INFECTION AND IMMUNITY, June 1994, p. 2202–2207
0019-9567/94/$04.00+0

Vol. 62, No. 6

Intracellular Expression of Toxic Shock Syndrome Toxin 1 in *Saccharomyces cerevisiae*

ROBERT L. DERESIEWICZ,[1,2,3]* JESSE A. FLAXENBURG,[1] MELVIN CHAN,[4] ROBERT W. FINBERG,[3,4] AND DENNIS L. KASPER[1,3,5]

Channing Laboratory[1] and Division of Infectious Diseases,[2] Brigham and Women's Hospital, Laboratory of Infectious Diseases, Dana-Farber Cancer Institute,[4] Division of Infectious Diseases, Beth Israel Hospital,[5] and Department of Medicine, Harvard Medical School,[3] Boston, Massachusetts 02115

Received 10 January 1994/Returned for modification 26 February 1994/Accepted 9 March 1994

In order to search for an occult cytotoxic enzymatic activity of the toxic shock syndrome toxin 1 (TSST-1), we placed the gene encoding TSST-1 (*tstH*) under the control of an inducible promoter in the eukaryotic yeast *Saccharomyces cerevisiae*. Under similar circumstances, the known bacterial enzymatic cytotoxins Shiga-like toxin and diphtheria toxin are both highly lethal to the yeast host. Although full-length stable TSST-1 was demonstrated within the yeast cells and although it retained mitogenicity for human T cells, it had no apparent effect on the yeast cells' growth kinetics or on their gross morphology. Retrieval and sequencing of the toxin gene revealed the wild-type sequence throughout, thus demonstrating that the apparent lack of toxicity for the yeast cells was not due to a serendipitous attenuating mutation within the coding region of the toxin gene. Similar results obtained after a second transformation of the same strain and after transformation of an unrelated strain demonstrate that neither chance permissive host mutation nor intrinsic host resistance was likely to have obscured an existing cytotoxic property of TSST-1. We conclude that TSST-1 probably does not possess a discrete enzymatic property cytotoxic for eukaryotic cells.

The toxic shock syndrome (TSS) is an acute multisystem disease characterized by fever, erythroderma, profound hypotension, end-organ failure, and late desquamation. The majority of cases are associated with either colonization or superficial infection by strains of *Staphylococcus aureus* that elaborate an exoprotein called toxic shock syndrome toxin 1 (TSST-1) (17). It is the effect of that exoprotein, either alone or in concert with other factors, that is deemed to be ultimately responsible in most cases for the clinical syndrome of TSS (16).

TSST-1 is a single-chain polypeptide and is secreted in mature form following cleavage of a typical bacterial signal sequence (1, 19). Mature TSST-1 has a molecular mass of ~22 kDa and an isoelectric point of 7.2. It contains no disulfide bonds. It is a member of a larger group of related pyrogenic staphylococcal and streptococcal exotoxins that have been implicated in a variety of disease processes, including, among others, staphylococcal food poisoning, staphylococcal scalded-skin syndrome, and invasive group A streptococcal infection. TSST-1 shares with these toxins certain properties, including the abilities (i) to stimulate large numbers of T cells, not according to the epitope specified by the variable region of the T-cell receptor, but according to the receptor's V_β haplotype (thus, TSST-1 is a V_β-restricted T-cell mitogen); (ii) to provoke the release of the cytokines interleukin 1, tumor necrosis factor, and interferon by leukocytes; and (iii) to enhance the lethality of injected endotoxin for test animals. Although a precise mechanism has not yet been defined, some or all of these properties are considered to be important in the pathogenesis of TSS (2).

No conventional (i.e., enzymatic) toxic property has ever been ascribed to TSST-1. Nevertheless, speculation persists that in addition to its immunomodulatory properties, TSST-1 may be directly cytotoxic to certain cell types. Moreover, this putative cytotoxicity has been postulated to be responsible for a key feature of TSS: the massive capillary leak that underlies the hypotension and perhaps also the end-organ damage associated with this illness. Indeed, TSST-1 has been found to bind with high affinity to cultured human epithelial cells and to be rapidly internalized by them (11). While it is not cytotoxic for most of the cell lines against which it has been tested, TSST-1 also binds cultured porcine aortic endothelial cells (PAECs) and kills them in a time- and dose-dependent fashion. Finally, it enhances the permeability of PAEC monolayers to albumin flux, again in a time- and dose-dependent fashion (14). Might TSST-1 have an as-yet-unrecognized enzymatic property that accounts for this apparent cytotoxicity?

In order to address this question, we engineered the *tstH* gene for inducible intracellular expression in the eukaryotic yeast *Saccharomyces cerevisiae*. We reasoned that if TSST-1 is cytotoxic to eukaryotic cells by virtue of an unrecognized enzymatic property and if its cytotoxicity has eluded widespread recognition because it is predicated on the presence of a particular receptor that is not broadly expressed in mammalian cells, then that cytotoxicity might be unmasked in this fashion. In this report we show that, in fact, intracellular expression of TSST-1 is well tolerated in *S. cerevisiae*, and therefore we argue that TSST-1 probably does not possess a discrete enzymatic property cytotoxic for eukaryotic cells.

