Identification of Class II Major Histocompatibility Complex and T Cell Receptor Binding Sites in the Superantigen Toxic Shock Syndrome Toxin 1

By J. Michael Hurley,* Richard Shimonkevitz,‖ Adrienne Hanagan,‖ Kristin Enney,‖ Eric Boen,‖ Sharon Malmstrom,‖ Brian L. Kotzin,*‡§ and Masazumi Matsumura‖

*From the Departments of *Pediatrics and ‡Medicine, National Jewish Center for Immunology and Respiratory Medicine, Denver, Colorado 80206; the §Departments of Immunology and Medicine, University of Colorado Health Sciences Center, Denver, Colorado 80262; and ‖Supragen, Incorporated, Lakewood, Colorado 80214*

Summary

Superantigens, in association with class II major histocompatibility complex (MHC) molecules, activate T cells bearing particular β chain variable domains of the T cell receptor (TCR). Unlike conventional peptide antigens, superantigens bind as intact proteins to TCR and MHC molecules outside their peptide binding sites. To characterize these interactions at the molecular level, random point mutations were generated in the gene encoding toxic shock syndrome toxin 1, a bacterial superantigen associated with toxic shock syndrome. Functionally impaired mutants were identified based on their lack of murine and human T cell stimulatory activities, and experiments analyzing binding to human histocompatibility leukocyte antigen–DR molecules differentiated residues involved in MHC from TCR binding. The results showed that the great majority of mutations are clustered in two distinct regions of the toxic shock syndrome toxin 1 molecule. The class II MHC binding site is located in the hydrophobic region of the NH_2-terminal domain, and the TCR binding site is primarily in the major central groove of the COOH-terminal domain. These studies provide insight into the interactions necessary for superantigen-mediated disease in humans.

Staphylococcus aureus and group A streptococci secrete a number of exotoxins expressing superantigen activity, including toxic shock syndrome toxin (TSST)[1]-1, staphylococcal enterotoxins A to E, and streptococcal pyrogenic exotoxins A and C (1, 2). TSST-1 has been shown to be the major cause of toxic shock syndrome (2–4). The development of this disease appears to be dependent on superantigen-induced T cell stimulation and consequent systemic release of cytokines such as TNF and IL-2 (2, 5). TSST-1 activates human T cells bearing β chain variable domains (Vβ)-2, which represent ~10% of the total T cell repertoire (5–7), and stimulation is dependent upon antigen-presenting cells expressing HLA-DR molecules (8, 9). Although x-ray crystallographic studies have suggested that TSST-1 consists of two separate domains (10, 11), the TCR and MHC binding sites have not yet been defined. The present study used a comprehensive mutagenesis of TSST-1 to functionally define these binding sites.

[1] *Abbreviations used in this paper:* SEB, staphylococcal enterotoxin B; TSST, toxic shock syndrome toxin; Vβ, β chain variable domain.

Materials and Methods

Cloning of the Tst-1 Gene from S. Aureus. Total genomic DNA was extracted from several strains of TSST-1–producing *S. aureus* (kindly provided by Dr. John James and Dr. Jim Todd, Children's Hospital, Denver, CO) using a protocol (12) modified for small bacterial cultures. For cloning, the wild-type *tst-1* gene (13) was amplified by PCR using flanking primers A and B shown in Table 1. Forward primer A anneals to the first seven codons of the mature wild-type *tst-1* gene of *S. aureus*. Preceding these codons in primer A is an initiation codon (ATG), a ribosome binding site (GGAGG) and a spacer region (A_8) for efficient expression in *Escherichia coli* (14), along with a KpnI restriction site for cloning. Reverse primer B anneals to the final 10 codons of *tst-1* (including the *ochre* stop codon TAA) and also contains an XbaI restriction site. After agarose gel electrophoresis and purification by GeneClean (Bio 101, Inc., Vista, CA), the PCR product was digested with KpnI and XbaI and cloned into *E. coli* as described (15). Several wild-type clones were sequenced, confirming the published sequence (13) as corrected by Lee et al. (16).

