A Toxic Shock Syndrome Toxin 1 Mutant That Defines a Functional Site Critical for T-Cell Activation

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Toxic shock syndrome toxin 1 (TSST-1), a superantigen produced by *Staphylococcus aureus*, is a causative agent of toxic shock syndrome (TSS). This superantigen is a potent stimulator of T cells and macrophages/monocytes, resulting in the release of cytokines that are implicated in the pathogenesis of TSS. This study characterizes a mutant TSST-1, derived by site-directed mutagenesis, that has an alanine substitution at histidine 135 (mutant 135). This single-amino-acid change results in a mutant toxin that has lost mitogenic activity for T cells. In contrast to wild-type TSST-1, this mutant does not induce T cells to express interleukin-2, gamma interferon, or tumor necrosis factor beta (TNF- β). The inability of mutant 135 to activate T cells is not due to a lack of binding to the class II major histocompatibility complex receptor. In addition, the mutant TSST-1 does not induce expression of TNF- α , which plays a role in the development of lethal shock. The lack of TNF- α induction by mutant 135 is likely due to its inability to activate T cells. These data suggest that the mutation at histidine 135 in TSST-1 affects toxin interactions with the T-cell receptor rather than the class II major histocompatibility complex receptor.

Toxic shock syndrome toxin 1 (TSST-1) is a 22-kDa exotoxin produced by *Staphylococcus aureus* (4, 35). This toxin has been implicated in the development of menstrually associated TSS (4, 11, 12, 30, 34, 35). This protein has profound effects on murine and human immune systems, precipitating massive T-cell stimulation (32). In humans, this activation can lead to shock and in some cases death. It has been described, along with other staphylococcal enterotoxins, as a superantigen (22).

Superantigens differ from conventional antigens according to several criteria. Unlike conventional antigens, processing is not required for T-cell activation (13, 22, 29). Binding to the class II major histocompatibility complex (MHC) receptor at a site outside the conventional peptide binding groove is necessary prior to recognition by T cells (19, 22). Superantigens stimulate a larger proportion of the T-cell population than do conventional antigens because the specificity of interaction is largely determined by the V β element of the T-cell receptor (TCR) (22). The high frequency of activated T cells leads to overproduction of cytokines, such as interleukin-2 (IL-2) (24, 37) and gamma interferon (IFN- γ) (20) from T cells and IL-1 (17, 18) and tumor necrosis factor (TNF) (14, 18) from macrophages and monocytes. These cytokines have been implicated in the pathogenesis of TSS and other superantigen-induced diseases (22, 25, 26). The capacity of this toxin to engage both the class II MHC molecule and TCR suggests the existence of separate functional domains within the molecule. Distinct sites on the toxin molecule that interact with both receptors have been proposed from the recently described crystal structure of TSST-1 (1, 33).

In order to gain a better understanding of the molecular mechanisms by which superantigens cause disease, a structure-function study of TSST-1 was undertaken. Previous mutagenesis of TSST-1 had yielded a mutant toxin (mutant 135) which

was totally devoid of mitogenic activity and did not cause lethal shock in a rabbit model of TSS (8). This mutant toxin contained a single-amino-acid (histidine-to-alanine) substitution at position 135. This paper addresses the basis for the effects of this mutation on this toxin. We present evidence that mutant 135 is defective in its interactions with the TCR rather than the class II MHC molecule. The resulting loss of T-cell activation and lack of cytokine production account for the loss of biologic activities in mutant 135.

