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Production of human and murine interleukin-2 by toxic shock syndrome toxin-1*

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SUMMARY

Toxic shock syndrome toxin-1 (TSST-1), isolated from *Staphylococcus aureus* strains associated with toxic shock syndrome (TSS), is known as a potent mitogen and interleukin-1 inducer. The potential of TSST-1 as an interleukin-2 (IL-2) inducer was tested on human peripheral blood lymphocytes (HPBL) and murine spleen lymphocytes (MSL). These cells were incubated with TSST-1 and the supernatants analysed for IL-2 production. Preincubation of IL-2-dependent indicator cells (IC) with a monoclonal antibody specific for murine IL-2 receptors inhibited their proliferation by supernatants of TSST-1-treated MSL, thus strongly suggesting that they contain IL-2. The concentrations of TSST-1 required for HPBL or MSL to produce IL-2 ranged between 10^{-1} and 10^{-4} μg/ml. The amount of IL-2 units/ml varied little from one experiment to another. In contrast, IL-2 production by PHA-stimulated HPBL or Con A-stimulated MSL showed great variability and dependence on mitogen concentration. T-cell depleted MSL exposed to TSST-1 produced less IL-2. Experiments with germ-free mice and TSST-1-primed mice demonstrated that IL-2 production is not related to TSST-1 antigenicity.

INTRODUCTION

Interleukin-2 (IL-2) represents an important lymphokine which is formed among others at the cellular level during immunological events. Pioneer work associated IL-2 with T-cell proliferation (Morgan, Ruscetti & Gallo, 1976; Gillis *et al.*, 1978; Smith *et al.*, 1979). Since then, intensive studies have demonstrated the role of IL-2 in phenomena such as enhancement of the *in vitro* plaque-forming cell response of B-cell enriched spleen cultures, induction of primary cytotoxic T-cell responses, induction of helper activity in spleen cells of athymic mice, maturation of different cell types, secretion of gamma interferon, and production by T cells of a B-cell growth factor (for reviews see Smith & Ruscetti, 1981; Farrar *et al.*, 1982; Robb, 1984). The production of IL-2 can be triggered in T cells in response to either the specific signals provided by antigens (Smith, 1980) or the non-specific signals of mitogens (Gillis *et al.*, 1978).

In the past few years, substances of bacterial origin have also been reported to be mitogenic. Among them, staphylococcal enterotoxins (SE) have been shown to act as very potent mitogens for murine and human T cells, both *in vitro* (Peavy, Adler & Smith, 1970; Langford, Stanton & Johnson, 1978) and *in vitro* (Archer, Smith & Peeler, 1980; Zehavi-Willner, Shenberg & Barnea, 1984). One of the several SE, namely the staphylococcal enterotoxin A (SEA), was also shown to stimulate IL-2 production in human peripheral blood lymphocytes (Clark *et al.*, 1984).

More recently, a new toxin named toxic shock syndrome toxin-1 (TSST-1) (Bergdoll & Schlievert, 1984) has been identified and purified (Bergdoll *et al.*, 1981; Schlievert *et al.*, 1981; Reiser *et al.*, 1983; Igarashi *et al.*, 1984) from cultures of *Staphylococcus aureus* strains isolated from patients with toxic shock syndrome (TSS) (Todd *et al.*, 1978). TSST-1 is a single-chain protein of about 210 amino acid residues (24,000 MW) and contains no glycosidic residues. It is different from SE since this toxin neither has a disulphide bond (Micusan *et al.*, 1985), nor elicits emesis in cynomolgus and rhesus monkeys. Injected into rabbits, TSST-1 primarily affects the lymphoid tissue (Best *et al.*, 1984) and induces symptoms such as fever, diarrhoea, low blood pressure, erythroderma and respiratory distress (Rasheed *et al.*, 1985). In spite of the above symptoms, which are very