MATERIALS AND METHODS

Plasmids and strains. pRN6550 contains the intact *tstH* gene on a 1,575-bp *Mnl*I fragment in the *Nru*I site of pBR322 and was kindly provided by Barry Kreiswirth and Richard Novick (Public Health Research Institute of the City of New York, New York, N.Y.). YEp52 is a 2μm-based multicopy yeast shuttle and expression plasmid (18) and was the gift of James Broach (Princeton University, Princeton, N.J.). It contains the *LEU2* gene and the *GAL10* promoter for selection

* Corresponding author. Mailing address: Channing Laboratory, 180 Longwood Ave., Boston, MA 02115. Phone: (617) 432-2643. Fax: (617) 731-1541. Electronic mail address: bobderes@warren.med.harvard.edu.

and expression in *S. cerevisiae*, as well as *ori* and *bla* from pBR322 for selection and propagation in *Escherichia coli*. DNA cloning and single-stranded DNA production were carried out with *E. coli* TG1 [F′ *traD36 lacI*^{q} Δ(*lacZ*)*M15 proA*$^+$*B*$^+$/ *supE* Δ(*hsdM-mcrB*)*5* (r_K^- m_K^- McrB$^-$) *thi* Δ(*lac-proAB*)]. Yeast plasmids were recovered into *E. coli* DH5α [F$^-$/*endA1 hsdR17*($r_K^-m_K^+$) *supE44 thi-1 recA1 gyrA* (Nal^r) *relA1* Δ(*lacZYA-argF*)*U169 deoR* (ϕ80 d*lac*Δ(*lacZ*)*M15*)]. Yeast experiments were performed with *S. cerevisiae* Fy69 (**a** *leu2D1*), the gift of Fred Winston (Harvard Medical School, Boston, Mass.), and NKY879 (α *leu2 trp1 ura3-52 prb1-1122 pep4-3*), the gift of Janet Lindsley (Harvard University, Cambridge, Mass.).

Oligonucleotides. All oligonucleotides used in this study were synthesized on a model 381A DNA synthesizer (Applied Biosystems, Foster City, Calif.) and purified with the manufacturer's oligonucleotide purification cartridges.

Construction of yeast expression vector encoding TSST-1. PCR was used to create a *Hin*dIII-*Xba*I *tstH* cloning cassette. Oligonucleotide primers 5′-CCGCAAGCTTATGTCTACAAACGATAAT ATA-3′ and 5′-GACTTCTAGAAATTAATTTCTGC-3′ were prepared. The first oligonucleotide contains a four-base leader followed by a *Hin*dIII site (underlined), an ATG start codon (double underlined), and the 18 nucleotides that begin the sequence encoding mature TSST-1. The second oligonucleotide contains a four-base leader followed by an *Xba*I site (underlined) and 18 nucleotides complementary to the distal end of the TSST-1 coding sequence, including the complement of the TAA stop codon (double underlined). Twenty cycles of amplification (denaturation at 94°C for 1 min, annealling at 45°C for 2 min, and polymerization at 72°C for 3 min) were carried out with a model 480 thermal cycler (Perkin Elmer-Cetus, Norwalk, Conn.) and the manufacturer's GeneAmp kit as recommended, beginning with 5 ng of pRN6550 template DNA and 100 nmol of each primer. The resultant *tstH* cassette was digested with both *Hin*dIII and *Xba*I and recovered in the phage vector M13mp18. PCR fidelity was verified by complete sequencing of the *tstH* portion with the Sequenase kit (U.S. Biochemical, Cleveland, Ohio). The cassette was then excised and subcloned between the *Hin*dIII and *Xba*I sites of YEp52 to create the yeast expression plasmid pRD600 (Fig. 1).

Yeast transformation and maintenance and plasmid recovery. Yeast cells were transformed by the lithium chloride method (20). Transformants were selected on synthetic complete medium lacking leucine and containing 2% glucose and 2% agar. Strains were maintained as glycerol stocks at −80°C. The yeast plasmids were recaptured in *E. coli* for analysis by the modified smash-and-grab procedure, as previously described (4). Once back in *E. coli*, the plasmids were examined by restriction digestion and then by complete sequencing of the *tstH* portion.

Qualitative assessment of TSST-1 production by transformed yeast cells. Yeast cells bearing either the TSST-1 expression plasmid pRD600 or the control plasmid YEp52 were grown up overnight in 5 ml of synthetic complete broth lacking leucine and containing 2% galactose. To assess whether intracellular production of TSST-1 had occurred, the induced cells were collected by centrifugation and solubilized in sodium dodecyl sulfate (SDS) sample buffer (15 mM Tris [pH 6.8], 0.8% SDS, 1.25% β-mercaptoethanol). They were heated for 2 min in boiling water, immediately chilled on ice for 5 min, and then centrifuged for 2 min. The resulting supernatant fluid is referred to as whole-cell yeast extract.

