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Study of the biological activities of toxic shock syndrome toxin-1: II. Induction of the proliferative response and the interleukin 2 production by T cells from human peripheral blood mononuclear cells stimulated with the toxin

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SUMMARY

Toxic shock syndrome toxin-1 (TSST-1) is an exotoxin produced by *Staphylococcus aureus* isolated from patients with toxic shock syndrome. We investigated the proliferative response of human lymphocytes and their interleukin 2 (IL-2) production after stimulation with TSST-1 *in vitro*. Human cord blood mononuclear cells (HCBM) and human peripheral blood mononuclear cells (HPBM) could proliferate with TSST-1 stimulation. T cell-depleted HPBM showed only a marginal response to this toxin. A IL-2-like factor with a molecular weight of 15–18 kD was obtained from the supernatants of TSST-1-stimulated HPBM cultures. The factor was absorbed by CTLL-2 cells but not by T cell-depleted murine spleen cells, indicating that it is IL-2. HPBM are very sensitive to TSST-1: a low concentration of TSST-1 (0·01 ng/ml in 36 h stimulation) and a short period of stimulation (8 h at 10 ng/ml of the toxin) were fully effective for HPBM to produce substantial amounts of IL-2. Removal of T cells abrogated the TSST-1-induced IL-2 production by HPBM. Reconstituted cell cultures of nylon wool column-passed T cells and macrophages produced IL-2 by TSST-1 stimulation and, furthermore, the accessory activity of the macrophages could be partially replaced by a macrophage-derived factor containing interleukin 1. These findings indicate that T cells require macrophages or IL-1 for TSST-1-induced production of IL-2. The roles of lymphokines, including IL-2, in the development of this illness are discussed.

Keywords TSST-1 human lymphocytes IL-2 production

INTRODUCTION

Toxic shock syndrome (TSS) is an acute and systemic illness caused by infection with a certain

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Staphylococcus aureus strain (Todd *et al.*, 1978; Davis *et al.*, 1980; Shands *et al.*, 1980). Symptoms include high fever, scarlatini-form rash, subcutaneous oedema, vomiting and diarrhoea, hypotension, and dysfunction of the kidney, liver and the blood coagulation system (Todd *et al.*, 1978; Shands *et al.*, 1980; Larkin *et al.*, 1982), suggesting that a toxin or toxins rather than simple bacteraemia are involved as causative agents of this illness. An exotoxin, toxic shock syndrome toxin-1 (TSST-1), was found to be produced by *S. aureus* isolated from TSS patients (Bergdoll *et al.*, 1981; Schlievert *et al.*, 1981; Bonventre *et al.*, 1983) and biological effects of the toxin on experimental animals and on lymphoid cells *in vitro* have been investigated extensively. For example, rabbits implanted with diffusion chambers which contained TSST-1-producing staphylococci showed a high mortality rate, and the fatal cases of these rabbits showed histopathological findings markedly similar to those of the TSS patients: inflammatory lesions in several organs and pathological changes in lymph nodes with the minimal signs of bacterial invasion (Rasheed *et al.*, 1985). These observations suggest that this toxin is a causative agent of this illness and that the responses of the immune and reticuloendothelial systems to the toxin take part in the development of this illness. Actually, it has been reported that TSST-1 is mitogenic to murine and human T lymphocytes (Calvano *et al.*, 1984; Poindexter & Schlievert, 1985; Uchiyama *et al.*, 1986) and induces human macrophages to produce interleukin 1 (IL-1) (Ikejima *et al.*, 1984). In addition, we recently found that TSST-1 induces murine splenic T cells to produce IL-2, one of T cell-derived lymphokines (Uchiyama *et al.*, 1986). In view of the reports showing that lymphokines manifest various side effects when these were injected into human patients (Lotze *et al.*, 1985; Gutterman *et al.*, 1980), we consider that lymphokines including IL-2 may play important roles in the development of TSS. Therefore, it seems necessary to investigate the mode of action of TSST-1 on IL-2 production by human lymphocytes, as a step to understanding the aetiology of TSS.