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Abbreviations: CCM, complete culture medium; Con A, concanavalin A; CTLL, IL-2-dependent cytotoxic murine T-cell line; FCS, fetal calf serum; [^{3}H]-TdR, tritiated thymidine; HPBL, human peripheral blood lymphocytes; IC, IL-2-dependent indicator cells; IL-2, interleukin-2; IL-2RR, IL-2 reference reagent; MSL, mouse spleen lymphocytes; PHA, phytohaemagglutinin; SD, standard deviation; SE, staphylococcal enterotoxins; SEA, staphylococcal enterotoxin A; TSS, toxic shock syndrome; TSST-1, toxic shock syndrome toxin-1.

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similar to those observed in TSS patients, there is no complete proof that TSST-1 is the cause of TSS. It has been shown that TSST-1 has important mitogenic and immunosuppressive activities (Schlievert *et al.*, 1981; Poindexter & Schlievert, 1985), being also an inducer of human interleukin-1 (IL-1) (Ikejima *et al.*, 1984; Parsonnet *et al.*, 1985).

In this report, we present evidence to suggest that TSST-1 can induce IL-2 production in human peripheral blood lymphocytes (HPBL) and in murine spleen lymphocytes (MSL), therefore revealing the complexity of the immunomodulating properties of this toxin.

MATERIALS AND METHODS

Animals

The C57BL/6 mice used (Charles River, St Constant, Quebec) were of both sexes and were between 8 and 10 weeks old. In one experiment, the mice received intraperitoneal injections of 10 μg of TSST-1 in complete Freund's adjvant (CFA) 10 and 3 days before the experiment. Presence of anti-TSST-1 antibodies in the serum of immunized mice was identified by conventional ELISA method using anti-mouse IgG conjugated with peroxidase (Amersham Canada Ltd, Oakville, Ontario). Germ-free and conventional mice of the non-inbred CDI strain were obtained from the Charles River Breeding Laboratories Inc. (Wilmington, MA).

Human blood donors

Human blood was obtained from healthy male volunteers, 25–40 years of age.

Mitogens

Phytohaemagglutinin-M (PHA) (Gibco, Chagrin Falls, OH) and concanavalin A (Con A) (Calbiochem-Behring, La Jolla, CA) were used throughout this study. TSST-1 was prepared as previously described (Reiser *et al.*, 1983). Briefly, the purification procedure involved the absorption of the toxic protein from culture supernatants of *S. aureus*, strain FRI-1169, with Amberlite CG-50 (Mallinckrodt Chemical Workers, St Louis, MO), ion exchange chromatography on CM-Sepharose CL-6B and gel filtration on Sephacryl S-200 (Pharmacia Fine Chemicals, Piscataway, NJ).

Lymphocyte suspensions

Murine spleen lymphocytes (MSL) were obtained from spleens aseptically removed, minced and washed in RPMI-1640 (Flow Laboratories Inc., Mississauga, Ontario). Red cells were eliminated by brief osmotic shock and, after three more washings, the MSL were resuspended in RPMI-1640 supplemented with 10% fetal calf serum (FCS), 100 IU of penicillin G, 10 μg of streptomycin, 10 mM HEPES (Flow Laboratories Inc., Mississauga, Ontario) and 5×10^{-5} M 2-mercaptoethanol. We refer to this medium as 'complete culture medium' (CCM). Viable cells were counted by trypan blue exclusion and the cell concentration was adjusted.

Human peripheral blood lymphocytes (HPBL) were separated from the blood collected in 15 ml heparinized tubes and centrifuged at 600 ***g*** for 20 min. The buffy coat was resuspended in 15 ml RPMI-1640 and HPBL were obtained by density gradient centrifugation on Ficoll–Paque (Pharmacia Canada, Dorval, Quebec). After three washings in RPMI-1640, the HPBL were resuspended in CCM, counted for viability and adjusted to the appropriate concentration.