Generation of Mutant TSST-1 Proteins. PCR primers carrying random mutations (Table 1) were used to generate mutant TSST-1 molecules. The primers were synthesized such that, at each position, the three incorrect nucleotides were incorporated at a frequency of 0.25–1.0% (17). Mutations were introduced into *tst-1*

Table 1. *TSST-1 Flanking and Mutagenic PCR Primers Used in This Study*

A	CGG GGT ACC CCG AAG GAG GAA AAA AAA ATG TCT ACA AAC GAT AAT ATA AAG
1)	TCT ACA AAC GAT AAT ATA AAG GAT TTG CTA GAC TGG TAT AGT AGT GGG TCT
2)	GGG TCT GAC ACT TTT ACA AAT AGT GAA GTT TTA GAT AAT TCC TTA GGA TCT
3)	GGA TCT ATG CGT ATA AAA AAC ACA GAT GGC AGC ATC AGC CTT ATA ATT TTT
4)	ATT TTT CCG AGT CCT TAT TAT AGC CCT GCT TTT ACA AAA GGG GAA AAA GTT
5)	AAA GTT GAC TTA AAC ACA AAA AGA ACT AAA AAA AGC CAA CAT ACT AGC GAA
6)	AGC GAA GGA ACT TAT ATC CAT TTC CAA ATA AGT GGC GTT ACA AAT ACT GAA
7)	ACT GAA AAA TTA CCT ACT CCA ATA GAA CTA CCT TTA AAA GTT AAG GTT CAT
8)	GTT CAT GGT AAA GAT AGC CCC TTA AAG TAT TGG CCA AAG TTC GAT AAA AAA
9)	AAA AAA CAA TTA ACT ATA TCA ACT TTA GAC TTT GAA ATT CGT CAT CAG CTA
10)	CAG CTA ACT CAA ATA CAT GGA TTA TAT CGT TCA AGC GAT AAA ACG GGT GGT
11)	GGT GGT TAT TGG AAA ATA ACA ATG AAT GAC GGA TCC ACA TAT CAA AGT GAT
12)	AGT GAT TTA TCT AAA AAG TTT GAA TAC AAT ACT GAA AAA CCA CCT ATA AAT
13)	ATA AAT ATT GAT GAA ATA AAA ACT ATA GAA GCA GAA ATT AAT
B	TGC TCT AGA GCA TTA ATT AAT TTC TGC TTC TAT AGT TTT TAT

The primers A and B are forward and reverse flanking oligonucleotide primers, respectively. The 13 indicated primers, which were randomized with incorrect nucleotides as indicated in the text, were used for mutagenesis of the entire *tst-1* gene. Underlined nucleotides represent regions of overlap between consecutive primers to ensure mutagenesis at the residues. Basepair mismatches near the 3′ end of mutagenic primers might perturb the primer-template junction and hence not be incorporated into the initial PCR product.

via the "megaprimer" PCR method (18, 19). The first PCR reaction was performed using 1 of the 13 mutagenic primers indicated in Table 1 paired with reverse primer B and with the cloned wild-type *tst-1* gene as template. After agarose gel electrophoresis and purification, this PCR product (megaprimer) was used directly in a second PCR reaction with primer A and *tst-1* template to reconstruct the full-length gene. Finally, using gel-purified full-length product, a third PCR reaction was performed with flanking primers A and B to generate sufficient material for cloning. Full-length *tst-1* genes with mutations were then cloned into either pTZ18R or pUC18 plasmids and transformed into *E. coli* for production and screening of mutant proteins (15).

Quantitation of TSST-1. Individual colonies of *E. coli* transformants harboring mutated *tst-1* genes were grown in 96-well plates containing 0.2 ml of 2× YT liquid medium (15) and carbenicillin. The production of recombinant TSST-1 was induced by the addition of 1 mM isopropyl-β-D-thiogalactoside, and bacterial lysates were prepared as described (17). To measure the amount of toxin in each lysate, a sandwich ELISA was developed using mAbs to TSST-1, produced by standard methods from BALB/c mice immunized multiple times with TSST-1. The properties of these antibodies will be described in detail elsewhere (Shimonkevitz, R. S., E. Boen, S. Malmstrom, E. Brown, J. M. Hurley, B. Kotzin, and M. Matsumura, manuscript submitted for publication). Two of these mAbs specific for different sites on TSST-1 were adsorbed overnight to microtiter plates, each at a concentration of 1.0 μg/ml in PBS. After washing to remove unbound antibody, lysates or known concentrations of TSST-1 were added to the microtiter wells and incubated for 2 h. After a second wash, a horseradish peroxidase–conjugated rabbit anti-TSST-1 (Toxin Technology, Sarasota, FL) was used as a secondary reagent to detect bound toxin. The color reaction was developed using *o*-phenylene-diamine and detected using a microplate ELISA reader.