In the present study, we investigated the mode of action of TSST-1 on proliferative response and IL-2 production of human lymphocytes, using an *in vitro* culture system. The results showed that TSST-1 is mitogenic for human cord blood mononuclear cells (HCBM) and T cells in human peripheral blood mononuclear cells (HPBM), and is a potent inducer of IL-2 production for T cells in HPBM. The results also showed that T cells in HPBM require the presence of macrophages or a macrophage factor, presumably IL-1, in the TSST-1-induced IL-2 production.

MATERIALS AND METHODS

Preparations of human mononuclear cells. Human mononuclear cells were isolated from the peripheral blood of healthy donors and the cord blood, using the Ficoll-Hypaque density gradient. T cell-depleted HPBM were obtained by the S-2-aminoethylisothiouronium (Nakarai Chemicals, Japan)-treated sheep red blood cell (AET-SRBC) rosette procedure (Falkoff, Peters & Fauci, 1982). Briefly, AET-SRBC rosettes were prepared by mixing 20×10^7 HPBM and 20×10^9 AET-SRBC in 40 ml RPMI medium containing 2·5% fetal calf serum (FCS, Filtron, Australia). The cell suspension was divided into four centrifuge tubes, incubated at 37°C for 15 min, spun at 400 *g* at 4°C, kept on ice for 1 h, and resuspended gently. The rosette forming cells (RFC) were removed by centrifugation on the Ficoll-Conray density gradient. The cells recovered at the interface of the gradient contained less than 2·5% RFC. T cells were obtained by passing HPBM twice over nylon wool columns (2×10^8 cells/1·5 g dry nylon wool) (Julius, Simpson & Herzenberg, 1973). The nylon wool column-passed HPBM contained more than 98% RFC. Adherent macrophage monolayers as accessory cells were prepared by incubating T cell-depleted HPBM (0·5 and 2×10^6/plate) in 35 mm Petri dishes (Nunc Products, Denmark), as reported previously (Uchiyama, Kamagata & Yoshioka, 1984).

Reagents and culture medium. TSST-1 was obtained from the culture fluid of *S. aureus* FRI 1169 by a combination of ion exchange chromatography, chromatofocusing and gel filtration, as reported previously (Igarashi *et al.*, 1984). TSST-1 did not show any mitogenic effect on IL-2-dependent CTLL 2 cells used in assay of IL-2 activity in the present study, as reported previously (Uchuiyama *et al.*, 1986). The macrophage factor containing IL-1 was obtained as a culture supernatant of adherent macrophages from HPBM which had been stimulated with 10 μg/ml of

lipopolysaccharide (LPS, Difco Laboratories, USA) for 48 h. This culture supernatant showed a marked mitogenic effect at a 1:50 dilution on peanut agglutinin-nonagglutinable murine thymus cells, but not to IL-2-dependent CTLL 2 cells. Recombinant human IL-2 (10,000 units/ml) was purchased from Biogen SA (Geneva, Switzerland). RPMI 1640 culture medium (GIBCO Laboratories, USA) used in the present study contained 100 μg/ml of streptomycin, 100 U/ml of penicillin, 5×10^{-5} M of 2-mercaptoethanol and 5% FCS.

Preparation of culture supernatants of TSST-1 stimulated cells. Whole or T cell-depleted HCBM and HPBM ($2{\cdot}5 \times 10^6$/plate) were cultured in 1 ml volumes in 35 mm Petri dishes at 37°C in a 5% CO_2 incubator and stimulated in duplicate with various concentrations of TSST-1 for various periods. T cells (2×10^6/plate) were cultured in 1 ml volumes in 35 mm Petri dishes containing macrophages or several dilutions of the macrophage factor and stimulated in duplicate with 1 ng/ml of TSST-1 for 35 h. Cell-free culture supernatants of duplicate cultures were combined into one in each experimental group and assayed for IL-2 activity. For the sample of the gel chromatographic analysis, whole HPBM ($7{\cdot}5 \times 10^6$/plate) were stimulated in 1·5 ml volumes in 35 mm Petri dishes with 1 ng/ml of TSST-1 for 40 h. Cell-free culture supernatants from 40 plates were pooled and concentrated to 4 ml on an Amicon UM-10 membrane and applied on a Sephacryl S-200 column.