MATERIALS AND METHODS

Construction of mutant 135 and expression and purification of recombinant toxins. Site-directed mutagenesis was used to construct mutant 135 in M13 vectors as previously described (7). The sequence of the mutagenic primer was 5'-ATTCGTCGCTCAGTAACT3'. Underlined bases indicate the mutation that was introduced to result in the alanine substitution at position 135. The toxin gene (encoding from the serine residue at position +1 of the mature protein through the end of the C terminus) was introduced into a pT7-7-based expression vector as previously described (9). Plasmids were transformed into *Escherichia coli* BL21(DE3) and selected for ampicillin resistance. Expression of recombinant toxins was assessed by Coomassie brilliant blue staining of sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) gels of periplasmic contents from overnight cultures of *E. coli*. Purification of toxins was performed with a C₁₈ reverse-phase high-pressure liquid chromatography column (Waters Associates, Milford, Mass.) as previously described (7). Recombinant wild-type TSST-1 (rTSST-1) and mutant 135 eluted as a single, symmetrical peak. Fractions that contained toxins were dialyzed extensively against phosphate-buffered saline and were pure, as assessed by a single visible band on Coomassie brilliant blue-stained SDS-PAGE gels. A Bradford protein assay (Bio-Rad Laboratories, Richmond, Calif.) was used for quantitation of purified toxins.

All subsequent experiments utilized recombinant toxins that had been purified from *E. coli*. These toxins were indistinguishable from those that had been prepared from *S. aureus* (8) in their reactivities with murine spleen cell and TSST-1-specific monoclonal antibodies (MAb) and polyclonal antibodies on immunoblots.

Toxin binding assay. Measurement of toxin binding to the class II MHC molecule was done with the murine B-lymphoma cell line, A20. Purified toxin at various concentrations was incubated with 10⁵ A20 cells in Hanks' balanced salt solution (Gibco-BRL, Bethesda, Md.) with 20 mM HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid), 5% fetal calf serum (Gibco-BRL), and 0.01% sodium azide at 4°C for 30 min. Cells were washed with this Hanks' buffer three times to remove unbound toxins. Cells were incubated as described before with polyclonal antibody specific for TSST-1 (Toxin Technology, Sarasota, Fla.) (1/400 dilution; 100 μ l per tube), and specifically bound polyclonal antibody was detected with fluorescein isothiocyanate-conjugated goat anti-rabbit antibody

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(Zymed, South San Francisco, Calif.). Fluorescence intensity was measured by flow cytometry (FACScan; Becton Dickinson). Background fluorescence was determined by incubating A20 cells with anti-TSST-1 polyclonal antibody and the fluorescein-labeled secondary antibody without toxin. Data are representative of several experiments.

To ascertain that toxins bound through the class II MHC receptor, an anti-class II monoclonal antibody (M5/114; Boehringer Mannheim Biochemicals, Indianapolis, Ind.) was utilized. For these experiments, 2 μ g (determined to be optimal) of antibody was incubated (30 min at 4°C) with A20 cells prior to the addition of toxin. As a control, rat immunoglobulin (Sigma Chemical Company, St. Louis, Mo.) that had been added to A20 cells prior to the addition of toxin had no effect on toxin binding (data not shown).

IL-2 assay. Homogenized spleens from BALB/c mice (Harlan Sprague Dawley, Indianapolis, Ind.) were dispensed at 10^6 cells per well into 96-well flat-bottom tissue culture plates (Corning Glass Works, Corning, N.Y.) with purified toxins at various concentrations. Polymyxin B sulfate (Sigma) was added to all cultures at a final concentration of 15 μ g/ml to neutralize the effect of lipopolysaccharide (LPS), although its addition had no effect on mitogenesis results. After incubation for 48 h (37°C, 5% CO₂), culture supernatants were collected and stored frozen at -70°C until assayed. To measure IL-2, 100 μ l of thawed supernatant was added to 5×10^3 IL-2-dependent CTLL-2 cells in 200 μ l total volume. Cells were incubated for 24 h, pulsed for 8 h with 1 μ Ci of [³H]thymidine (35 Ci/mmol; ICN Radiochemicals, Irvine, Calif.) per well, and harvested, and [³H]thymidine incorporation was measured by liquid scintillation counting. Data are the means of triplicate determinations $\pm$ standard deviations.

