST1 has no cysteine residues, disulfide linkage between TSST1 and cell surface proteins was facilitated through incorporating a cysteine onto the 3′ or 5′ end of the TSST1 coding region. The primers were as follows: upstream for cysts-TSST1, 5′-ccccatatgtgcctgcgtcgtgtaaatttc-3′; downstream for TSST1-cys, 5′-ccggatccatggatcagaatgacc-3′.

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In vitro cytotoxicity assay

The ability of H-TSST1 and H-TSST1 (88–194) to induce cytotoxic activity of PBLs against the tumor cell line MA 148 was evaluated in a 4-h 31Cr release assay. PBLs (1 × 106/ml) were pretreated with H-TSST1 (0.1 μg/ml) or H-TSST1 (88–194) (1 μg/ml) for 4 days. On day 4, MA 148 cells were labeled with 51Cr (300 μCi/× 106 cells) for 3 h, rinsed, and plated at 4000 cells/well in 96-well plates. PBLs were thoroughly rinsed of free protein, serially diluted (1/3), and added at different E:T ratios. No effector cells were added to background lysis control wells, and 0.2% Triton X-100 was added to 100% lysis control wells. After 4 h at 37°C, radioactivity released into supernatants was measured in a gamma counter.

Comparison of TCR Vβ-specific proliferation

The TCR Vβ-specific proliferation stimulated by H-TSST1 or H-TSST1 (88–194) was determined by flow cytometry. PBLs were incubated (1 × 106 cells/ml) with H-TSST1 (5 ng/ml) or H-TSST1 (88–194) (500 ng/ml) for 24 h, washed, and then resuspended in an equal volume of fresh RPMI 1640 containing 50 U/ml IL-2. After 24 h, cells were prepared for flow cytometry. TCR Vβ2, Vβ5, or Vβ8 elements were detected with appropriate primary Abs (2 μg/test) diluted in medium containing 3% normal goat serum. Bound primary Abs were detected with FITC-labeled goat anti-mouse polyvalent Ig (1:250). Cells were then incubated with phycoerythin-labeled mouse anti-human CD3 (1:20). Data are presented as the percentage of CD3+ positive lymphocytes or blasts, as indicated by forward and side scatter, that also stain positive for TCR Vβ2, Vβ5, or Vβ8. Background values of positively staining cells in the presence of only secondary Ab were subtracted before making the calculations.

Measurement of TNF-α and TNF-β release

PBL TNF-α and TNF-β release elicited upon H-TSST1 or H-TSST1 (88–194), or H-TSST1 (1–87) stimulation was measured using immunoassay kits. PBLs were incubated (1 × 106 cells/ml) with H-TSST1 (5 ng/ml), H-TSST1 (88–194) (500 ng/ml), H-TSST1 (1–87) (240 ng/ml), or H-TSST1 + H-TSST1 (88–194) in RPMI 1640 containing 10% FBS and 15 μg/ml polymyxin B sulfate for 72 h. Supernatants were clarified of cellular debris and diluted threefold before quantifying TNF release by ELISA, following the protocols recommended by the manufacturer.

Cross-linking cyt-TSST1, TSST1-cys, or H-TSST1 (88–194) onto LLC cells

LLC cells (6.25 × 106 cells/ml) were incubated in HBSS containing 1 mM Sulfo-Lo-SCMPT for 1 h at 37°C. Cells were thoroughly rinsed of unbound cross-linker and then incubated for 5 h at 37°C at the same cell concentration with 16 μM cysts-TSST1, TSST1-cys, or H-TSST1 (88–194) in serum-, methionine-, and cysteine-free high glucose DMEM. Negative control cells were incubated in serum-free high glucose DMEM to allow disulfide exchange with cysteines. Cells were rinsed of unbound protein 4 × 15 ml, irradiated (5000 rad), and directly prepared for injection into mice. Approximately 32 μg H(cys)-TSST1 or 20 μg H-TSST1 (88–194) (1.3 nmol) was linked to 5 × 106 cells.