Assay for the mitogenic activity of TSST-1. Whole or T-cell depleted HCBM and HPBM (5×10^5/well) were cultured in 0·2 ml volumes in round bottom 96-well microtitre plates (Nunc Products) and stimulated in triplicate with graded doses of TSST-1 for various periods. Cultures were pulsed with 1 μCi ^{3}H-thymidine for the final 16 h of stimulation period and incorporation of ^{3}H-thymidine of the harvested cells was measured with a liquid scintillation counter (Aloka, Tokyo). The data are presented as the average of triplicates and standard error (SE) of counts per minute (CPM).

Assay for IL-2 activity. The units of IL-2 activity of culture supernatants were determined by the method of Gillis *et at.* (1978), using IL-2-dependent CTLL-2 cells as the IL-2-reactive cells, and recombinant human IL-2 as the standard IL-2, respectively. Details of the procedure were described previously (Uchiyama *et al.*, 1986). For the assay of IL-2 activity of the column-chromatographed fractions or the absorbed culture supernatants, CTLL-2 cells (3×10^3/well) were stimulated in triplicate with 10% concentration of each fraction or with 50% concentration of several dilutions of the absorbed culture supernatants for 24 h and ^{3}H-thymidine incorporation for the last 4 h of stimulation period was measured. The data were presented as the average of triplicates and s.e. of ct/min.

Gel filtration chromatography. Four millilitres of the concentrated culture supernatant of TSST-1-stimulated HPBM were applied on a $2{\cdot}5 \times 90$ cm Sephacryl S-200 column equilibrated in phosphate buffered saline (pH 7·4). A flow rate of 23·3 ml/h was used and 3·5 ml fractions were collected. The column was calibrated by marker proteins of known molecular weight, such as egg albumin (45 kD), chymotrypsinogen (25 kD), and cytochrome c (12·4 kD).

Absorption of the TSST-1-induced factor. Various numbers of CTLL-2 cells or T cell-depleted murine spleen cells were incubated in 1 ml volumes in 35 mm Petri dishes in a culture medium containing 20% purified TSST-1-induced factor or recombinant human IL-2 (2 units/ml) for 5 h at 37°C. The cell-free culture supernatants were then removed and used for the assay of IL-2 activity.

RESULTS

Mitogenic activity of TSST-1 for human lymphocytes. The mitogenicities of TSST-1 for HCBM and HPBM were investigated (Fig. 1). HCBM were used as a source of lymphocytes which had never been immunologically sensitized to TSST-1. When HCBM or HPBM were stimulated with graded doses of TSST-1 for 72 h, proliferative responses were seen in both cell cultures, and the magnitudes of responses were dependent on the dose of TSST-1. The response of HCBP was slightly lower than that of HPBM. In contrast, only marginal responses were obtained in T cell-depleted HPBM cultures stimulated with a wide range of doses of the toxin. The kinetics of the response of HPBM for 48–96 h are shown in Fig. 2. The responses induced with 100 or 1 ng/ml of TSST-1 reached the maximum after 72 h of stimulation. The results of these experiments indicate that the toxin acts on human T cells as an intrinsic polyclonal T cell mitogen.