Purification of Mutant TSST-1 Proteins. Typically, 1 liter of *E. coli* was grown overnight and the production of TSST-1 was induced for 24 h as described above. Cells were harvested by centrifugation, suspended in 20 ml of 10-mM Tris-HCl/1-mM EDTA buffer, pH 7.4, and disrupted by ultrasonication. The bacterial extract was centrifuged and dialyzed overnight against 10 mM potassium phosphate buffer, pH 6.1. The TSST-1 protein was then purified by HPLC using a cation exchange column (SP-5-PW; Bio-Rad Laboratories, Hercules, CA) with a linear NaCl gradient from 0 to 500 mM in 10 mM potassium phosphate buffer, pH 6.1. The wild-type TSST-1 was eluted at ~120 mM NaCl. This method yielded ~10 mg of purified TSST-1 protein/liter of bacterial culture. The wild-type and mutant TSST-1 proteins prepared by this method were judged to be >95% pure by analysis on gels after SDS-PAGE.

Assays for T Cell Stimulatory Activity. Bacterial lysates were screened for superantigen activity by stimulation of a murine T cell hybridoma as described (17). Since murine T cells responding to TSST-1 are contained within the subset expressing Vβ15, a hybridoma cell line expressing Vβ15 (KOX 15-4.95) was used as responder cells. Various dilutions of lysate in 0.2 ml culture medium (RPMI 1640 or IMDM containing 10% fetal bovine serum, 2 mM L-glutamine, 2 mM pyruvate, 10 mM Hepes, 50 U/ml penicillin, and 50 mg/ml streptomycin) were added to microtiter culture wells containing ~1 × 10^5 T hybridoma cells and 5 × 10^4 HLA-DR–expressing antigen-presenting cells (either LG2, a human B lymphoma cell line homozygous for DR1, or Raji-DR, described below). T cell stimulation was assayed after the overnight release of IL-2 using the IL-2–dependent HT-2 cell line bioassay.

Human PBL were prepared by Ficoll-Hypaque centrifugation, and 1 × 10^5 cells were added to microtiter wells containing various dilutions of the wild-type or mutant TSST-1 proteins in 0.2 ml culture medium (described above). Cultures were incubated at 37°C for 3 d, pulsed overnight with 0.5 μCi tritiated thymidine, and harvested. Cellular proliferation was measured by the amount of incorporated radioactivity as quantitated with a beta scintillation counter.

In selected experiments, stimulation of a human Vβ2$^+$ cell line was also studied. Human PBL were stained for Vβ2 and CD4 as described (20) and sorted on a cytofluorograph. Double-positive cells were expanded by culture at 2 × 10^5/ml in the presence of 10^6 unseparated autologous PBL (previously irradiated to 4,000 rad) and 1 μg/ml PHA-P (Murex Diagnostics, Ltd., Dartford, UK). After 5 d of culture, PHA was washed out, and IL-2 was added for an additional 10 d before using the cells in a stimulation assay. For response to wild-type and mutant TSST-1 proteins, Vβ2$^+$ T cells were cultured at 5 × 10^4 cells per well in 0.2 ml of the culture medium described above with 1 × 10^5 LG2 cells.

Class II MHC Binding Assay. Mutant proteins were tested for binding to class II MHC using the Raji-DR cell line (American Type Culture Collection, Rockville, MD), which expresses a high and homogeneous level of cell surface HLA-DR detected by immunofluorescence. HPLC-purified wild-type and mutant TSST-1 protein were biotinylated, and various concentrations were incubated in the presence of 5 × 10^5 Raji cells on ice for 2 h in HBSS containing 0.1% BSA and 0.1% sodium azide. After washing, bound TSST-1 was detected by incubation with PE-avidin, and fluorescence intensity was analyzed on a cytofluorograph. At least 2 × 10^4 viable cells were evaluated for binding and compared to cells

incubated without TSST-1, and then stained and analyzed in a similar manner.

Results and Discussion

To identify the class II MHC and TCR binding sites on TSST-1, we generated random mutations throughout the entire 194-residue mature protein. The initial screening for mutant proteins was based on the ability of a murine Vβ15-expressing T cell hybridoma to secrete IL-2 in response to the wild-type recombinant TSST-1 expressed in *E. coli*. Lysates of individual bacterial clones that failed to stimulate IL-2 production were then screened by ELISA for the presence of toxin. Clones that failed to produce toxin or produced toxin at <25% of the wild-type levels were eliminated from further analysis.