IFN- γ assay. Murine spleen cells were prepared as described above for the IL-2 assay and stimulated for 72 h (determined to be optimal) with toxins at various concentrations. IFN- γ was quantitated with a commercial enzyme-linked immunosorbent assay (ELISA) kit (Genzyme, Cambridge, Mass.).

Preparation of TNF supernatants. Murine spleen cells were used to generate supernatants as described above for the IL-2 assay. Experiments included 15 μ g of polymyxin B sulfate per ml to neutralize any effects of contaminating LPS. Cells were stimulated with toxins at various concentrations for 48 h, and plates were frozen at -70°C. As a positive control for TNF induction, LPS (Sigma) was used at 1 μ g/ml in the absence of polymyxin B sulfate. The TSST-1-specific MAb 8-5-7 (10) was used to show that TNF was specifically induced by TSST-1. For these experiments, toxin was preincubated with purified antibody for 30 min at 37°C prior to the addition of toxin to spleen cell cultures. Thawed culture supernatants were used as described below for TNF quantitation.

For T-cell depletion experiments, single-cell spleen suspensions (10^8 cells per ml) were incubated for 30 min on ice with either undiluted immunoglobulin M anti-Thy 1.2 antibody, HO-13-4-9 (23), or medium. After being washed, cells were incubated with a 1/10 dilution of Low-Tox rabbit complement (Accurate Chemical and Scientific Corp., Westbury, N.Y.) at 37°C for 30 min. T-cell-depleted and control cell suspensions were washed and plated on 96-well plates at 10^6 cells per well. To verify depletion of T lymphocytes and test for any possible toxicity in this treatment, aliquots of treated spleen cells were incubated with concanavalin A (Sigma) or LPS for 48 h. Then cultures were pulsed with 1 μ Ci of [³H]thymidine for 18 h and harvested. Cells that had been treated with complement only responded vigorously to concanavalin A, whereas anti-Thy 1.2-treated spleen cells did not. Both complement-treated and anti-Thy 1.2-depleted cells proliferated in response to LPS.

Quantitation of TNF. TNF in thawed supernatants was quantitated by using either an ELISA kit (Genzyme) or the L929 biologic assay (16). For L929 assays, 50 μ l of culture supernatant or a murine TNF standard (Genzyme) along with 750 ng of actinomycin D (Sigma) per well was added to 2×10^5 L929 cells on 96-well culture dishes. Assay plates were incubated overnight at 37°C, medium was removed, and 100 μ l of a 0.5% crystal violet-25% methanol mixture was added to each well. After 30 min at room temperature, the stain was decanted, plates were rinsed with water and air dried, and the stain was eluted by adding 100 μ l of 100% methanol. The optical density of extracted dye was read at 570 nm, and quantitation of TNF in supernatants was based on comparison to a standard curve. Data are means of triplicate determinations $\pm$ standard deviations. As a control for specificity of the L929 assay, the lytic activities of supernatants were neutralized with an anti-TNF antibody (Pharmingen, San Diego, Calif.). This antibody, diluted 1/2,000, was preincubated with supernatants for 30 min prior to its addition to L929 assay plates. The anti-TNF antibody completely neutralized the lytic activities of supernatants, indicating that the assay was specific for TNF. In addition, L929 cells showed no sensitivity when they were cultured directly with either toxin.

cDNA synthesis and PCR amplification. Murine spleen cells, prepared as described above for the IL-2 assay, were stimulated for 6, 8, 18, 24, and 48 h with toxin. The peak levels of TNF- α and TNF- β mRNA expression occurred at 24 h; therefore, the data for this time point are presented. Cells were stimulated with 100 ng of either rTSST-1, mutant 135, or culture medium per ml, as indicated. Cultures contained 15 μ g of polymyxin B sulfate per ml. RNA was prepared from cells with RNAzol (Tel-Test, Inc., Friendswood, Tex.). Single-stranded cDNA was prepared with approximately 5 μ g of total RNA-15 pmol of oligo(dT) (Boehringer Mannheim)-0.01 M dithiothreitol-200 mM deoxynucleoside triphosphates (dNTPs)-0.5 U of placental ribonuclease inhibitor (Gibco-BRL) with 200 U of Superscript reverse transcriptase (Gibco-BRL) in a 20- μ l volume. Reaction mixtures were incubated at 37°C for 1 h.