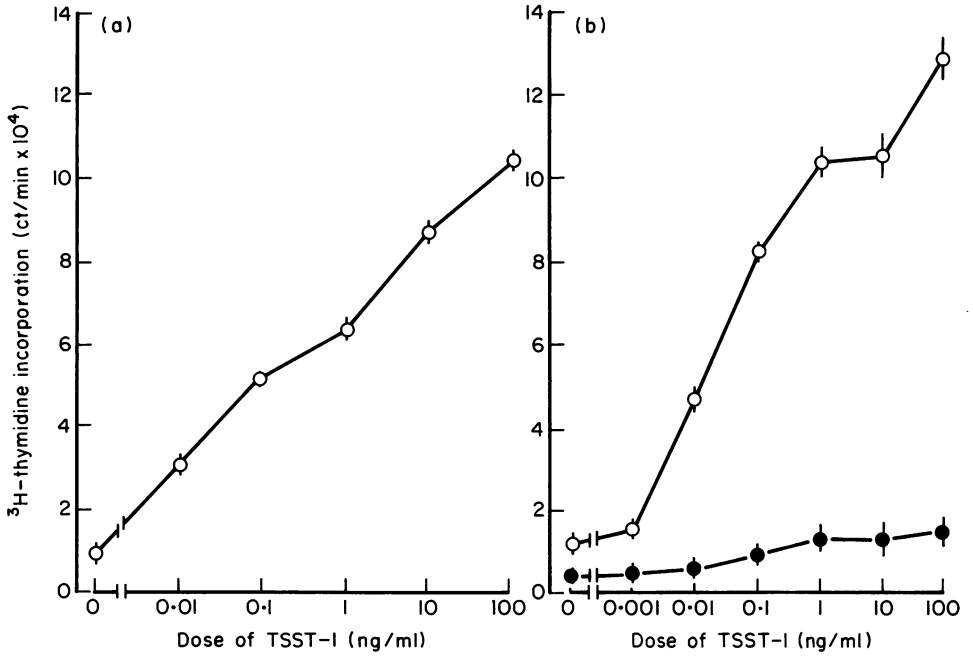

Fig. 1. The mitogenic effect of TSST-1 on HCBM and HPBM. (a) Whole HCBM (5×10^5/well) or (b) whole or T cell-depleted HPBM (5×10^5/well) were stimulated with graded doses of TSST-1 for 72 h. (○), Whole cells; (●), T cell-depleted cells.

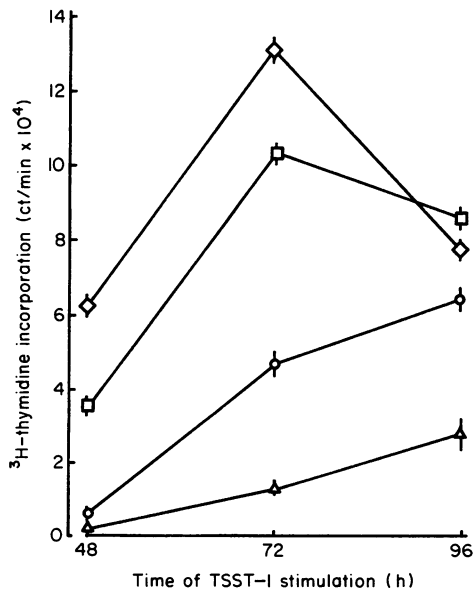

Fig. 2. Whole HPBM (5×10^5/well) were stimulated with several doses of TSST-1 for 48–96 h. (△), not stimulated; (○), 0·01 ng/ml; (□), 1 ng/ml; (◇), 100 ng/ml. Cultures were pulsed with 1 μCi ^{3}H-thymidine for the final 16 h of stimulation period.

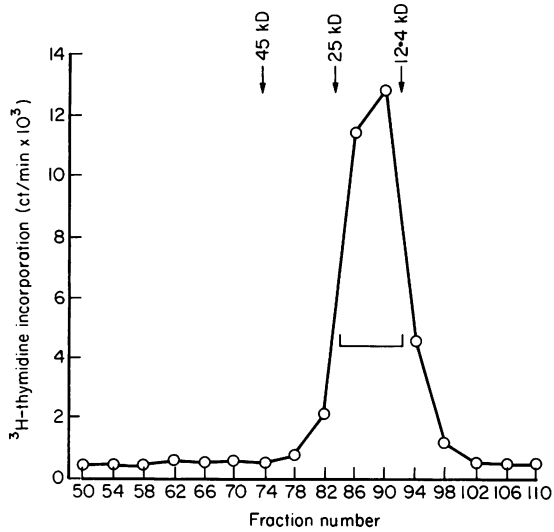

Fig. 3. Gel filtration chromatography of the culture supernatant of TSST-1-stimulated HPBM. HPBM ($7{\cdot}5 \times 10^6$/plate) were stimulated with 1 ng/ml of TSST-1 for 40 h. The cell-free culture supernatant (60 ml) was concentrated to 4 ml and applied to a Sephacryl S-200 column. Each fraction (3·5 ml) was assayed for the mitogenic activity for CTLL-2 cells.

