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Rapid Assay for Detection of Toxic Shock Syndrome Toxin 1 from Human Sera

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A noncompetitive enzyme-linked immunosorbent assay that enables the quantitation of toxic shock syndrome toxin 1 (TSST-1) to as little as 30 pg/ml and the detection of TSST-1 to 10 pg/ml in phosphate-buffered saline including 33% human serum or plasma was developed. It takes only 3 h to complete this assay after plate preparation. In this study, 64 human serum samples obtained from 30 patients with toxic shock syndrome or toxic shock syndrome-like symptoms were subjected to testing for the detection of TSST-1. With a cutoff level for TSST-1 of less than 100 pg/ml, 28 samples obtained from 12 patients were positive for TSST-1. The mean and maximum concentrations for these TSST-1-positive samples were 440 and 5,450 pg/ml, respectively. Of these 12 patients, 8 were *Staphylococcus aureus* culture positive, 3 were negative upon bacterial culturing, and 1 had no cultures done.

Toxic shock syndrome toxin 1 (TSST-1) (1, 13, 15), a staphylococcal exoprotein, is strongly implicated in some of the illnesses associated with *Staphylococcus aureus* infections. Therefore, various biological properties of TSST-1 have been studied. These properties include T lymphocyte mitogenicity (2, 18), enhancement of the lethality of or tumor necrosis factor synthesis induction by lipopolysaccharides (5, 7), and pyrogenicity in rabbits (3, 5). Moreover, TSST-1 production was demonstrated in over 90% of *S. aureus* strains associated with menstruation-related toxic shock syndrome (TSS) (1, 12, 15, 19). These results strongly suggest that TSST-1 is the major etiologic agent of TSS. The existence of TSS was first reported by Todd et al. in 1978 (17).

To detect and quantify TSST-1, reverse passive latex agglutination (6) or enzyme-linked immunosorbent assays (ELISA) (4, 9, 11, 14, 16, 20) have been used. However, reverse passive latex agglutination cannot be used with a high concentration of serum because of nonspecific agglutination. Using ELISA, Wells et al. (20) reported the detection of TSST-1 in the urine of clinically confirmed TSS patients, and Melish et al. (10) detected TSST-1 in the serum of TSS patients using a radioimmunoassay. However, for further studies of the biological effects of TSST-1 in clinical stages, a more sensitive assay for quantifying TSST-1 in serum is essential.

In this study, we developed a sensitive and specific ELISA that enables the quantitation of TSST-1 at concentrations of 30 pg/ml to 1 ng/ml and the detection of TSST-1 to 10 pg/ml in phosphate-buffered saline (PBS) including 33% human serum. Using this ELISA, we detected TSST-1 directly in serum derived from patients with TSS or TSS-like symptoms.

Sixty-four serum samples were obtained from 30 patients (20 males and 10 females; mean age, 28 years [range, 1 to 72]). These patients were suspected of having TSS, and samples from them were brought to the Tokyo Metropolitan Research Laboratory of Public Health for the detection of *S. aureus* exotoxins and the characterization of *S. aureus*. Samples were collected from 11 hospitals in Japan between September 1986 and April 1991.

Purification of TSST-1 for the standard antigen was carried out as reported by Igarashi et al. (8). Polyclonal anti-TSST-1 antibody purified from the serum of a TSST-1-immunized rabbit was immobilized as the capture antibody, and biotinylated mouse monoclonal anti-TSST-1 antibody was used as the indicator antibody. Purified rabbit anti-TSST-1 antibody (immunoglobulin G) was diluted in PBS (pH 7.2) at a concentration of 0.5 μg/ml, and 0.1-ml volumes were added to the wells of Immuno-module plates (Nunc, Roskilde, Denmark). The plates were incubated overnight at 4°C to allow binding of the antibody to the ELISA plates. Unbound rabbit antibody was removed by aspiration, and the plates were rinsed with PBS (pH 7.2)–0.05% Tween 20. The plates were then treated overnight at 4°C with 0.4 ml of PBS (pH 7.2)–0.05% Tween 20 containing 0.5% bovine serum albumin fraction V (BSA) (Seikagaku Kogyo Co. Ltd., Tokyo, Japan). Before use, the plates were rinsed once with PBS (pH 7.2)–0.05% Tween 20. Biotinylation of the mouse monoclonal anti-TSST-1 antibody was done by coupling the purified mouse antibody with sulfosuccinimidyl-6-(biotinamido) hexanoate (Pierce) at a 1:100 weight ratio.