The nonparticulate portion of each sample was mixed with 1/5 volume of 5× tracking dye (40% glycerol, 0.35% bromophenol blue), and the proteins therein were separated by electrophoresis through 15% polyacrylamide–SDS gels (SDS-PAGE). Low-range prestained molecular weight standards (Bio-Rad, Melville, N.Y.) and purified TSST-1 (kindly provided by Jeffrey Parsonnet, Dartmouth-Hitchcock Medical Center, Hanover, N.H.) were included on each gel. Electrophoretic transfer of the separated proteins to nitrocellulose was carried out according to standard procedures. Immunoreactive bands were visualized by sequential incubation with polyclonal rabbit anti-TSST-1 antiserum (kindly provided by Jeffrey Parsonnet) and 1 μCi of ^{125}I-protein A (Amersham Corp., Arlington Heights, Ill.), followed by autoradiography overnight at −80°C with Kodak XRP-1 film (Eastman Kodak, Rochester, N.Y.) and one Cronex intensifying screen.

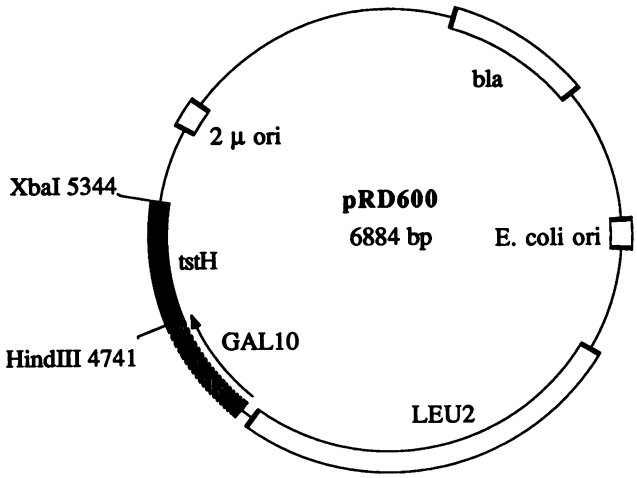

FIG. 1. Map of pRD600. pRD600 contains the mature *tstH* coding sequence, preceded by an ATG start codon, on a *Hin*dIII-*Xba*I cassette that was constructed by PCR. The cassette was cloned between the *Hin*dIII and *Xba*I sites of YEp52, just downstream of the inducible *GAL10* promoter. Also shown are *LEU2* and the 2 μm control element for selection and maintenance in *S. cerevisiae* and *bla* and *ori* for selection and maintenance in *E. coli*.

Growth properties of transformed yeast cells. For solid-phase growth experiments, single colonies of transformed yeast cells were streaked in duplicate onto leucine-deficient synthetic medium supplemented with either 2% glucose or 2% galactose. Growth was monitored for 48 h and scored as either positive or negative.

For measurement of growth kinetics in broth cultures, individual strains were grown up overnight in 15 ml of selective broth containing 2% glucose. The next day, 1 ml of each culture was diluted into a total volume of 200 ml of the same medium in a 2-liter Erlenmeyer flask. The cultures were incubated at 30°C and 275 rpm. When the cells had reentered log-phase growth, 180 ml was centrifuged, washed once each with cold water and with selective broth devoid of sugar, split, and resuspended in 90 ml of selective broth containing either 2% glucose or 2% galactose. Incubation was continued in parallel in 1-liter flasks. Aliquots were periodically withdrawn for measurement of optical density at 600 nm and CFU of per milliliter. CFU per milliliter was determined by plating 200 μl of 10-fold serial dilutions of the broths in 3 ml of leucine-deficient 2% glucose top agar (0.75% Bacto-agar) on leucine-deficient 2% glucose plates. Each measurement was performed in duplicate. Colonies were counted at 72 h, and the CFU per milliliter of the original culture was calculated.

Morphological assessment of transformed yeast cells. Yeast cells bearing either pRD600 or the control plasmid YEp52 were grown to saturation in leucine-deficient synthetic medium

supplemented with 2% galactose. Gram stains were prepared from 1 μl of each culture with standard reagents and procedures.