In vivo tumorigenicity assay

The ability of cell surface-attached cysts-TSST1, TSST1-cys, or H-TSST1 (88–194) to induce a local antitumor response was measured in an in vivo tumorigenicity assay. C57BL/6 mice were inoculated s.c. into the left side of the shaved back with 5 × 106 parental LLC and 5 × 106 coated, irradiated LLC. Biecting tumor diameters were measured with calipers every 2 to 4 days. Moribund mice, or mice with ulcerated tumors were killed. Because of morbidity, the experiment is considered ended on day 26 postinoculation. Surviving mice were still measured through day 32, and surviving mice were still measured through day 32, and killed. Because of morbidity, the experiment is considered ended on day 26 postinoculation. Surviving mice were still measured through day 32, and killed. Because of morbidity, the experiment is considered ended on day 26 postinoculation. Surviving mice were still measured through day 32, and killed. Because of morbidity, the experiment is considered ended on day 26 postinoculation. Surviving mice were still measured through day 32, and killed.

Lymphocyte proliferation assays

The biologic activity of H-TSST1, H-TSST1 (88–194), and H-TSST1 (1–87) was measured in proliferation assays using human PBLs. Gradient-isolated PBLs were plated at 2 × 104 in 0.2 ml RPMI 1640 containing 10% FBS, 15 μg/ml polymyxin B sulfate, and serial dilutions of protein. Cultures were incubated for 72 h, labeled with [3H]thymidine (83 Ci/mmol), harvested 18 h later, and incorporated radioactivity was measured in a scintillation counter.

Results

A description of the H-TSST1 constructs

All rTSST1 proteins used in this study were expressed with a histidine (H-) tag at the amino terminus and purified as described (20). The constructs are depicted in Figure 1. Using this system, we expressed native H-TSST1, the N-terminal H-TSST1 (1–87),
and the C-terminal H-TSST(88–194). We also expressed TSST1 and TSST(88–194) preceded by Kemptide, a specific PKA phosphorylation sequence that serves as a 32P radiolabeling site (21, 26). These proteins are referred to as HK-TSST1 and HK-TSST(88–194). Because native TSST1 has no cysteine residues, specifically positioned disulfide linkages between TSST1 and other proteins were achieved by the addition of a single cysteine residue to either the N or C terminus (cys-TSST1 or TSST1-cys). H-TSST(88–194), HK-TSST1, and HK-TSST(88–194) also have N-terminal cysteines.

H-TSST(88–194) does not bind MHC II molecules

To determine whether the C-terminal domain of TSST1 binds to MHC II molecules, we tested the ability of [32P]HK-TSST(88–194) to bind the MHC II-positive human B cell lymphoma cell line, Raji. We also evaluated [32P]HK-TSST1 binding to Raji. As a negative control, the binding of both labeled proteins to the Raji MHC II-deficient mutant, RJ2.2.5, was determined (17). Labeled [32P]HK-TSST1 clearly bound Raji cells, but did not bind to RJ2.2.5 cells. In contrast, labeled [32P]HK-TSST(88–194) did not bind to either Raji or RJ2.2.5 cells, even when present at 250 nM (Fig. 2A). In a separate experiment, H-TSST(88–194) did not detectably bind Raji cells when present at 800 nM (data not shown). These results suggest that H-TSST(88–194) does not bind MHC II molecules.

Effects of the individual TSST1 domains on PBL proliferation

TSST1 residues within 27–85 of the N-terminal domain have been shown to directly interact with MHC II molecules (10). Because a synthetic peptide corresponding to TSST1 residues 58–78 induced proliferation in vitro (27), we tested the ability of H-TSST(1–87) to elicit a proliferative response. The addition of up to 320 nM H-TSST(1–87) did not induce proliferation of PBLs (Fig. 2B). Because TSST1(1–87) did not induce proliferation and contains residues that directly interact with MHC II, we postulated that this molecule might inhibit TSST1-induced proliferation. Indeed, a 100-fold molar excess of H-TSST(1–87) incubated with H-TSST1 shifted the H-TSST1 concentration-response curve down by 10-fold, and reduced the maximum H-TSST1-induced proliferation by 30% (p < 0.001, Fig. 2B). These data suggest that H-TSST(1–87) does not induce proliferation and interferes with proliferation induced by intact H-TSST1.