IL-2 production by HPBM stimulated with TSST-1. Culture supernatants of HPBM stimulated with TSST-1 contained a marked stimulatory activity to IL-2-dependent CTLL-2 cells. To verify that the activity in the culture supernatants was due to IL-2, the following experiments were conducted. First, the culture supernatant of HPBM which had been stimulated with 1 ng/ml of TSST-1 for 40 h was concentrated and applied on a Sephacryl S-200 column to determine the molecular weight of the activity. Fractions eluted from the gel were assayed for the stimulatory activity to CTLL 2 cells (Fig. 3). The peak response in the stimulatory activity was observed in the fraction which corresponded with a molecular weight of 15–18 kD. This elution profile was very similar to that observed with human IL-2 (Gillis, Mochizuki & Conlon, 1982; Welte *et al.*, 1982). The peak response was not due to the toxin which was carried over into the culture supernatant, since the molecular weight of this toxin was known to be 24 kD (Igarashi *et al.*, 1984; Reiser *et al.*, 1983; Schlievert *et al.*, 1981) and the toxin had no effect on the proliferative responses of CTLL-2 cells (Uchiyama *et al.*, 1986). Secondly, the absorption test was done to examine the specificity of the TSST-1-induced factor. It was expected that, if this factor was IL-2, the activity of the purified factor should be absorbed by IL-2-reactive T cells, as shown by other investigators (Bonnard, Yasaka & Jacobson, 1979; Gillis *et at.*, 1978). The chromatographed fractions showing the stimulating activity to CTLL-2 cells were pooled as indicated by the bracket shown under the peak in Fig. 3, concentrated and dialysed against the culture medium. Then, this sample was diluted at 20% concentration by culture medium, mixed with various numbers of CTLL-2 cells and T cell-depleted murine spleen cells, and incubated for 5 h at 37°C. As a control sample, the medium containing recombinant human IL-2 (2 units/ml) was also incubated with these cells. After the incubation, the cells were removed and the supernatants of the samples were assayed for the stimulatory activity to CTLL-2 cells. As shown in Fig. 4A, the activity in the samples were reduced in proportion to the numbers of CTLL-2 cells used for absorption, whereas the absorption with T cell-depleted murine spleen cells (5×10^6/plate) had no detectable effect on the activity. This absorption profile was almost identical to that observed on the sample containing recombinant human IL 2 and being absorbed with the cells (Fig. 4B). Results observed in Fig. 3 and Fig. 4 indicate that the factor obtained is IL-2.

TSST-1 doses required for IL-2 production by HPBM. Whole and T cell-depleted HPBM were