FIG. 1. Flow cytometry analysis of toxin binding to class II MHC molecules on the surfaces of A20 cells and abrogation of binding with an anti-class II MAb. (A) rTSST-1; (B) mutant 135. Data are representative of four experiments.

The following sequences of primer sets for PCR amplification were used: TNF- α , 5'-CCAGACCCTCACACTCAGAT3' and 5'-AACACCCATTCCCTTCACAG3'; TNF- β , 5'-GCTTGGCACCCTCTGT3' and 5'-AAGTGTAGATGGGAGATGCCGT3' (2); β -actin, 5'-GTGGGCGCTCTAGGCACCA3' and 5'-CGGTTGGCCTTAGGGTTCAGGGGGG3'. Each target cDNA was amplified separately in a 50- μ l volume that contained 1.25 U of AmpliTaq DNA polymerase (Perkin-Elmer Cetus, Norwalk, Conn.), 200 mM each dNTP, and 0.5 mM each primer. Water controls were routinely performed for each primer set, and no amplification was observed. Samples were cycled through 94°C for 1 min, 58°C for 1 min, and 72°C for 1 min for 30 cycles. One-tenth of each reaction was resolved on 1.2% agarose gels and visualized with ethidium bromide staining. The predicted sizes of these PCR products were as follows: TNF- α , 497 bp; TNF- β , 533 bp; and β -actin, 240 bp. All amplifications were adjusted to contain equivalent amounts of starting cDNA, based on initial normalization with β -actin. Data are representative of several experiments with independently generated RNA samples.

RESULTS

Binding of mutant 135 to class II MHC receptors on A20 cells. Because engagement of both the class II MHC molecule and TCR are requirements for T-cell activation by superantigens, the defect in mutant 135 may be in its interaction with either cellular receptor. To examine if mutant 135 interacts with class II MHC molecules, the binding of recombinant toxins to A20 (a murine B lymphoma) was tested. Flow cytometry analysis shows that the mutant appears to bind to A20 cells as well as the wild-type toxin does at concentrations as low as 0.1 μ g/ml (Fig. 1). At all three concentrations of toxin tested, the

FIG. 2. Incorporation of [3 H]thymidine into IL-2-dependent CTLL-2 cells in response to supernatants that had been generated by stimulating splenic cultures with increasing amounts of mutant 135 and wild-type toxin (rTSST-1). Mutant 135 fails to induce expression of IL-2 from murine spleen cells, as indicated by the background level (52 ± 8 cpm) of [3 H]thymidine incorporation. Standard deviations are also shown.

level of both rTSST-1 and mutant 135 binding is dose responsive.

Because it has been shown that superantigens can stimulate MHC-independent T-cell responses (3), it was of interest to demonstrate that the binding of toxin on A20 cells occurred through the class II MHC receptor. Incubation of cells with a MAb to class II MHC (M5/114) prior to toxin addition reduces the binding of both rTSST-1 and mutant 135. When 10 μ g of toxin per ml was used, there was not a complete abrogation of toxin binding. However, at 1 and 0.1 μ g of toxin per ml, this antibody reduced the mean fluorescence to background levels for both rTSST-1 and mutant 135, indicating that binding interaction occurs through the class II MHC receptor.