Preparation of crude yeast protein extracts for functional assay of TSST-1. Yeast cells (80 ml) containing either YEp52 or pRD600 were grown to saturation in leucine-deficient synthetic medium supplemented with 2% galactose. The cells were sedimented at 4,000 × *g* and 4°C for 10 min in a Sorvall SS34 rotor. The pellets were washed once with 10 ml of extraction buffer [200 mM Tris (pH 8.0), 400 mM $(NH_4)_2SO_4$, 10 mM $MgCl_2$, 1 mM EDTA, 10% glycerol, 1 mM phenylmethylsulfonyl fluoride, 0.05% (wt/vol) β-mercaptoethanol], resuspended in 1.6 ml of extraction buffer, and divided to four microcentrifuge tubes. Acid-washed glass beads (0.3 g) were added to each tube, and the mixtures were agitated at maximum power in a vortex mixer at 4°C for seven 1-min increments. The samples were spun for 5 min in a microcentrifuge at 4°C, and the supernatant fluids were removed to fresh microcentrifuge tubes. The supernatants were respun in a microcentrifuge for 60 min at 4°C. The supernatants from the last spin were pooled, passed through a 0.2-μm-pore-size membrane, and filtered across a 100-kDa-cutoff ultrafiltration membrane (Centricon-100; Amicon Corp., Beverly, Mass.). Those filtrates were dialyzed extensively across a 10-kDa-cutoff ultrafiltration membrane, and the retentates were saved at −80°C. The products are referred to as crude yeast protein extracts.

The protein content of the crude yeast protein extracts was quantitated by the Micro-BCA assay (Bio-Rad) according to the manufacturer's recommendations with a solution of bovine serum albumin of known concentration as the standard.

Mitogenicity of yeast extracts. The mitogenicity of the crude yeast protein extracts was assessed in a 4-day lymphocyte proliferation assay using fractionated human peripheral blood leukocytes, as follows.

(i) Isolation of $CD4^+$ T cells. $CD4^+$ T cells were purified from leukopaks, i.e., the discarded leukocytes from platelet donations, as previously described (7). The cells were fractionated on Ficoll-Hypaque gradients (Sigma Chemical Co., St. Louis, Mo.), and a small portion was irradiated with 6.4 kilorads for use as feeder cells. The remaining cells were washed and passed through nylon wool (Cellular Products, Buffalo, N.Y.) to remove B cells and macrophages, which adhere to the wool, and then were treated for 30 min on ice with an antibody cocktail containing saturating amounts of LB3.1 (binds to any residual major histocompatibility complex II-positive cells), B73.1 (binds to NK cells), and OKT8 (binds to CD8-positive cells). They were next washed and incubated for 30 min at 4°C with goat anti-mouse immunoglobulin covalently coupled to magnetic beads (Advanced Magnetics Inc, Cambridge, Mass.) at a bead-to-cell ratio of 10:1. Finally, bead-bound cells were purged by exposing the mixture to a strong magnetic field. The unbound cells were harvested, and their purity was assessed by staining with various B-cell, T-cell, NK cell, and monocyte markers.

(ii) Proliferation assay. Irradiated feeder cells (10^5) were plated with 10^4 magnetic bead-purified $CD4^+$ T cells (thus giving a 10:1 ratio of feeders to responders) in 96-well U-bottom plates (Costar, Cambridge, Mass.) in Ultraculture (BioWhittaker, Walkersville, Md.), a completely defined serum-free medium that had been supplemented with 2 mM L-glutamine and with penicillin-streptomycin (Sigma). Various dilutions of the crude yeast protein extracts were added to the wells in quadruplicate, and the plates were incubated for 4 days at 37°C. Lymphocyte proliferation was assessed by measuring incorporation of methyl-[^{3}H]thymidine on the 4th day.

RESULTS

Construction of TSST-1 expression plasmid pRD600. PCR was used successfully to create a gene fragment encoding the sequence of mature TSST-1. Complete sequencing of the PCR-generated fragment in M13mp18 revealed three discrepancies at the nucleotide level relative to the published sequence of *tstH* available at that time (1). The published sequence has since been amended by the authors of the original publication (13); our sequence and the published sequence are now in agreement.

The PCR product was next cloned from M13mp18 into YEp52 to create the expression plasmid pRD600 (Fig. 1). The construct was verified by restriction analysis.

Intracellular expression of TSST-1 in *S. cerevisiae*. YEp52, the yeast shuttle vector used for this study, replicates to a moderate copy number in *S. cerevisiae*. Its *Hin*dIII site is located downstream of the native *GAL10* transcriptional start site but upstream of the *GAL10* translational start site (18). Thus, constructs cloned between the *Hin*dIII and *Xba*I sites are translated beginning with the first ATG codon in the cloned sequence. Accordingly, under inducing conditions (i.e., galactose present and glucose absent), pRD600 is expected to direct production of mature TSST-1, since the first ATG in the cloned segment is immediately upstream of the first codon of the mature protein. Because a signal sequence is not present, the product is expected to remain in an intracellular location. Under repressive conditions (i.e., glucose present and galactose absent), the cloned element should remain silent.

As shown in Fig. 2, whole-cell yeast extract from cells transformed with pRD600 and induced with galactose contained a single immunoreactive band at the expected position of mature TSST-1. Degradation products were not evident. No immunoreactive material was found under noninducing conditions or in extracts from the control strain [Fy69(YEp52)] under either condition.