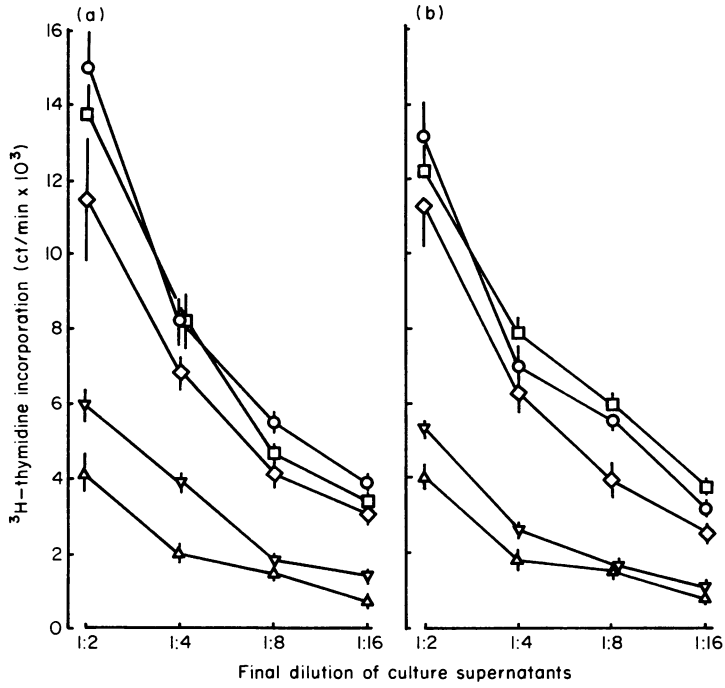

Fig. 4. Selective removal of the mitogenic activity of the culture supernatant of TSST-1-stimulated HPBM by CTLL-2 cells. Various numbers of CTLL-2 cells and T cell-depleted murine spleen cells were incubated in 35 mm Petri dishes containing 1 ml volumes of the purified TSST-1-induced factor or recombinant human IL-2 (2 units/ml) for 5 h at 37°C and the culture supernatants were assayed for the mitogenic activity to CTLL-2 cells. (a) the purified TSST-1-induced factor; (b), recombinant human IL-2. (○), cells not added; (△), 5×10^6 CTLL-2 cells/plate; (▽), 5×10^5 CTLL-2 cells/plate; (◇) 5×10^4 CTLL-2 cells/plate; (□), 5×10^6 T cell-depleted murine spleen cells/plate.

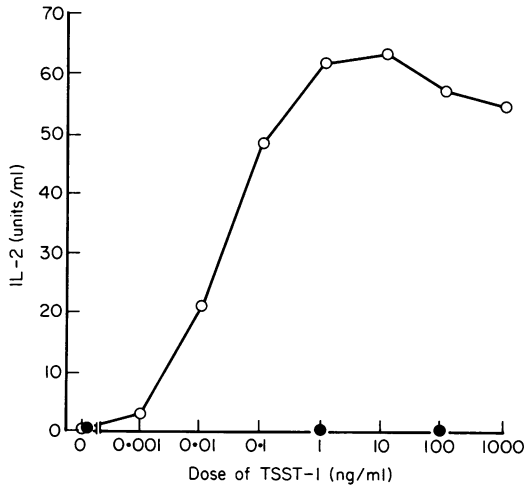

Fig. 5. Concentrations of TSST-1 required for T cells from HPBM to produce IL-2. Whole or T cell-depleted HPBM ($2{\cdot}5 \times 10^6$/plate) were stimulated with graded doses of TSST-1 for 36 h. Cell-free culture supernatants were assayed for IL-2 activity. (○), whole HPBM; (●), T cell-depleted HPBM.

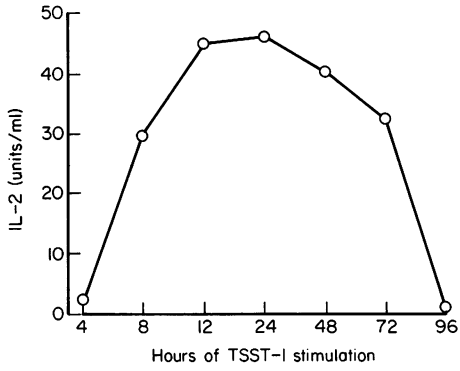

Fig. 6. Kinetics of IL-2 production by HPBM stimulated with TSST-1. HPBM ($2{\cdot}5 \times 10^6$/plate) were stimulated with 10 ng/ml of TSST-1 for various periods and the cell-free culture supernatants were assayed for IL-2 activity.

Table. 1. Effect of adherent macrophages and a macrophage factor on the IL-2 production by TSST-1-stimulated T cells in HPBM

T cells*	TSST-1†	Adherent macrophages prepared from	Macrophage factor‡	IL-2 (units/ml)
+	+	—	—	1·9
+	+	5×10^5 T cell-depleted HPBM	—	7·9
+	+	2×10^6 T cell-depleted HPBM	—	44·4
+	+	—	1:20	13·1
+	+	—	1:40	14·6
+	+	—	1:80	5·3
−	+	2×10^6 T cell-depleted HPBM	—	<0·1
+	−	—	1:20	<0·1

* Nylon wool column-passed T cells (2×10^6/plate) were stimulated with TSST-1 for 35 h.

† 1 ng/ml.

‡ A LPS-stimulated human macrophage culture supernatant containing IL-1.

cultured in the presence of graded doses of TSST-1 for 36 h, and the culture supernatants were assayed for IL-2 activity (Fig. 5). The IL-2 activity in the culture supernatants of whole HPBM was elevated sharply by increasing the doses of TSST-1 from 0·001 to 1 ng/ml. The maximum of the activity was observed at 10 ng/ml of TSST-1, but greater doses (100–1000 ng/ml) of TSST-1 were still capable of inducing the remarkable activity of IL-2. Furthermore, the TSST-1-induced IL-2 production by HPBM was completely abrogated by removing T cells from them. The results indicate that T cells in HPBM are essential for the production of IL-2 by TSST-1-stimulation.