Mitogenic activity

Direct mitogenic action of TSST-1 and Con A on MSL at different concentrations was determined in parallel with measurement of IL-2 production. MSL (3×10^6 cells/ml) were distributed in volumes of 100 μl into 96-well flat-bottomed tissue culture plates (Flow Laboratories Inc., McLean, VA) and incubated in the presence or absence of mitogens at 37° in a humid atmosphere containing 5% CO_2. The cells were cultured for 3 days, and 18 hr before the termination of culture the cells were pulsed with 1 μCi/well of [^{3}H]thymidine ([^{3}H]TdR) (New England Nuclear, Boston, MA; 15·1 Ci/mmol specific activity). The cell cultures were harvested using an automatic cell harvester (Titertek, Flow Laboratories Inc., Rockville, MD). After drying, the filters were counted in a liquid scintillation counter having 60% counting efficiency (Beckman LS 100, Beckman Instruments, Palo Alto, CA). All experiments were performed in triplicate and the mean values and standard deviations (SD) were calculated. The results are given after subtraction of background and appropriate control values.

IL-2-dependent indicator cells (IC)

As IC, both Con A-induced blast cells and a murine line of cytotoxic T lymphocytes (CTLL) were used. Con A-induced blast cells were prepared by an adaptation of a previously described method (Lafferty *et al.*, 1980). Briefly, MSL (1×10^6 cells/ml) were cultured in 10 ml of CCM in the presence of Con A, 5 μg/ml. The cells were cultured in 25 cm^2 tissue culture flasks at 37° in a humid atmosphere containing 5% CO_2. After 72 h of incubation, the blast cells were harvested, washed three times with RPMI-1640, counted and reseeded to a concentration of 4×10^5 cells/ml in CCM and used immediately. These cells have been shown to give results comparable to those obtained with established IL-2-dependent T-cell lines (Bertoglio *et al.*, 1984). The CTLL cells (kindly provided by Dr F. Dumont, Merck Institute, Rahway, NJ) were maintained in 10% FCS complete RPMI-1640 supplemented with 1 mM Na pyruvate and 1% non-essential amino acids 100× (Flow Laboratories Inc., McLean, VA), 5×10^{-5} M 2-mercaptoethanol and 5% IL-2 (Rat T-cell Polyclonal®, Collaborative Research Inc., Lexington, MA).

IL-2 production

The capacity of HPBL and MSL to produce IL-2 *in vitro* in the presence of TSST-1, Con A or PHA at different concentrations was studied by culturing 3×10^6 cells/ml in CCM. Volumes of 1 ml were dispensed in each of the 24 wells of a flat-bottomed culture plate (Flow Laboratories Inc., McLean, VA) and incubated at 37° in a humid atmosphere containing 5% CO_2. After 24 hr of incubation, the cells were centrifuged and the cell-free supernatants were collected under sterile conditions. These supernatants were stored at −20° until tested for IL-2 activity. As controls, different concentrations of TSST-1 were incubated without any lymphoid cells, and assessed for possible direct mitogenic action on IC.

Assay of IL-2 activity

Cell-free supernatants were assayed for IL-2 activity using 50 μl of supernatants, in triplicate, which were added to 50 μl of IC which were either Con A-induced blast cells (4×10^5 cells/ml) or

CTLL cells (8×10^4 cells/ml) and dispensed into 96-well flat-bottomed microtitre plates. The plates were incubated for 20 hr in standard conditions, pulsed with 1 μCi/well of [^{3}H]TdR and harvested 5 hr later. Processing and determination of [^{3}H]TdR incorporation were done as described above. A reference preparation of human IL-2, 99% pure, was kindly supplied by Dr G. Thurman (Biological Response Modifiers, Biological Resources Branch, NCI-FCRF, Frederick, MD) and is called herein 'IL-2 reference reagent' (IL-2RR). Different dilutions of IL-2RR were used with each sample of IC in parallel with our IL-2-containing supernatant samples. IL-2RR activity, expressed as percentage of maximum [^{3}H]TdR uptake, was plotted on probit/$\log_2$ paper (Gillis *et al.*, 1978). The results are given in IL-2 units/ml based on IL-2RR activity, which was 500 IL-2 units/ml. Controls of supernatants from cells without mitogens gave a [^{3}H]TdR uptake of the same magnitude as the background given by unstimulated IC.