Human plasma or serum samples were added in 33-μl volumes to test wells each containing 66 μl of dilution buffer (0.25% BSA–0.05% Tween 20 in PBS [pH 7.2]) and incubated for 1 h at 25°C. Unbound reagents were removed by aspiration, and the wells were then rinsed three times with PBS–0.05% Tween 20. The biotinylated mouse monoclonal antibody was diluted in dilution buffer to 0.1 μg/ml, and 0.1-ml volumes were added to all test wells. The plates were incubated for 1 h at 25°C. After the addition of 0.1 ml of avidin-horseradish peroxidase conjugate solution (0.625 μg/ml in PBS–0.05% Tween 20) to all test wells and incubation for 15 min at 25°C, the plates were rinsed three times. Substrate solution (0.09% hydrogen peroxide–0.2 mg of 3,3′,5,5′-tetramethylbenzidine per ml in 0.1 M citric acid [pH 3.5]) was then added in volumes of 0.1 ml and incubated for 30 min at 25°C. The enzyme reaction was terminated by the addition of 0.1 ml of 0.5 M sulfonic acid. ELISA plates were

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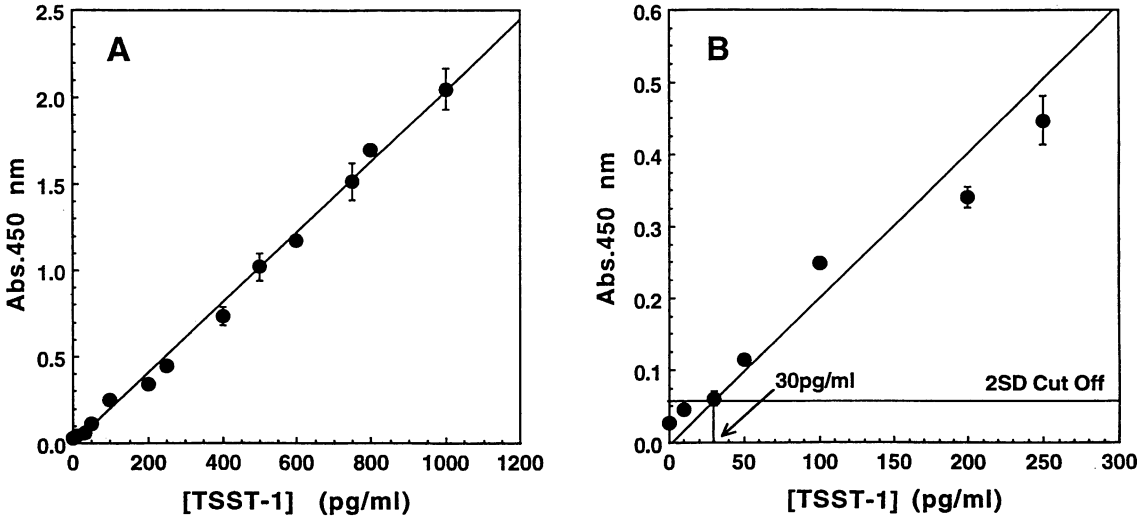

FIG. 1. Quantitative ELISA for TSST-1. The correlation coefficient was 0.996, and the lowest concentration for quantitation, as determined from the mean absorbance (Abs.) value + 2 standard deviations (SD), was 30 pg/ml. (A) Concentration range of 0 to 1,000 pg/ml. (B) Concentration range of 0 to 250 pg/ml.

read spectrophotometrically at 450 nm with a microplate reader (M-3550; Bio-Rad). Plasma from healthy humans and PBS without human plasma were used as the negative controls in each assay.

The standard curve for calibration was prepared by plotting the A_{450} versus the TSST-1 concentration over the range of 0 to 1 ng/ml. The standard series dilution and the calibration curve were prepared for each assay plate. The TSST-1 concentration in each plasma sample was calculated from the corresponding calibration curve.

S. aureus enterotoxins A, B, and C were substituted for TSST-1 specimens in the ELISA to determine whether their presence would contribute to false-positive results. Purified enterotoxins were diluted over a concentration range of 0 to 1 ng/ml in dilution buffer. Each diluent was incubated with the anti-TSST-1 antibody immobilized on an ELISA plate and the indicator antibody for TSST-1. The same procedures as those described above were used. To determine the effect of protein A, polyclonal chicken anti-protein A antibody (Immunsystem AB, Uppsala, Sweden) conjugated to biotin was substituted for the monoclonal anti-TSST-1 antibody as the indicator antibody.

The ELISA with the rabbit polyclonal anti-TSST-1 antibody as the capture antibody and the mouse monoclonal anti-TSST-1 antibody (immunoglobulin G2A)–biotin conjugate as the indicator antibody detected as little as 10 pg of TSST-1 per ml. The relationship between the optical density at 450 nm and the TSST-1 concentration was consistently linear, with a correlation coefficient of 0.996 at a concentration range of 0 to 1 ng/ml (Fig. 1A). A mean absorbance value + 2 standard deviations at a TSST-1 concentration of 0 pg/ml was obtained from 15 determinations on a single plate. On the basis of this value, as shown in Fig. 1B, this assay system is considered reliable for the quantitation of TSST-1 to as little as 30 pg/ml. Despite the greater variations in absorbance values at lower TSST-1 concentrations, these values were consistently higher than the background values, indicating that the assay is sensitive for detection to 10 pg/ml. Purified staphylococcal enterotoxins A, B, and C did not react with the ELISA for TSST-1 (Fig. 2); therefore, these toxins do not interfere with the TSST-1 assay. Also, the ELISA plates that were incubated with patient serum were treated with a biotinylated anti-protein A antibody; however, no reaction was observed (data not shown). This result suggests that protein A was neutralized by serum immunoglobulin G and that binding to the immobilized antibody was completely obviated.