Kinetics of IL-2 production. HPBM were cultured in the presence of 10 ng/ml of TSST-1 for various periods and the culture supernatants were then assayed for IL-2 activity (Fig. 6). Only minimal IL-2 activity was observed during the first 4 h, and the maximum IL-2 activity was observed between 12 and 24 h after TSST-1 stimulation. Thereafter, the activity progressively declined and a very low level of IL-2 activity remained after 96 h of TSST-1 stimulation.

Effects of adherent macrophages and a macrophage factor in the TSST-1-induced IL-2 production by purified T cells. Nylon wool column-passed T cells from HPBM were cultured with the adherent macrophages or a macrophage culture supernatant containing IL-1 activity and stimulated with 1 ng/ml of TSST-1 for 35 h. As shown in Table 1, only minimal amounts of IL-2 were produced, when

T cells were stimulated in the absence of both the macrophages and the macrophage factor. The IL-2 production was enhanced by increasing either the numbers of macrophages or the concentrations of the macrophage factor. The enhancing activity of macrophages was higher than that of the macrophage factor. These results suggest that macrophages are required for TSST-1-stimulated T cells to produce IL-2 and the function of macrophages can be replaced, although partially, by the factor that is presumably IL-1.

DISCUSSION

The mitogenic effect of TSST-1 on human T cells was reported by other investigators (Calvano *et al.*, 1984; Poindexter & Schlievert, 1985) and it was also confirmed by our experimental results showing that TSST-1 was mitogenic for whole HPBM, but not to T cell-depleted HPBM (Fig. 1 and 2). To understand the role of TSST-1 in the aetiology of TSS, it seems important to know which property of TSST-1, mitogenic or immunogenic, is more related to the activation of human lymphocytes. TSST-1 is mitogenic to HCBM (Poindexter & Schlievert, 1985; and Fig. 1 in the present study) and peanut-nonagglutinable thymus cells (Uchiyama *et al.*, 1986) both of which are thought to be never immunologically sensitized by the toxin. Our preliminary experiments with the limiting dilution analysis showed that one of 10–20 T cells in HPBM formed a colony to TSST-1 stimulation, indicating that the frequency of TSST-1-reactive T cells is markedly high. Micusan *et al.* (1986) reported that lymphocytes from germfree mice produced as much IL-2 as lymphocytes from conventional mice to TSST-1 stimulation and presensitization of mice with the toxin did not essentially influence the subsequent proliferative response of murine spleen cells to the toxin. These investigations lead us to the conclusion that TSST-1 acts on human T cells as an intrinsic polyclonal mitogen rather than an immunogenic antigen.

Previously we reported that TSST-1 is a potent inducer of IL-2 from T cells (Uchiyama *et al.*, 1986). A factor in culture supernatants of TSST-1-stimulated murine spleen cells which stimulated the proliferation of CTLL-2 cells was eluted in a fraction corresponding with a molecular weight of 30–35 kD from a gel chromatography, was absorbed with CTLL-2 cells but not with T cell-depleted murine spleen cells, and was produced by T cells. All of these experimental findings fitted for the criteria of murine IL-2. Recently Micusan *et al.* (1986) reported that culture supernatants of TSST-1-stimulated murine and human lymphocytes was stimulatory to IL-2-dependent T cells, suggesting that the toxin can induce human lymphocytes to produce IL-2. We consider, however, that much more analysis was necessary for conclusive evidence for IL-2 production by TSST-1-stimulated human lymphocytes in their study. In the present study, we showed that the toxin is also a potent inducer of IL-2 by human lymphocytes. First, the analysis using a gel chromatography showed that a factor stimulatory to CTLL-2 cells in a culture supernatant of TSST-1-stimulated HPBM was eluted in a peak corresponding with a molecular weight of 15–18 kD (Fig. 3). The molecular weight estimated by us was similar to that of human IL-2 reported by others (Gillis, Mochizuki, & Conlon, 1982; Welte *et al.*, 1982). Secondly CTLL-2 cells could absorb the stimulatory factor purified by gel chromatography, as shown by other investigators (Bonnard, Yasaka & Jacobson, 1979; Gillis *et al.*, 1978). The absorption profile of the factor was almost identical to that of the recombinant human IL-2 (Fig. 4). Thirdly T cells produce the factor (Fig. 5 and Table 1). These experimental results fit for the criteria of human IL-2.