Inhibition of IL-2-dependent IC proliferation

A rat monoclonal antibody (7D4) directed against murine IL-2 receptors (Malek, Robb & Shevach, 1983) was kindly donated by Dr T. R. Malek, NIH, Bethesda, MD. The inhibition experiments were done by incubating 50 μl of IC (4×10^5 cells/ml) with 50 μl of 7D4 monoclonal antibody for 1 hr at 37°. After the incubation period, 50 μl of supernatants of TSST-1 or Con A-treated MSL, as well as 50 μl of IL-2 from a commercial source (Boehringer-Mannheim, Dorval, Quebec) were added and the IL-2 activity on IC proliferation was assayed as described above. As control, culture medium was added instead of the 7D4 antibody.

T-cell depletion

T-cells were eliminated by the treatment of MSL with mouse monoclonal anti-Thy 1.2 serum (Cedarlane Laboratories, Hornby, Ontario). Briefly, MSL (1×10^7 cells/ml) were incubated for 1 hr at 4° with different dilutions of anti-Thy 1.2 serum. After centrifugation, the cells were resuspended to the original volume in 1:10 diluted agarose-absorbed guinea-pig complement and incubated at 37° for 1 hr. The cells were then washed twice with RPMI-1640 and tested for proliferation and for TSST-1 or Con A-induced IL-2 production.

RESULTS

IL-2 production as a function of TSST-1 concentration

In a first experiment, 3×10^6 cells/ml of HPBL from two donors or MSL from C57BL/6 mice were reacted with different concentrations of TSST-1 ($1\text{–}1 \times 10^{-5}$ μg/ml). As shown in Fig. 1a, all concentrations, except 1 μg/ml which was inhibitory, induced substantial IL-2 production, as measured by [^{3}H]TdR uptake. The c.p.m. values given by IC in response to different concentrations of TSST-1 alone was always negligible in comparison with that of TSST-1 incubated with either HPBL or MSL. This kind of control has been repeated in all the other experiments and has always produced comparable results. In subsequent experiments, using HPBL from three other donors (Fig. 1b), the IC response to TSST-1 alone has always been subtracted from the IC response (in c.p.m.) to TSST-1-treated cell culture supernatants and the IL-2 activity expressed in units/ml. The results showed that TSST-1 concentrations as low as

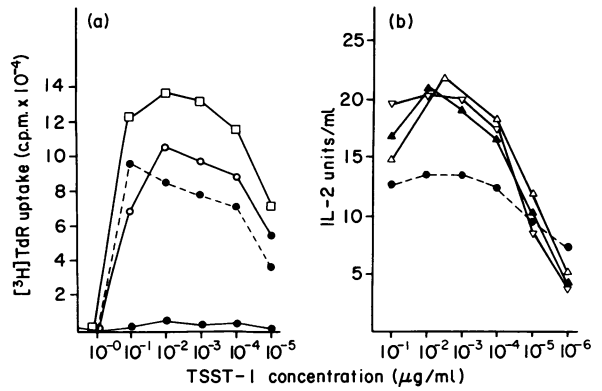

Figure 1. IL-2 production by HPBL and MSL (3×10^6 cells/ml) incubated for 24 hr with different concentrations of TSST-1. Cell-free supernatants (50 μl) were added to 50 μl of IC (4×10^5 cells/ml) and [^{3}H]TdR incorporation over 5 hr was measured after 20 hr incubation. (a) IL-2 production expressed as [^{3}H]TdR uptake, or (b) as IL-2 units/ml; (□,○,△,▽,▲) different donors; (● - - - ●) C57Bl/6 mice; (●——●) control of IC response. Each point represents the mean of triplicate wells.