Growth properties of transformed yeast cells. As shown in Fig. 3, there was no difference between the growth kinetics of Fy69(pRD600) and those of the control strain Fy69(YEp52) in either glucose broth or galactose broth. Both strains showed the expected lag phase following a substrate shift from glucose to galactose. They resumed growth concurrently and saturated at a similar cell density. Analagous results were obtained when cells were grown on solid media (data not shown).

Yeast cells bearing pRD600 and harvested from late time points in the galactose arm of the liquid-phase growth curve contained full-length immunoreactive TSST-1, thereby establishing that the vitality of that culture was not due to any of a variety of conceivable mutations that might have prevented production of full-length TSST-1. Resequencing of the *tstH* cassette recovered from Fy69(pRD600) confirmed the presence of the wild-type sequence, proving as well the absence of a chance attenuating point mutation within the toxin's coding sequence.

In order to establish that a chance permissive host mutation was not responsible for the apparent lack of toxicity of intracellular TSST-1 for strain Fy69(pRD600), the strain was assembled a second time. Induction of the cloned element was again well tolerated. To establish that intrinsic host resistance was also not responsible for the apparent lack of toxicity, the *tstH*-bearing plasmid pRD600 and the control plasmid YEp52 were each cloned into NKY879, a yeast strain unrelated to Fy69. NKY879(pRD600) and NKY879(YEp52) grew equally well on galactose plates (data not shown).

Morphological assessment of transformed yeast cells. By Gram stain, there were no evident morphological differences

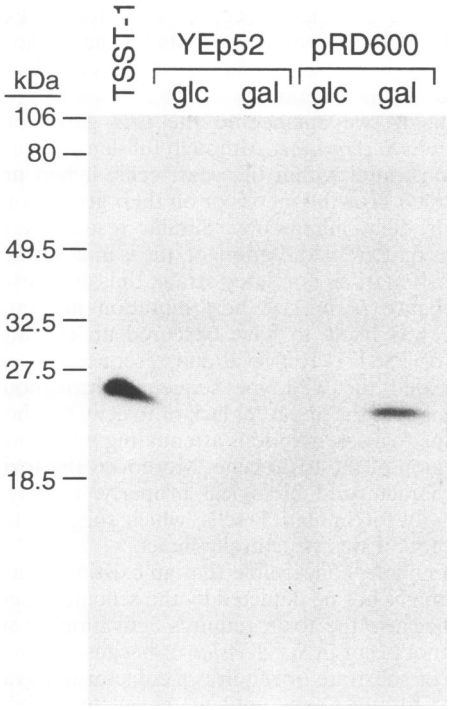

FIG. 2. Immunoblot of whole-cell yeast extracts from the control strain Fy69(YEp52) or the experimental strain Fy69(pRD600) that had been grown in the presence of either glucose (glc) or galactose (gal). Proteins were separated by SDS-PAGE under reducing conditions and electroblotted to nitrocellulose. The blot was developed with rabbit anti-TSST-1 serum followed by ^{125}I-protein A. The positions of the molecular mass markers and of the TSST-1 standard are also shown.

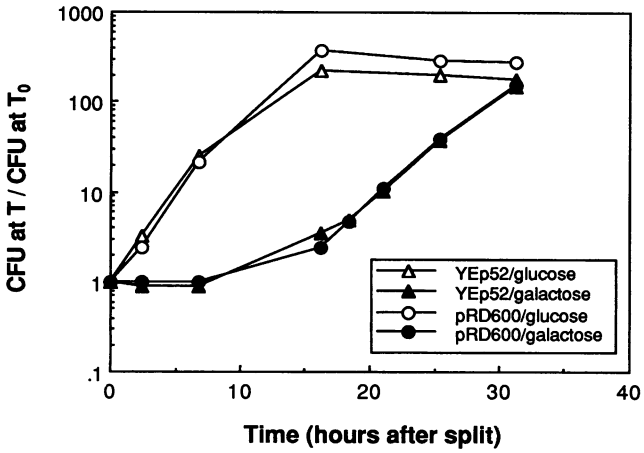

FIG. 3. Growth in various sugars of Fy69 cells bearing either YEp52 or pRD600. At time zero, early-log-phase cultures that had been growing in glucose broth were harvested, washed, and split to either glucose or galactose broth. Incubation was resumed. Datum points reflect the CFU per milliliter in each culture at time *T* divided by the CFU per milliliter in that same culture at time zero.