Expression of IL-2 and IFN- γ from murine spleen cells that have been stimulated with mutant 135. Binding data indicate that mutant 135 maintains the ability to interact with the class II MHC receptor, therefore suggesting that failure to activate T cells is due to a defect in interaction with the TCR. Signaling through the TCR can lead to cytokine production in the absence of T-cell proliferation (6, 27). This can result in the differential loss of specific cytokine production. The expression of cytokines from T lymphocytes in response to mutant 135 was measured to examine these possibilities. rTSST-1 consistently induces substantial levels of IL-2 at all of the concentrations tested (Fig. 2). In contrast to wild-type toxin, mutant 135 has a major defect in its ability to induce IL-2. The level of IL-2 production induced at high concentrations of mutant 135 (1,000 ng/ml) is substantially lower than that induced by a 1,000-fold-lower concentration of wild-type toxin. The lack of IL-2 production is consistent with loss of mitogenic activity.

rTSST-1 toxin induces levels of IFN- γ that are approximately 25-fold greater than the lowest detectable background level at a concentration of 1 ng/ml (Table 1). The induction of IFN- γ by rTSST-1 is dose responsive and shows maximum expression (approximately 19,000 pg/ml) at 1 μ g of toxin per ml. In contrast, IFN- γ induction by mutant 135 is reduced by greater than 99%. Thus, the loss of mitogenic activity by mutant 135 is concomitant with the loss of both IL-2 and IFN- γ induction.

TABLE 1. Expression of IFN- γ from murine spleen cells, as measured by ELISA

Concn of toxin (μ g/ml)	Mean IFN- γ $\pm$ SD (pg/ml) ^a	
	rTSST-1	Mutant 135
10	16,667 $\pm$ 2,723	476 $\pm$ 51
1	19,866 $\pm$ 2,203	<128
0.1	14,667 $\pm$ 2,603	<128
0.01	9,733 $\pm$ 1,222	<128
0.001	3,480 $\pm$ 1,185	<128

^a Medium control value was <128 pg/ml.

Attempts were made to measure IL-4 induction from both rTSST-1- and mutant 135-stimulated splenic cultures. However, expression of IL-4 was not detected, as measured, for an IL-4-dependent cell line (CT.4S; sensitivity was 1.25 U of recombinant IL-4) in response to either rTSST-1 or mutant 135. Most likely, the production of IFN- γ inhibited the TH2 pattern of cytokine production in these cultures.

Mutant 135 fails to induce expression of TNF by murine splenocytes. Previous reports have indicated that TSST-1 stimulates macrophages and monocytes to produce TNF as a result of direct interaction between this toxin and the class II MHC receptor (14, 18). Thus, mutant 135 binding to the class II MHC receptor could potentially stimulate TNF expression. However, the role of T cells in TNF induction by TSST-1 is unclear. In one report, TNF production from a highly purified preparation of human monocytes that had been stimulated with TSST-1 required the presence of T cells (36). In addition, the requirement for T cells did not involve IFN- γ . Consistent with the latter observation, TNF production from thioglycolate-induced peritoneal macrophages that had been stimulated with TSST-1 (0.1 to 100 μ g/ml) was not observed (unpublished data). The addition of IFN- γ (100 to 500 U/ml) did not alleviate the requirement for T cells, as has been reported for other superantigens (15, 21). Thus, a murine spleen cell culture system was used to characterize induction of TNF by both wild-type rTSST-1 and mutant 135.

rTSST-1 induces TNF from spleen cells in a dose-responsive manner over the background level (<50 pg/ml) (Fig. 3A). TNF expression is reduced by the addition of the TSST-1-specific MAb 8-5-7 during incubation, indicating that TNF production is TSST-1 specific (Fig. 3B). TNF induction by LPS is unaffected by the addition of MAb 8-5-7. In contrast to rTSST-1, mutant 135 does not induce levels of TNF over background levels, even at concentrations of 10 μ g/ml (Fig. 3A). These results provide indirect evidence that the activation of T cells in these splenic populations was required for TNF induction by TSST-1.

To test more directly whether T cells were required for TNF induction by TSST-1, spleen cell populations were depleted of T cells by using an anti-Thy 1.2 antibody and complement-mediated lysis. When rTSST-1 was used to stimulate cultures that had been depleted of T cells, TNF expression was no longer evident (Table 2). These results provide further evidence that T cells are required for production of TNF by TSST-1 and that the lack of T-cell activation by mutant 135 results in the loss of TNF induction.