The frequency of mutations resulting in nonstimulatory toxins varied greatly in different regions of the molecule (Table 2). Two functionally important regions were revealed. The first region, defined by libraries 1–3, corresponds to residues 1–47 of the mature TSST-1 protein. Of 846 transformants screened within this region, 35 clones (4.1%) failed to stimulate the murine T cell hybridoma. The second region comprises residues 91–152 (libraries 7–10). Here, 155 of 1,646 clones (9.4%) were negative for murine T cell hybridoma activation. We found no mutants affecting T cell stimulation between residues 46 and 92 and only one mutant between residues 151 and 194.

Clones of interest were analyzed by DNA sequencing to identify mutations and then tested for their capability to stimulate human PBL (Table 3). Since TSST-1 stimulates human Vβ2$^+$ T cells, many of the mutants were also tested against a human Vβ2$^+$ T cell line with results identical to those obtained with human PBL (data not shown). Although many mutants that were negative in the murine T cell hybridoma stimulation assay showed partial to full activity with human responding PBL, several of the mutant toxins were completely devoid of human T cell stimulatory activity. These included mutants with amino acid substitutions of Tyr13 → Leu and Ser15 → Trp (designated Y13L/S15W), G16V, G31S/S32P, all mutants at residues H135 and L137V, and several at residue Q139.

The differences in murine and human T cell stimulation by certain TSST-1 mutants may reflect the extensive amino acid polymorphism between the human Vβ2 and murine Vβ15 TCR (5) and/or differences in the two assay systems used to measure T cell proliferation. A similar observation was made for a number of staphylococcal enterotoxin B (SEB) mutants that failed to stimulate any murine T cell hybridomas (17) but strongly stimulated human T cells (Kappler, J. W., and B. L. Kotzin, unpublished work). The structural similarities between murine Vβ15 and human Vβ2 that allow subsets expressing these Vβs to be selectively targeted by TSST-1 are not currently known.

To further characterize the mutations that abolished human PBL stimulatory activity and to dissect the effects of the mutations on TCR and MHC binding, several mutant proteins of interest (Y13L/S15W, G16V, I45V, G31S/S32P, T128S/V88A, H135Q, H135R, L137V, Q139K, and Q139P) were purified by ion-exchange HPLC. A quantitative proliferation assay of the purified mutant toxins with responding human PBL revealed that all of these mutants were 10^4–10^6-fold less active than the wild-type TSST-1 (Fig. 1 *A*), except for I45V, which showed 100-fold decreased activity (Fig. 1 *B*). Interestingly, even very conservative side-chain changes such as G16V, H135Q, and L137V in TSST-1 dramatically decreased its T cell–stimulatory activity.

Table 2. *Frequency of Mutant TSST-1 Proteins Unable to Stimulate Murine Vβ15$^+$ T Cells*

Library number	TSST-1 residues subject to mutation	Murine Vβ15 stimulation	Percentage
		Functionally defective/total studied	
1	1–17	10/282	3.5
2	16–32	17/282	6.0
3	31–47	8/282	2.8
4	46–62	0/94	0.0
5	61–77	0/94	0.0
6	76–92	0/60	0.0
7	91–107	6/282	2.1
8	106–122	7/282	2.5
9	121–137	35/429	8.2
10	136–152	107/653	16.4
11	151–167	0/94	0.0
12	166–182	1/107	0.9
13	181–194	0/35	0.0

Table 3. *Functional Effects of TSST-1 Mutations*

Murine Vβ15 nonstimulatory mutants	Stimulation of human PBL*	Binding to HLA-DR‡
L10Q/D11V	+ + + +	
Y13L/S15W	−	+ + + +
S14I	+ +	
S15G	+ + + +	
G16V	−	+ + + +
T21R	+ + + +	+ + + +
G31S/S32P	−	−
I45V	+ + +	+ + +
P97L/L102V	+ + + +	
L102S	+ + + +	
L113F/K114T	+ + + +	
W116R, S	+ + +	
S127F	+	
T128S/V88A§	+	+ + +
F131I, L, V, Y	+ + + +	+ + + + (F131Y)
E132A, G, I, P, R, V, W	+ + (E132W), + + + +	
E132A/Q136L	+ +	+ + + +
R134L	+	
H135A, D, I, Q, R	−	+ + + + (H135Q, R)
Q136R	+ + +	+ + + +
L137V	−	+ + + +
T138I	+	+ + + +
Q139E, H, K, L, P, R	+ +, − (Q139K, P)	+ + + + (Q139K, P)
Q139E/L143S	−	+ + + +
Q139E/S147R	+	
Q139L/Y144F	−	+ + + +
Q139L/Y144N	+	
I140K, R, T, V	+ + + +	+ + + + (I140R, T)
H141D, L, N, P, R, Y	+ + + +	+ + + + (H141N)
H141L/S147G	+ + + +	
R145H, P, S	+ + + +	+ + + + (R145H)
T150P	+ + + +	