HPBM are quite sensitive to TSST-1, since a low concentration of TSST-1 (0·01 ng/ml of TSST-1 in 36 h stimulation) (Fig. 5) and a short period of the stimulation (8 h at 10 ng/ml) are fully effective for HPBM to produce substantial amounts of IL-2 (Fig. 6). By contrast, our previous study showed that much higher concentrations of the toxin and much longer periods of stimulation were required for murine spleen cells to produce substantial amounts of IL-2 (Uchiyama *et al.*, 1986). This different sensitivity between human and murine lymphocytes to the toxin may partially explain an observation that human beings are susceptible to the toxin, though mice do not show any apparent symptoms, when injected with the toxin (unpublished data).

The mechanism through which the accessory cells participate in the T cell activation induced by polyclonal T cell mitogens is still debatable. The results in the present study showed that the IL-2

production by TSST-1-stimulated human T cells require the presence of macrophages or a macrophage factor. The activity supporting the IL-2 production by T cells was higher in the macrophages than in the macrophage factor (Table 1). The present results are at variance with our previous report on the accessory cell requirement in that IL-2 production by TSST-1-stimulated murine splenic T cells was dependent on the presence of macrophages, irrespective of the presence of exogenously added factor (Uchiyama *et al.*, 1986), suggesting that T cells require the contact with accessory cells in murine cell system. For the discrepancy between human and murine cell system, two explanations may be available. First, T cells in HPBM in comparison with murine T cells may be relatively independent of the accessory cells and may be ready to produce IL-2 by co-stimulation with TSST-1 and the macrophage factor. The second explanation is that a small number of accessory cells might contaminate the T cell preparation and the contaminated macrophages might participate in the T-cell activation by TSST-1 in cooperation with the exogenously added macrophage factor. The macrophage culture supernatant used in the present study may have contained biologically active products beside IL-1. Further analysis of the roles of macrophage factor and accessory cells in the TSST-1-induced T cell activation is in progress, with the use of purified IL-1 preparation.

The present study showing that TSST-1 is a potent inducer of IL-2 production by human T lymphocytes suggests a possibility that the toxin may also induce the production of other lymphokines. Actually we found that macrophage-activating factor(s) (MAF), one of which might be interferon (IFN), were produced by TSST-1-stimulated T cells in both murine spleen cells and HPBM (manuscript in preparation). The present study and our unpublished observations on the production of MAF by human T cells suggest that in TSS patients the lymphokines including IL-2, MAF and IFN are presumably produced in excess over the physiologically required amounts by TSST-1 stimulation. It has been reported that recombinant human IL-2 has various dose-related side effects such as chill, high fever, malaise, headache, nausea and vomiting, diarrhoea, weight gain due to fluid retention, hypotension and dyspnea, when it was administered into patients with a variety of malignancies (Lotze *et al.*, 1985). Some of these side effects were also induced by IFN (Gutterman *et al.*, 1980). During continuous IL-2 infusion, lymphocyte counts in peripheral blood often decreased (Lotze *et al.*, 1985). It seems noteworthy that some of these clinical signs or symptoms are observed in TSS patients. Although it has been thought that lymphokines are essential in the physiological regulation and the acquisition of resistance to microbial infection (Dumonde & Hamblin, 1983), in view of these abnormal signs and symptoms that are observed in patients administered with lymphokines, lymphokines produced by TSST-1 stimulation in TSS patients may act as the factors which develop the harmful responses in this complicated illness. The similarities of the histopathological findings in several organs of the fatal cases between human TSS patients and rabbits with experimentally induced TSS described in the section of introduction may support this consideration.

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