PCR was used to analyze expression of TNF- α and TNF- β mRNAs from spleen cells that had been stimulated with toxins to further test the effects on cytokine production by mutant 135. TNF- α is primarily expressed by macrophages and monocytes, whereas TNF- β is expressed by T cells (5, 31). Figure 4 indicates that after 24 h, rTSST-1-stimulated cells show PCR

FIG. 3. Expression of TNF in supernatants that had been generated by stimulating splenic cultures with mutant 135 and rTSST-1, as measured by ELISA. (A) Production of TNF by rTSST-1 (hatched bars) and mutant 135 (solid bars). Mutant 135 shows background levels (<50 pg/ml) of TNF at all of the concentrations tested. (B) TSST-1-specific MAb 8-5-7 abrogated expression of TNF from rTSST-1-stimulated cells but did not affect LPS-induced TNF expression. Standard deviations are also shown in each panel.

products of the sizes predicted for both TNF- α and TNF- β . In contrast, both control medium- and mutant 135-stimulated cells show no visible PCR products for either TNF- α or TNF- β . This is consistent with our TNF ELISA data and provides further evidence that mutant 135 does not induce expression of TNF- α or TNF- β mRNA from either macrophages or T cells.

DISCUSSION

In this report, we have shown that a single-amino-acid change (histidine 135 to an alanine residue) results in a recombinant toxin that has greatly altered biologic activities. On the basis of the ability of mutant 135 to interact with a TSST-1-specific MAb on Western blots (immunoblots) and in competitive ELISAs (8), combined with its binding to class II MHC molecules, we conclude that no gross conformational change in the structure of mutant 135 can account for the loss of mito-

FIG. 4. Mutant 135 fails to induce expression of TNF- α and TNF- β mRNAs, as measured by PCR. Splenic cultures were stimulated for 24 h (determined to be optimal) with medium (control), rTSST-1, or mutant 135. Ethidium bromide-stained agarose gel of PCR products is shown. Lanes: 1, 3, and 5, TNF- α ; 2, 4, and 6, TNF- β ; 7, 8, and 9, β -actin. Molecular sizes (in base pairs) are given on the right.

genic activity. A more likely explanation is that the histidine-to-alanine substitution results in a localized change in the toxin molecule that precludes toxin interaction with the TCR, not the class II MHC receptor.

A previous report (8) showed that mutant 135 had lost mitogenic activity for murine spleen cells. The binding profiles of mutant 135 and rTSST-1 to A20 cells were identical. This shows that the histidine-to-alanine substitution at amino acid 135 does not detectably affect the binding of this toxin to the class II MHC receptor. Therefore, the loss of binding to class II MHC molecules does not account for the loss of mitogenic activity by mutant 135. The possibility that mutant 135 is defective in an interaction with class II MHC molecules that is not detected by binding studies cannot be excluded. However, the most likely explanation is that this mutation affects interaction between this toxin and the TCR.

The pattern of cytokine induction by mutant 135 was tested since T cells are able to produce cytokines after stimulation through the TCR in the absence of T-cell proliferation (6, 27). There is no evidence for this type of T-cell stimulation by mutant 135, as shown by its inability to induce either IL-2, IFN- γ , or TNF- β from T cells. In addition, the lack of mitogenic activity of mutant 135 with T cells is consistent with the lack of IL-2 production.

It was important to ascertain whether mutant 135 maintains the ability to induce TNF since this cytokine has a critical role in superantigen-induced lethal shock (25, 26). While a substantial amount of TNF was induced by rTSST-1, mutant 135 did not induce TNF production over background levels. This result is consistent with the observation that mutant 135 is nontoxic in a rabbit infection model of TSS (8).

This work also establishes that T cells are crucial for TNF induction by TSST-1. Murine splenocytes that had been depleted of T cells lost the ability to produce TNF in response to TSST-1. This corroborates the work of See et al. (36), which showed that TNF production from human peripheral blood monocytes requires the presence of T cells. Reports of TNF production from macrophages in response to TSST-1 may be due to contaminating T cells or perhaps LPS contamination of toxin preparations.