Although H-TSST(88–194) does not engage MHC II molecules, it induces proliferation of PBLs in vitro. The maximum proliferation of PBLs stimulated by H-TSST(88–194) was equal to that induced by H-TSST1, but 100-fold greater molar concentrations were required (Fig. 2B). H-TSST(88–194) induced maximum PBL proliferation at 65 nM, a concentration 12-fold less than the maximum concentration tested (800 nM) to demonstrate its inability to bind MHC II (Fig. 2B and data not shown). These results suggest that H-TSST(88–194) stimulates PBL proliferation independent of MHC II binding.

H-TSST(88–194) induces antitumor cytotoxicity in vitro

Superantigenic staphylococcal enterotoxins A (SEA) and B can induce NK- and T cell-mediated cytotoxicity against tumor cell lines independent of target cell MHC II expression (6, 7). We compared the abilities of H-TSST1 and H-TSST(88–194) to elicit cytotoxicity in vitro against the human ovarian carcinoma cell line, MA 148 (18), using a 51Cr release assay. Human PBLs prestimulated with either H-TSST1 or H-TSST(88–194) were cytotoxic against MA148 cells; however, about a fivefold greater E:T ratio was required of H-TSST(88–194)-stimulated PBLs to elicit a comparable response (Fig. 2C).
**Table I. T cell receptor Vβ-specific proliferation stimulated by H-TSST1 and H-TSST(88–194)**

<table>
<thead>
<tr>
<th>Stimulus</th>
<th>Vβ2 (%)</th>
<th>Vβ5 (%)</th>
<th>Vβ8 (%)</th>
<th>Vβ2 (%)</th>
<th>Vβ5 (%)</th>
<th>Vβ8 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>9.6</td>
<td>2.3</td>
<td>4.1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>H-TSST1</td>
<td>5.1 (0.5)</td>
<td>2.0 (0.9)</td>
<td>4.1 (1.0)</td>
<td>33.0 (3.4)</td>
<td>4.5 (2.0)</td>
<td>4.6 (1.1)</td>
</tr>
<tr>
<td>H-TSST(88–194)</td>
<td>5.8 (0.6)</td>
<td>3.2 (1.4)</td>
<td>4.2 (1.0)</td>
<td>29.0 (3.0)</td>
<td>5.4 (2.4)</td>
<td>5.2 (1.3)</td>
</tr>
</tbody>
</table>

* PBLs were incubated with 5 ng/ml H-TSST1 or 500 ng/ml H-TSST(88–194) for 72 h, rinsed, and then incubated in medium containing 50 U/ml IL-2 for 24 h. Percentages of cells staining positive with addition of only secondary Ab have been subtracted.

$^{b}$ Fold differences vs unstimulated controls in parentheses. Calculated as the percentage of lymphocytes or blasts that bound an anti-Vβ Ab after stimulation divided by the percentage of lymphocytes that bound the anti-Vβ before stimulation.

**Table II. TNF-α and TNF-β released upon stimulation with H-TSST1, H-TSST(88–194), or H-TSST(1–87)**

<table>
<thead>
<tr>
<th>Stimulus</th>
<th>TNF-α (pg/ml)</th>
<th>TNF-β (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>&lt;4.4$^{b}$</td>
<td>&lt;16</td>
</tr>
<tr>
<td>H-TSST1 (0.2 nM)</td>
<td>1271 (±22)c</td>
<td>1269 (±14)</td>
</tr>
<tr>
<td>H-TSST(1–87) (0.5 nM)</td>
<td>741 (±5)$^{d}$</td>
<td>192 (±14)$^{d}$</td>
</tr>
<tr>
<td>H-TSST(1–87) (40 nM)</td>
<td>437 (±14)</td>
<td>&lt;16</td>
</tr>
<tr>
<td>H-TSST(88–194) (0.5 nM)</td>
<td>295 (±12)</td>
<td>&lt;16</td>
</tr>
<tr>
<td>H-TSST(88–194) (16 nM)</td>
<td>1364 (±27)</td>
<td>1704 (±44)</td>
</tr>
<tr>
<td>H-TSST(88–194) (0.1 nM)</td>
<td>&lt;4.4</td>
<td>&lt;16</td>
</tr>
</tbody>
</table>

* PBLs were incubated with indicated concentrations of different H-TSST1 constructs for 84 h. The supernatants were clarified of cellular debris, and released TNF-α or TNF-β was measured by ELISA.