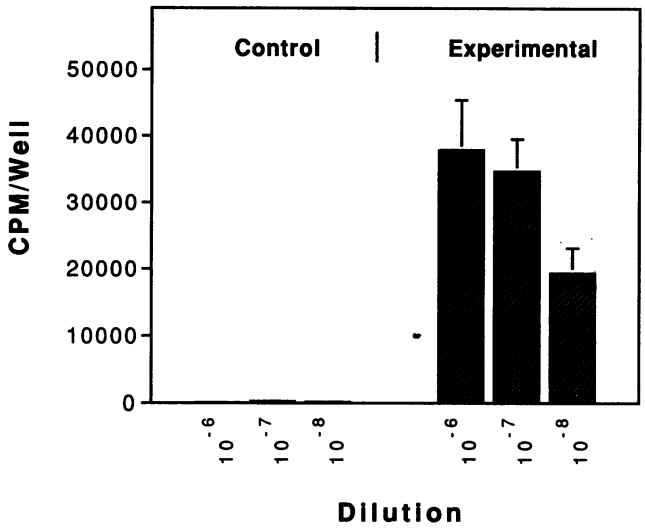

FIG. 4. Mitogenicity of crude yeast protein extracts for human lymphocytes. Crude extracts from the control strain [Fy69(YEp52)] or the experimental strain [Fy69(pRD600)] were plated at dilutions of 10^{-6}, 10^{-7}, and 10^{-8} with irradiated class II-positive feeder cells and $CD4^+$ responders as described in the text. Lymphocyte proliferation was assessed by measuring incorporation of methyl-[^{3}H]thymidine on the 4th day and is depicted as mean counts per minute per well for quadruplicate wells.

between Fy69(YEp52) cells and Fy69(pRD600) cells that had been grown to saturation in galactose broth. Both cell types stained heterogeneously gram positive. They exhibited a similar range of sizes and a similar frequency of budding. The contours of their cell walls appeared to be identical. Morphologically aberrant cells were not visible.

Crude protein extracts from transformed yeast cells. In order to verify that the intracellular TSST-1 produced in *S. cerevisiae* retained one of its known functional properties, namely, mitogenicity, we partially purified total cellular protein as described above. The total yield of protein was ~9.5 mg/liter of culture. The crude extracts were then adjusted to a concentration of ~5.1 mg/ml; 25 μg was separated by SDS-PAGE. The banding patterns of the control and experimental extracts appeared to be similar on Coomassie blue-stained gels, except for the presence of an additional ~22-kDa band in the experimental extract only, which presumably corresponds to TSST-1. As in the whole-cell yeast extracts, the band was visible on a Western blot (immunoblot) stained with polyclonal rabbit anti-TSST-1. No immunoreactive product was present in the control extract. On the basis of its appearance on the immunoblot, the TSST-1 content of the experimental extract was estimated to be ~200 μg/ml. (Thus, ~5×10^7 molecules of TSST-1 were isolated per yeast cell.)

Mitogenicity of crude protein extracts from transformed yeast cells. As shown in Fig. 4, the crude protein extract from yeast cells bearing the *tstH* expression plasmid and grown in galactose broth was mitogenic for human T cells at a dilution as low as 10^{-8}. In contrast, the protein extract from the control yeast cells was not mitogenic even at a dilution of 10^{-6}.

DISCUSSION

Since the discovery of TSST-1 in 1981, much effort has been directed at understanding the pathophysiological mechanisms by which it causes disease. It is now well established that

TSST-1 is a potent immunomodulator. It binds directly and without prior processing to HLA class II molecules on the surfaces of antigen-presenting cells. In that context, it causes a broad polyclonal expansion of T lymphocytes by stimulating T cells, not according to their idiotype, as do conventional antigens, but according to their V_β regions (15). The consequence is the induction and release of several cytokines, among them interleukin 1, tumor necrosis factor alpha, and gamma interferon, by macrophages, and interleukin 2 and tumor necrosis factor beta, by lymphocytes (2). It is that cytokine release and the sequelae thereof that are probably responsible for many of the clinical features of TSS. The mitogenic properties of TSST-1 can be demonstrated at concentrations as low as 0.1 pg/ml (4a).

There are bits of evidence that, in addition to having the above-described properties, TSST-1 penetrates and possibly is also directly cytotoxic to certain cell types. In a study by Drumm et al. (5), for example, it caused cellular architectural distortion and subsequent detachment of chicken embryo cells in tissue culture at a toxin concentration of 0.2 μg/ml. The effect was potentiated by bacterial lipopolysaccharide and was dependent on the means by which the target cells were prepared. No such effect on a variety of other cell types tested, including cells from both primary cultures and established lines, was seen.

In studies by Kushnaryov et al. (10, 11), TSST-1 was shown to bind specifically and saturably to normal human conjunctival epithelial cells in tissue culture, to be internalized by them in coated pits, and to decrease their net growth rate by ~64% at a toxin concentration of 20 μg/ml.

More recently, Lee et al. (12) developed evidence that toxin-induced vascular leakage, rather than toxin-induced T-cell proliferation, may ultimately be responsible for the profound shock so integral to the lethality of TSS. They also showed that TSST-1 binds cultured PAECs and kills them in a time- and dose-dependent fashion and that it enhances permeability of PAEC monolayers to albumin flux, also in a time- and dose-dependent fashion (14). Those effects began at toxin concentrations of 2 and 1 μg/ml, respectively. The mechanisms by which the toxin exerts those effects, and presumably also by which it causes capillary leakage in vivo, are not understood but may involve a direct toxic property of the protein for the endothelial cell. The goal of this study was to search for evidence of such a property.