All mutant proteins listed here, except for mutations at residues 132, failed to stimulate the murine Vβ15 T cell hybridoma yet produced wild-type levels of TSST-1 proteins as determined by ELISA. Mutant proteins with more than two amino acid substitutions were eliminated from the table.
*Toxin concentrations ranging from 2.5 pg to 250 ng/ml were tested. Data from the 2.5-ng/ml dose (see Fig. 1) are presented as: + + + +, 75–100%; + + +, 50–75%; + +, 25–50%; +, 10–25%; and −, <10% of the response to wild-type toxin.
‡Toxin concentrations from 0.1 ng to 100 μg/ml were tested, with data obtained using the 0.1 μg/ml dose presented as: + + + +, 75–100%; + + +, 50–75%; + +, 25–50%; +, 10–25%; and −, <10% of the binding obtained using wild type TSST-1. Blank spaces indicate that those mutants were not tested for binding to HLA-DR.
§Although only residues 121–137 were targeted for mutagenesis, a second mutation (V88A) was found by DNA sequencing, presumably due to a PCR error.

HLA-DR binding studies using biotinylated TSST-1 directly demonstrated that the G31S/S32P mutations markedly diminished class II MHC binding, whereas the I45V mutation had only a slight effect (Fig. 2). The residual MHC binding of the G31S/S32P mutant may account for its residual T cell–stimulatory activity at high concentration on human PBL (Fig. 1 *B*). To dissect the effect of mutations at residues 31 and 32 on MHC binding, single amino acid mutants G31S and S32P were generated. The former mutant stimulated human PBL similarly to the wild-type TSST-1,

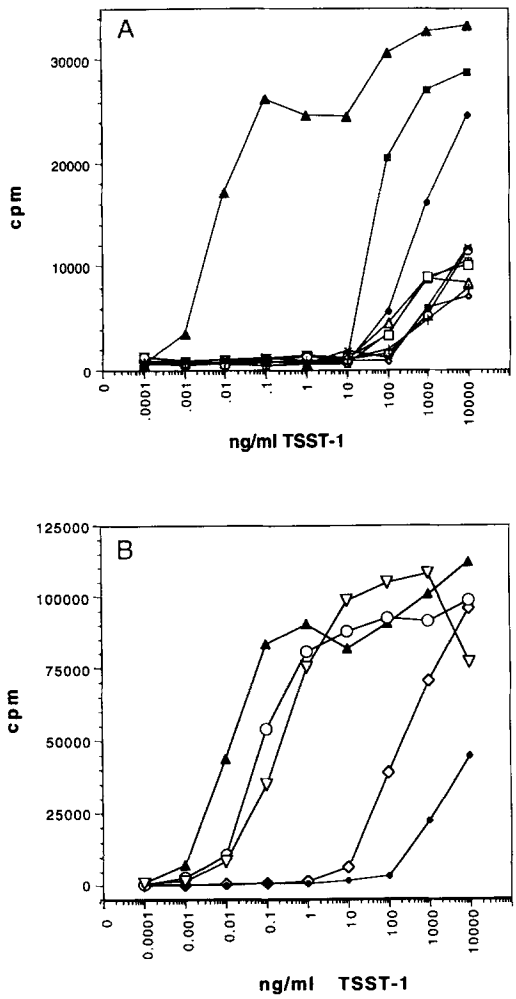

Figure 1. Proliferative response of human PBL to HPLC-purified mutant and wild-type TSST-1 proteins. Stimulation assays were performed as described in Materials and Methods. (*A*) Comparison of mutants that affect TCR binding (except for G31S/S32P) —◇—, Y13L/S15W; —△—, G16V; —◆—, G31S/S32P; —■—, T128S/V88A; —⊞—, H135Q; —□—, H135R; —○—, L137V; —x—, Q139K; —+—, Q139P; —▲—, wild-type rTSST-1. (*B*) Comparison of mutants that affect MHC binding. —○—, G31S; —◇—, S32P; —◆—, G31S/S32P; —▽—, 145V; —▲—; wild-type rTSST-1.