$^{b}$ The minimum detection limit was 4.4 pg/ml for TNF-α and 16 pg/ml for TNF-β.

$^{c}$ SD of triplicate measurements are in parentheses.

$^{d}$ p < 0.0001, two-tailed Student’s t test.

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**H-TSST(88–194) stimulates Vβ-specific T cell proliferation**

Superantigens induce selective proliferation of T cells bearing certain TCR Vβ elements (3). Primarily, human T cells that express TCR Vβ2 proliferate upon exposure to TSST1 (28). We compared TCR Vβ expansion profiles of H-TSST1- and H-TSST(88–194)-stimulated PBLs using flow cytometry and mAbs specific for Vβ2 (Table I). For negative controls, mAbs specific for Vβ5 or Vβ8 were used. PBLs gated for expression of CD3 and Vβ2, Vβ5, or Vβ8 elements stained equivalently, whether stimulated with H-TSST1 or H-TSST(88–194). The percentage of CD3$^+$ blasts expressing Vβ2 tripled vs nonstimulated controls, and a correlating decrease of TCR Vβ2$^+$ cells remained in the resting CD3$^+$ lymphocyte population. This result implies that the majority of T cells bearing Vβ2 were stimulated to become blasts upon exposure to H-TSST1 or H-TSST(88–194) (29). By comparison, the percentage of CD3$^+$ blasts expressing Vβ5 doubled, and the proportions of Vβ5-expressing CD3$^+$ elements did not appreciably change, when stimulated with either H-TSST1 or H-TSST(88–194) (Table I). Nominal expansion of Vβ5-expressing T cells upon exposure to TSST1 has been previously observed (29, 30). These results show that H-TSST1 and H-TSST(88–194) stimulated T cells similarly.

**TNF release stimulated by TSST1(1–87) or TSST(88–194)**

TSST1 potently induces release of TNF-α and TNF-β (5, 31), which can cause lethal TSS (32). We measured release of both TNF isoforms into cell culture supernatants using ELISA. Because TSST1(1–87) did not induce proliferation by itself, but inhibited TSST1-induced proliferation, we tested whether TSST1(1–87) could induce TNF release or inhibit TSST1-induced TNF release. Unexpectedly, PBL cultures incubated with as little as 0.5 nM H-TSST(1–87) elicited nearly 300 pg/ml TNF-α, but even 40 nM H-TSST(1–87) did not induce detectable amounts of TNF-β release (Table II). However, when incubating a 200-fold molar excess of H-TSST1(1–87) with H-TSST1, concentrations of H-TSST1-induced TNF-α and TNF-β were reduced by 40 and 85%, respectively ($p < 0.0001$ for inhibition of both TNF isoforms, Table II).

We measured H-TSST(88–194)-elicited TNF-α and TNF-β release in vitro at a concentration in which it induces proliferation (32 nM), and at concentrations in which H-TSST1 induces proliferation (0.5 and 0.1 nM). Human PBLs incubated with an H-TSST(88–194) concentration that induces proliferation released large amounts of both TNF-α and TNF-β (Table II), comparable with H-TSST1-induced release. At 100-fold lesser concentrations, in which only H-TSST1 is maximally active, H-TSST(88–194) did not elicit detectable amounts of TNF-α or TNF-β production. Together, these results demonstrate that H-TSST(88–194) stimu-

**FIGURE 3.** H-TSST(88–194) induces antitumor cytotoxicity in vivo. Antitumor response induced by LLC cells cross-linked to Hcys-TSST1 or H-TSST(88–194) against coincubated untreated LLC cells. Control mice were coincubated with irradiated, cysteine-coated LLC cells. The outgrowth of untreated tumor was followed, and the data are presented as the average biecting tumor diameters. Tumor incidence on day 48 per the total number of mice injected in each group is in parentheses. All three test groups were significantly different from cysteine-coated negative controls when the experiment was ended on day 26 ($p < 0.02$, Wilcoxon Rank Sum test). Tumors of surviving mice were measured through day 32, when all control mice were killed.