With this assay, we determined that TSST-1 concentrations in healthy human plasma were less than 100 pg/ml from measurements of 30 samples. Among 64 samples obtained from 30 patients with TSS or TSS-like symptoms, 28 samples from 12 patients (40%) were TSST-1 positive; these samples had higher TSST-1 concentrations than healthy human plasma (Fig. 3). Each datum point in Fig. 3 represents the concentration in one sample. Therefore, when serum was obtained twice from a single patient, on different days, two points are shown. One patient for whom bacterial culturing

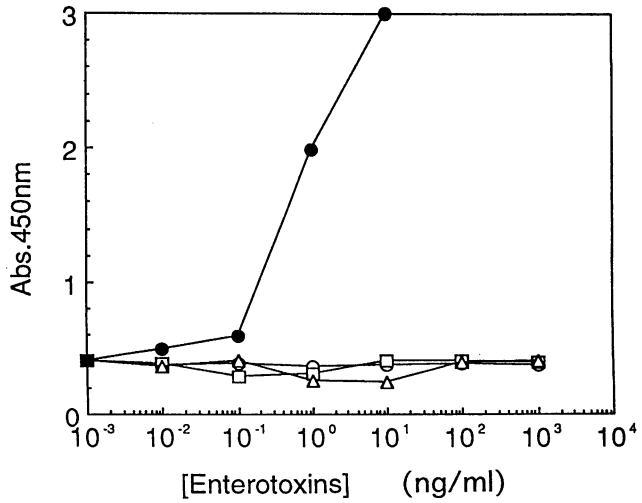

FIG. 2. Specificity of the ELISA in relation to other *S. aureus* enterotoxins (A [○], B [□], and C [△]). ●, TSST-1. Abs., absorbance.

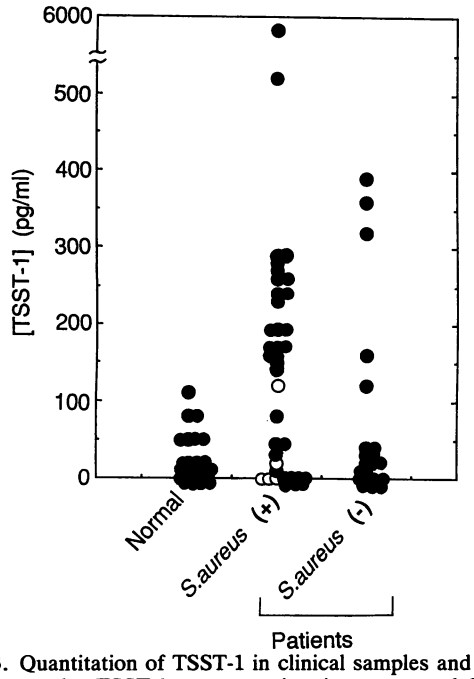

FIG. 3. Quantitation of TSST-1 in clinical samples and correlation between the TSST-1 concentration in serum and bacterial culturing. Symbols: ●, TSST-1-producing strains [in the *S. aureus* (+) column]; ○, non-TSST-1-producing strains.

was not done is not represented (the TSST-1 concentration was 220 pg/ml). While the mean concentration of TSST-1 in healthy human plasma was approximately 30 pg/ml (under the quantitation limit), the mean and maximum concentrations of TSST-1 in patient samples were 440 pg/ml and approximately 5,450 pg/ml (over the quantitation limit), respectively.

These results suggest that the degradation of TSST-1 does not completely occur locally. The toxin therefore circulates systematically and acts as the pathogenic factor for systemic toxic shock symptoms. In addition, bacterial culturing was done for blood, sputum, stool, or lochia samples from 20 patients, and the correlation between bacterial culturing and TSST-1 concentrations is also shown in Fig. 3. Occasionally, bacterial culturing may be negative because of the influence of antibiotics. However, antibiotics should have no effect on the detection of bacterial products. For this reason, some culture-negative patient specimens were included with TSST-1-positive specimens. Since our results showed that TSST-1 was detected in the body fluids of a high percentage of TSS patients without the effect of antibiotics, we believe our assay will have diagnostic value for the rapid detection of *S. aureus* infections. Some assumptions may be made regarding the 60% *S. aureus*-positive patients who were TSST-1 negative. One possibility is that the anti-TSST-1 antibody which was observed in some patient sera (data not shown) prevented the binding of TSST-1 to ELISA plates. Another is that these isolates of *S. aureus* were TSST-1-negative isolates (indicated by open circles in Fig. 3). In these latter cases, TSS-like symptoms would be caused by other staphylococcal enterotoxins, such as A, B, and C. Therefore, the detection of other staphylococcal enterotoxins would be expected. Finally, it is possible that *S. aureus* does not produce TSST-1 all the time. The timing of blood sampling may then be important.

As described in this report, the ELISA is a rapid and simple method for the detection of TSST-1. For a more reliable diagnosis of TSS, we are now developing quantitative approaches to staphylococcal enterotoxin A, B, and C detection and methods to measure antistaphylococcal toxin antibodies, including anti-TSST-1 antibody.

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