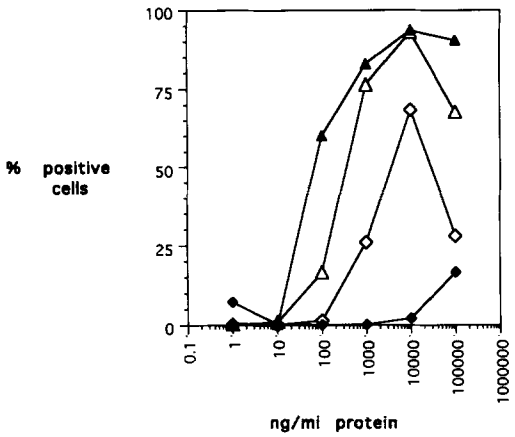

Figure 2. The binding of G31S/S32P, S32P, I45V, and wild-type TSST-1 proteins to Raji cells that express class II MHC on the cell surface. A mutant Raji cell line, which does not express class II MHC, demonstrated undetectable binding of the wild-type TSST-1 at concentrations as high as 100 μg/ml (data not shown). In the experiment shown, binding was detected using biotinylated mutant and wild-type TSST-1 proteins and streptavidin-PE as secondary reagent. In separate experiments, identical results were obtained using unlabeled toxins and a biotinylated anti-TSST-1 mAb as detecting reagent (data not shown). Presented data show the percentage of cells staining positively over background controls. In addition, fluorescence intensity, assessed by the increase in mean channel fluorescence over background, was negligible for the G31S/S32P double mutant, ~threefold for the S32P single mutant, and 15-fold for wild-type TSST-1 at optimal binding concentrations. —▲—, TSST-1^{wt}:biotin; —◆—, G31S/S32P:biotin; —◇—, S32P:biotin; —△—, 145V:biotin.

whereas the latter was 10^4-fold less active than the wild-type protein (Fig. 1 *B*). Likewise, binding experiments showed that S32P was impaired in HLA-DR binding by four orders of magnitude (Fig. 2), whereas G31S showed little difference from wild type (data not shown). Thus, residue 32 accounts for most, but not all, of the impaired interaction with class II MHC observed with the double mutant.

Fig. 3 illustrates the locations on the three-dimensional structure of TSST-1 (11) of mutations that had the greatest effect on human T cell–stimulatory activity (see mutants indicated as negative for human PBL-stimulatory activity in Table 3). These mutations are unambiguously clustered within two sites: a TCR binding site comprising both the proximal NH_2- and COOH-terminal domains (residues 13–16 and 135–139, respectively) and a class II MHC binding site within the NH_2-terminal domain (residues 31–45). As shown in Table 3, additional mutants that knocked out murine but not human T cell stimulation were identified. If these mutations are also considered, the TCR binding site consists of the central long α helix (residues 127 to 150) and the NH_2-terminal short α helix along with its subsequent loop (residues 10–16). In addition, a portion of the loop between the β7 and β8 sheets (residues 97–116) influences TCR binding. Thus, the TCR binding site identified by murine T cell responses precisely overlaps with the region comprising the major groove in the TSST-1 molecule (10, 11). Our results indicate that amino acid residues at positions 13 and/or 15, 16, 135, 137, and 139 appear to be the most important for TCR binding, because mutations in these positions almost completely abolished the stimulatory activity of TSST-1 for both murine and human T cells. Most notably, the residues at positions 16, 135, and 139 are located on the protein surface (10, 11), and therefore these residues might directly interact with the TCR. On the other hand, residue 137 is buried within the protein and, therefore, the effect of this mutation on TCR binding would be indirect, presumably because of a distortion of local structure.