**H-TSST(88–194) induces antitumor cytotoxicity in vivo**

Ab-anchored SEA has been shown to direct superantigen-dependent cytotoxic responses against tumor cells in vivo (33, 34). We determined the ability of cell-anchored H-TSST(88–194) to induce an antitumor response in an in vivo tumorigenicity assay. Irradiated LLC cells cross-linked to equimolar amounts of cysteine-TSST1, TSST1-cys, or H-TSST(88–194) were s.c. coinjected with an equal number of parental LLC cells. Outgrowth was compared with control mice coinjected with irradiated cysteine-coated LLC. The average diameter values reflect a reduced incidence and hindered outgrowth of tumor in the treatment groups. Tumor cells coated with cys-TSST1, TSST1-cys, or H-TSST(88–194) significantly inhibited the outgrowth of coincubated parental tumor vs control mice ($p < 0.02$ on day 26, Fig. 3). The antitumor response was independent of N- or C-terminal TSST1 attachment or the presence of the MHC II binding domain (Fig. 3).
Discussion

We describe in this work the separate expression of the TSST1 N- and C-terminal domains, and the evaluation of their biologic activities on human PBLs. The N-terminal domain did not induce proliferation or TNF-β release, but low concentrations elicited TNF-α release. Molar excesses of the N-terminal domain inhibited proliferation and TNF release induced by intact TSST1. The C-terminal domain did not bind MHC II, but stimulated Vβ-selective proliferation, antitumor cytotoxicity, and release of both TNF isoforms.

Biologic activities of the N-terminal domain

Culturing PBLs with up to 320 nM H-TSST(1–87) did not induce proliferation in vitro. Others have demonstrated that a synthetic peptide corresponding to TSST1(58–78) elicited proliferation in vitro contingent upon MHC II binding; however, a minimum concentration of 750 nM was required for a significant response (27, 35). We tested the activities of H-TSST(1–87) in vitro within a biologically relevant concentration range for its application as an antagonist to H-TSST1. It is possible that H-TSST(1–87) might also induce proliferation if cultured at greater concentrations. Although H-TSST(1–87) did not elicit a proliferative response below 320 nM, concentrations as low as 0.5 nM did induce TNF-α release. Nevertheless, culturing PBLs with H-TSST1 and a molar excess of H-TSST(1–87) not only inhibited H-TSST1-induced proliferation, but significantly lowered TNF-α release, and nearly abolished TNF-β release. Although we do not explicitly show binding of H-TSST(1–87) to MHC II molecules, these results suggest that H-TSST(1–87) directly interacts with monocytes, but does not stimulate T cells. If this hypothesis is true, then H-TSST(1–87) most likely interferes with H-TSST1-induced T cell proliferation and TNF release through preventing H-TSST1 presentation by MHC II molecules.

Since reported body fluid concentrations of TSST1 range from 0.02 to 1.8 nM in patients suffering from TSST1-precipitated TSS (36–38), it is conceivable that a nonstimulatory version of TSST1 with an intact MHC II binding domain, such as H-TSST(1–87), could serve as a specific antagonist during the acute phase of this disease. There are many reports describing nonstimulatory TSST1 mutants (12, 13, 15, 39–41). Mutating TSST1 at Asp 132/Gln 136 or His 135 yielded proteins that were nonmitogenic and nonlethal in rabbit infection models for TSS (12, 41). Both of these mutants have intact MHC II binding domains, indicating that TSST1-induced lethality is not a necessary consequence of MHC II binding. Indeed, His 135-mutated TSST1 bound MHC II molecules (16) and induced in vitro PBL TNF-α release at concentrations comparable with H-TSST(1–87) (42). This TNF-α release should not be considered trivial; however, His 135-mutated TSST1 did not precipitate lethal toxic shock in rabbits (41) nor in d-galactosamine-sensitized mice (43). These results merit testing the potential of a nonstimulatory, MHC II-binding version of TSST1 as a specific antagonist in acute TSST1-precipitated TSS.