TNF- α and TNF- β mRNA expression in spleen cell cultures was also measured by PCR. TNF- β , which is predominantly

TABLE 2. Ablation of rTSST-1-induced expression of TNF from murine spleen cells after T-cell depletion

Concn of toxin (μ g/ml)	Mean TNF $\pm$ SD (pg/ml) ^a	
	Anti Thy 1.2 + complement	Complement only
10	<6.24	232.5 $\pm$ 66
1	<6.24	177 $\pm$ 96
0.1	<6.24	96.3 $\pm$ 8.9
0.01	<6.24	60 $\pm$ 9.1
0.001	<6.24	71.7 $\pm$ 9.2
0	<6.24	<6.24

^a As measured by L929 assay.

expressed by T cells (31), was induced by rTSST-1, but not by mutant 135. This result was expected and is consistent with the inability of mutant 135 to stimulate production of other T-cell-derived cytokines. In addition, mutant 135 did not induce TNF- α transcription in spleen cell cultures, despite its ability to bind class II MHC molecules. Collectively, these results imply that mutant 135 is not able to activate the T cells which are necessary for TNF- α production.

Several lines of evidence support the assertion that amino acid 135 is located in a TSST-1 domain that interacts with the TCR. In a previous report, we have shown that mutations at amino acids 115 and 141 result in toxins that are only partially mitogenic, compared with wild-type toxin (7). In addition, Murray et al. (28) have shown that mutations at amino acids 132 and 140 also reduce, but do not totally abolish, the mitogenic activity of TSST-1. These partial mutants must maintain the ability to interact with both the TCR and class II MHC molecules since this is a requisite for T-cell activation. The fact that both mutants 115 and 141 retain normal class II MHC binding (unpublished data) suggests that modification of either of these amino acids also affects normal toxin-TCR interactions. Although the binding profiles of mutants 132 and 140 were not reported (28), the close proximity of amino acids 115, 132, 135, 140, and 141 in the three-dimensional structure of TSST-1 (1, 33) suggests that these mutants also bind normally to the class II MHC receptor and constitute part of the TCR binding domain. However, amino acid 135 appears to be involved in a more critical interaction with the TCR than are residues 115, 132, 140, and 141, since its substitution totally ablates mitogenic activity while the other mutations yield toxins which retain partial mitogenic activities. The clustering of these mutations between amino acids 115 and 141 on the three-dimensional structure of TSST-1 (1, 33), all of which modify mitogenic activity, likely defines a biologically active domain in this toxin that interacts with the TCR. Furthermore, Acharya et al. report that amino acids 115, 132, 135, and 140 are solvent accessible (1), which is consistent with the ability of these residues to interact with the TCR. This site is distinct from the proposed class II MHC binding site on the TSST-1 molecule (1) and is in agreement with the binding data for mutant 135. It is not clear, however, from our results or the crystal structure of TSST-1 (1, 33) whether the mutation at residue 135 affects the local conformation of the TCR binding site or the residue is directly involved in binding the TCR.

Mutant 135 does not induce the production of cytokines IFN- γ , IL-2, TNF- α , and TNF- β , which is consistent with our previous report that mutant 135 is nontoxic in a rabbit infection model of TSS (8). It is clear from our previous studies (7, 8) and the more recent one of Murray et al. (28) that TSST-1 mutants which maintain partial T-cell-activating capacity are capable of causing death in rabbit models of TSS. Mutant 135, however, does not cause lethal shock in rabbits (8) or D-galactosamine-sensitized mice (9), even though it binds normally to the class II MHC receptor. It is likely that mutant 135 is nontoxic because of its failure to activate T cells to produce directly or to stimulate other cells to produce cytokines. This study and our previous studies (7, 8) support the notion that T-cell activation is a requirement for lethality in superantigen models of shock.

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