We adapted a system that had previously been used to find attenuated mutants of the enzymatic portions (A chains) of the known cytotoxins ricin toxin, Shiga-like toxin I (4, 8), and diphtheria toxin (9a). Each is a potent protein synthesis inhibitor: the ricin toxin and Shiga-like toxin I A chains are RNA *N*-glycosidases that specifically depurinate one adenine residue in eukaryotic 28S rRNA (6), while diphtheria toxin A chain is an ADP-ribosyl transferase that ADP-ribosylates a modified histidine residue on eukaryotic elongation factor 2 (3). If any of the three toxins gains access to the interior of a eukaryotic cell, either by receptor-mediated endocytosis as occurs naturally or by intracellular expression in the laboratory, the effect is death of the target cell. In the case of diphtheria toxin, that effect can be demonstrated with even a single molecule of the toxin (21). Intracellular expression of each of the three toxins in the eukaryote *S. cerevisiae* reduced by several orders of magnitude the number of viable cells in broth culture. Permissive host mutants were rarely seen with diphtheria toxin A chain and were never seen with either the ricin toxin or Shiga-like toxin I A chains. We reasoned that if TSST-1 also has an enzymatic activity, which has perhaps eluded widespread recognition because it is predicted on the presence of a particular receptor not broadly expressed in mammalian cells and which accounts for the apparent cytotoxicity of TSST-1 as described above, then that activity might be unmasked in this fashion.

Accordingly, we engineered the *tstH* gene for inducible expression in *S. cerevisiae*. Although full-length, stable TSST-1 was demonstrated within the yeast cells, it had no apparent effect on their growth kinetics or on their gross morphology as assessed by light microscopy. Similar results were obtained after a second transformation of the same strain and after transformation of an unrelated strain, thus demonstrating that neither chance permissive host mutation nor intrinsic host resistance was likely to have obscured an existing cytotoxic property of TSST-1. Retrieval and resequencing of the toxin gene revealed the wild-type sequence throughout, thereby establishing that the apparent lack of toxicity for the yeast cells was not due to a serendipitous attenuating mutation within the coding region of the toxin gene. Moreover, the toxin retained its best-characterized biological property, namely, its mitogenic activity for human T cells, which suggests that by that criterion, too, it was structurally intact.

It is, of course, conceivable that an existing toxic enzymatic property might not be detected by the scheme described here, perhaps because the toxin requires activation by some event that does not occur in *S. cerevisiae* or because the toxin acts on a process or substrate or requires a cofactor not available to it there. The former seems unlikely, given the toxin's lack of a disulfide bond that might be easily reduced and given its well-known resistance to proteolysis. The latter is possible but would distinguish this putative enzymatic toxin from several of the classic bacterial exotoxins that act on highly conserved eukaryotic targets (3, 6) or that require highly conserved eukaryotic cofactors (9).

It is also possible that the apparent cytotoxicity of TSST-1 for PAECs and for the other cell types against which it was suggested to be cytotoxic was due not to an enzymatic property of the toxin but to a nonenzymatic cytotoxic mechanism that cannot manifest itself in yeast cells. Finally, it is possible that the apparent cytotoxicity was artifactual and due to the relatively high concentrations of toxin needed to elicit it in all of those experiments (concentrations in the microgram-per-milliliter range), concentrations that contrast markedly to those at which mitogenicity can be demonstrated (a concentration as low as 0.1 pg/ml).

We conclude that TSST-1 probably does not possess a discrete enzymatic property cytotoxic for eukaryotic cells.

ACKNOWLEDGMENTS

We gratefully acknowledge Barry Kreiswirth and Richard Novick for the gift of plasmid pRN6550, James Broach for plasmid YEp52, Fred Winston for yeast strain Fy69, Janet Lindsley for yeast strain NKY879, and Jeffrey Parsonnet for purified TSST-1 and rabbit anti-TSST-1.

This work was supported by grant 80917 from Tambrands Incorporated (to D.L.K. and R.L.D.) and by grant 91018 from Sandoz Incorporated (to R.W.F.).

REFERENCES

1. **Blomster-Hautamaa, D. A., B. N. Kreiswirth, J. S. Kornblum, R. P. Novick, and P. M. Schlievert.** 1986. The nucleotide and partial amino acid sequence of toxic shock syndrome toxin-1. J. Biol. Chem. **261**:15783–15786.
2. **Bohach, G. A., D. Fast, R. D. Nelson, and P. M. Schlievert.** 1990. Staphylococcal and streptococcal pyrogenic toxins involved in toxic shock syndrome and related illnesses. Crit. Rev. Microbiol. **17**:251–272.
3. **Collier, R. J.** 1975. Diphtheria toxin: mode of action and structure. Bacteriol. Rev. **39**:54–85.