The MHC binding site localizes to the region in the vicinity of the NH_2-terminal portion of the second β strand (Fig. 3). The five β strands in this region form a structure

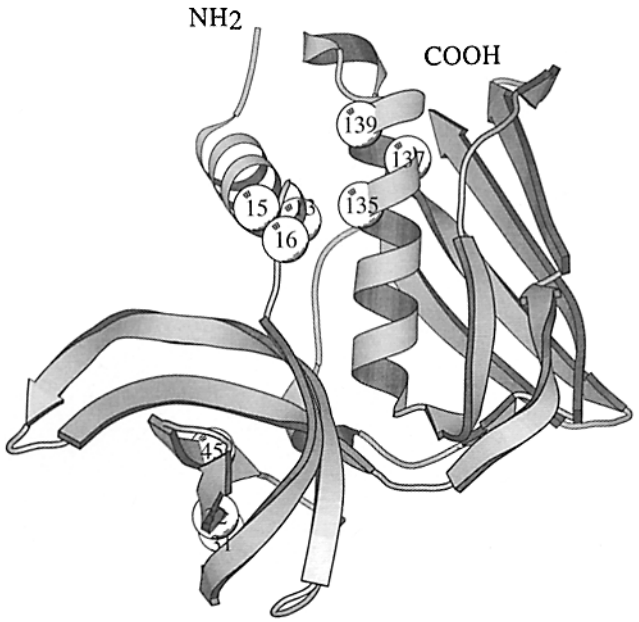

Figure 3. A ribbon diagram of TSST-1 showing the locations of mutations that eliminated human T cell stimulatory activity (mutants indicated as negative in Table 3) by preventing interactions with either class II MHC molecules or with the TCR Vβ chain. Although the I45V mutant does not completely satisfy these criteria, it is included in the figure because of its effect on MHC binding. The figure was kindly prepared by Dr. Douglas Ohlendorf (University of Minnesota, Minneapolis, MN).

known as a β–barrel motif (10, 11). Mutations G31S/S32P, which greatly impair HLA-DR binding, are located in a solvent-exposed hydrophobic surface formed by the concave face of β–barrel strands 1, 2, and 3 in the NH_2-terminal domain. The β–barrel motif in the NH_2-terminal domain of TSST-1 is similar to that in the corresponding domain of SEB. In SEB, nonpolar residues F44, L45, and F47 form a ridge that protrudes from the loop between β-strands 1 and 2 in the NH_2-terminal domain, and this ridge interacts with a hydrophobic depression in the HLA-DR1 molecule (21, 22). Mutations in SEB at F44 disrupt binding to HLA-DR (17). Similarly, our study suggests that this hydrophobic region of TSST-1 may form the MHC binding site. The I45V mutation (on β-strand 3) also affects MHC binding, although its effect is not as significant as G31S/S32P (Fig. 1 *B* and Fig. 2). In contrast to the mutations of G31S and S32P, which locate on the protein surface, the isoleucine at position 45 is within the protein interior (10, 11). It is likely, therefore, that the I45V mutation indirectly affects MHC binding by perturbing the conformation of the hydrophobic surface.

Binding of both TSST-1 and SEB to HLA-DR are affected by the same mutations in the DR α-chain, most notably at residue K39 (23, 24). Although we found only a single MHC binding site in TSST-1, mutational analysis of SEB predicted two class II MHC binding sites on opposite sides of the molecule (17). This may suggest that, whereas TSST-1 and SEB interact with the same region of the HLA-DR1 α-chain, SEB can use a second distinct binding site.

Our findings are consistent with and greatly extend previous studies of TSST-1 mutations that affect T cell mitogenicity and toxicity (16, 25–27). For example, TSST-ovine is a naturally occurring variant that is different from TSST-1 at seven amino acids, including E132K and I140T. These changes have been predicted to be responsible for its reduced toxicity in rabbits (25). Furthermore, the mutant H135A was identified in a previous study as lacking murine T cell–stimulatory activity (27). Our studies show that these mutations are centrally located in the TCR binding site.

TSST-1 has been implicated in ~90% of menstruation-related and nearly half of nonmenstrual cases of toxic shock syndrome (2, 4). In addition, some studies have suggested that TSST-1 may be an etiologic agent in some cases of Kawasaki syndrome (20, 28), an inflammatory syndrome of children, although this remains controversial (29). Different superantigens have also been suggested as triggers for other autoimmune diseases (30–32). The present studies provide a basis for designing immunomodulatory molecules that interfere with superantigen activity and vaccines that prevent the harmful effects of these potent immunostimulatory molecules.

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Address correspondence to Dr. Masazumi Matsumura, Supragen, Inc., 1670 Pierce Street, Lakewood, CO 80214.

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