Biologic activities of the C-terminal domain

Residues within the TSST1 C-terminal domain are critical to the superantigenic properties of this molecule (12, 13, 15). Edwin and Kass demonstrated that a fragment of TSST1 generated by papain digestion and corresponding to amino acids 88–194 was immunostimulatory (14). The crystallographic structure of TSST1 later revealed that these amino acids comprise nearly the entire C-terminal domain (8, 9). rH-TSST(88–194) did not bind MHC II molecules, but induced proliferative and cytotoxic responses in vitro and in vivo (Figs. 2 and 3). That H-TSST(88–194) did not bind MHC II molecules agrees with the crystallographic structure of TSST1 bound to a human MHC II (DR1) molecule (10). In contrast, Soos et al. showed that a synthetic peptide corresponding to TSST1 residues 155–194 effectively competed with 125I-labeled TSST1 binding to MHC II; however, molar concentrations in excess of 1 μM were required for significant displacement. (44). We tested the MHC II-binding ability of HK-TSST(88–194) at molar concentrations up to 12-fold greater than that required to elicit a maximal proliferative response, thereby remaining in a biologically relevant concentration range. At concentrations greater than 1 μM, H-TSST(88–194) might also bind MHC II molecules. Nevertheless, [125I]HK-TSST(88–194) labeled to a high sp. act., did not detectably bind Raji cells at concentrations up to 800 nM (data not shown). Our results support the postulate that the biologic actions of H-TSST(88–194) are independent of MHC II binding.

An MHC II-independent response does not necessarily exclude the involvement of APC and a myriad of other potential binding molecules. Indeed, APC involvement is probably required. Dennig et al. demonstrated that TSST1 could induce T cell proliferation in the complete absence of MHC II molecules as long as APCs were present (45). In agreement with our observations, about 100-fold greater concentrations of TSST1 were required for an equivalent proliferative response in the absence of MHC II presentation (45). H-TSST(88–194) induced PBL release of equimolar amounts of TNF-α and TNF-β, produced primarily by monocytes and T cells, respectively. This indicates the probable involvement of both of these cell types upon H-TSST(88–194) stimulation. In addition, cross-linking CD28 molecules with Abs to provide costimulatory signaling, in the event that H-TSST(88–194) did not efficiently encourage T cell:APC cognate interaction, did not markedly improve the concentration-response curve (data not shown). Together, these results suggest that H-TSST(88–194)-induced biologic activity is independent of MHC II binding, but involves direct contact between T cells and APCs, and perhaps an alternative, low affinity receptor. Others have suggested that MHC II-independent superantigenic activation may involve binding to as yet undefined molecules (6, 46, 47).

Intact superantigens generally direct T cell-mediated cytotoxicity against MHC II-positive cells on which they are bound (48, 49). Immunostimulatory superantigens unable to engage MHC II, such as H-TSST(88–194), are necessary for feasible development of superantigen-based antitumor therapies to avoid directing undesirable toxicity against normal MHC II-positive cells. Our results and the results of others support the possibility of using superantigens unable to bind MHC II to stimulate antitumor responses. Hansson et al. demonstrated that an Ab-directed SEA molecule with compromised MHC II-binding capacities effectively induced an antitumor response with reduced toxicity in vivo (50). In agreement with these results, when we administered equimolar amounts of tumor cell-attached H(cys)-TSST1 or H-TSST(88–194), comparable inhibition of tumor outgrowth was observed (Fig. 3). Convinced attachment of a superantigen to the surface of a cell, whether through cross-linking or Abs, may substitute for MHC II presentation. Indeed, Ab-immobilized TSST1 or SEA stimulated the proliferation of purified human T cells in vitro (11, 51). In further support of this hypothesis, an SEA mutant unable to bind MHC II molecules induced equivalent MHC II-dependent cytotoxicity when added at 100-fold greater molar concentrations than wild-type SEA (52). When anchored onto the cell surface through an Ab, however, equimolar amounts of SEA and the mutant elicited equivalent cytotoxic responses in vitro (52).

Conclusions

Our data suggest that the individual domains of TSST1 may have therapeutic potential. The N-terminal β-barrel, containing residues
shown to directly interact with MHC II molecules, might serve as a specific antagonist to ameliorate the acute phase of TSST1-induced TSS. The C-terminal domain, containing residues critical to the superantigenicity of TSST1, may be effectively used in anti-cancer therapies once attached to the surface of tumor cells.

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References