4. **Deresiewicz, R. L., S. B. Calderwood, J. D. Robertus, and R. J. Collier.** 1992. Mutations affecting the activity of the shiga-like toxin I A-chain. Biochemistry **31:**3272–3280.
4a. **Deresiewicz, R. L., et al.** Unpublished data.
5. **Drumm, A., J. C. S. de Azavedo, and J. P. Arbuthnott.** 1989. Damaging effect of toxic shock syndrome toxin 1 on chick embryo cells in vitro. Rev. Infect. Dis. **11:**S275–S280.
6. **Endo, Y., K. Tsurugi, T. Yutsudo, Y. Takeda, T. Ogasawara, and K. Igarashi.** 1988. Site of action of a vero toxin (VT2) from *Escherichia coli* O157:H7 and of Shiga toxin on eukaryotic ribosomes: RNA N-glycosidase activity of the toxins. Eur. J. Biochem. **171:**45–50.
7. **Finberg, R. W., W. White, and A. Nicholson-Weller.** 1992. Decay-accelerating factor expression on either effector or target cells inhibits cytotoxicity by human natural killer cells. J. Immunol. **149:**2055–2060.
8. **Frankel, A., D. Schlossman, P. Welsh, A. Hertler, D. Withers, and S. Johnston.** 1989. Selection and characterization of ricin toxin A-chain mutations in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **9:**415–420.
9. **Fu, H., J. Coburn, and R. J. Collier.** 1993. The eukaryotic host factor that activates exoenzyme S of *Pseudomonas aeruginosa* is a member of the 14-3-3 protein family. Proc. Natl. Acad. Sci. USA **90:**2320–2324.
9a. **Fu, H., and R. J. Collier.** Personal communication.
10. **Kushnaryov, V. M., M. S. Bergdoll, H. S. MacDonald, J. Vellinga, and R. Reiser.** 1984. Study of staphylococcal toxic shock syndrome toxin in human epithelial cell culture. J. Infect. Dis. **150:**535–545.
11. **Kushnaryov, V. M., H. S. MacDonald, R. Reiser, and M. S. Bergdoll.** 1984. Staphylococcal toxic shock toxin specifically binds to cultured human epithelial cells and is rapidly internalized. Infect. Immun. **45:**566–571.
12. **Lee, P. K., J. R. Deringer, B. N. Kreiswirth, R. P. Novick, and P. M. Schlievert.** 1991. Fluid replacement protection of rabbits challenged subcutaneously with toxic shock syndrome toxins. Infect. Immun. **59:**879–884.
13. **Lee, P. K., B. N. Kreiswirth, J. R. Deringer, S. J. Projan, W. Eisner, B. L. Smith, E. Carlson, R. P. Novick, and P. M. Schlievert.** 1992. Nucleotide sequences and biologic properties of toxic shock syndrome toxin 1 from ovine- and bovine-associated *Staphylococcus aureus*. J. Infect. Dis. **165:**1056–1063.
14. **Lee, P. K., G. M. Vercellotti, J. R. Deringer, and P. M. Schlievert.** 1991. Effects of staphylococcal toxic shock syndrome toxin 1 on aortic endothelial cells. J. Infect. Dis. **164:**711–719.
15. **Marrack, P., and J. Kappler.** 1990. The staphylococcal enterotoxins and their relatives. Science **248:**705–711.
16. **Parsonnet, J.** 1989. Mediators in the pathogenesis of toxic shock syndrome: overview. Rev. Infect. Dis. **11:**S263–S269.
17. **Parsonnet, J., and D. L. Kasper.** 1992. Toxic shock syndrome: clinical developments and new biology, p. 3–14. *In* J. D. Wilson, E. Braunwald, K. J. Isselbacher, J. B. Martin, A. S. Fauci, and D. L. Kasper (ed.), Harrison's principles of internal medicine, 12th ed., suppl. 1. McGraw-Hill, New York.
18. **Rose, A. B., and J. R. Broach.** 1990. Propagation and expression of cloned genes in yeast: two micron circle-based vectors. Methods Enzymol. **185:**234–279.
19. **Schlievert, P. M., K. N. Shands, B. B. Dan, G. P. Schmid, and R. D. Nishimura.** 1981. Identification and characterization of an exotoxin from *Staphylococcus aureus* associated with toxic-shock syndrome. J. Infect. Dis. **143:**509–516.
20. **Sherman, F., G. R. Fink, and J. B. Hicks.** 1986. Laboratory course manual for methods in yeast genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
21. **Yamaizumi, M., E. Mekada, T. Uchida, and Y. Okada.** 1978. One molecule of diphtheria toxin fragment A introduced into a cell can kill the cell. Cell **15:**245–250.