1×10^{-6} μg/ml still induced measurable amounts of IL-2. In this experiment, HPBL responded with almost no variation to TSST-1 stimulus, in terms of IL-2 production. In both experiments, HPBL generally produced more IL-2 than MSL.

Inhibition of IL-2-dependent IC proliferation by a rat monoclonal anti-murine IL-2 receptor antibody

Preincubation of IL-2-dependent IC with a rat monoclonal anti-murine IL-2 receptor antibody (7D4) and subsequent addition of supernatants from TSST-1-treated MSL led to a 77% inhibition of their proliferation as compared with controls. A similar rate of inhibition was also obtained when supernatants from Con A-stimulated MSL or a commercial IL-2 preparation were added to antibody-treated IC (Table 1).

Comparison of IL-2 production after stimulation with TSST-1, PHA or Con A

In two separate experiments, IL-2 production by HPBL as well as by MSL was stimulated with TSST-1, PHA or Con A at different concentrations. The results are shown in Fig. 2. Again, TSST-1, in the concentration range of 1×10^{-1} to 10^{-4} μg/ml, elicited large amounts of IL-2, both with HPBL and MSL. In contrast, MSL responded poorly to PHA stimulation and exhibited a sensitive, concentration-dependent response to Con A. HPBL responded poorly to Con A and, in the case of PHA stimulation, these responses exhibited some variation from one experiment to another.

Thymus-dependence of TSST-1 stimulation

In order to test the thymus-dependence of TSST-1 stimulation, T cells from MSL were depleted by pretreating them with monoclonal anti-Thy 1.2 serum and guinea-pig complement. MSL treated with complement alone were used in parallel as control. Both the direct [^{3}H]TdR uptake as response to mitogenic action and the IL-2 production were assayed on these cells, using TSST-1 and Con A as inducers (Fig. 3). The results show

Table 1. Inhibition of IL-2-dependent IC proliferation following their preincubation with anti-murine IL-2 receptor antibody, in the presence of supernatants from TSST-1, Con A-treated MSL and commercial IL-2 ([^{3}H]TdR uptake ± SD)

	Source of IL-2		
	TSST-1	Con A	Commercial IL-2
Preincubation of IC with culture medium	47,551 ± 3969	79,990 ± 8005	76,513 ± 2933
Preincubation of IC with anti-murine IL-receptor antibody	10,898 ± 1845	26,512 ± 1449	24,745 ± 3262
% inhibition	77	66	67

Supernatants were obtained after the incubation of 4×10^5 MSL/ml for 20 hr with 10^{-2} μg/ml of TSST-1 or 5 μg/ml of Con A. Commercial IL-2 was added undiluted.

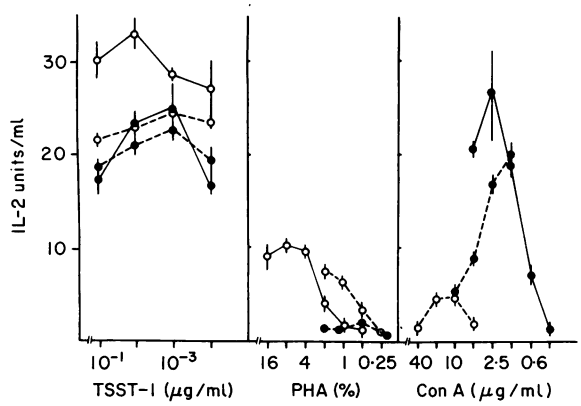

Figure 2. IL-2 production by HPBL and MSL (3×10^6 cells/ml) incubated for 24 hr with different concentrations of TSST-1, PHA and Con A, and [^{3}H]TdR incorporation by IC over 5 hr was measured after 20 hr incubation. (○) HPBL; (●) MSL; (——) Experiment 1; (- - -) Experiment 2. Vertical bars represent SD.

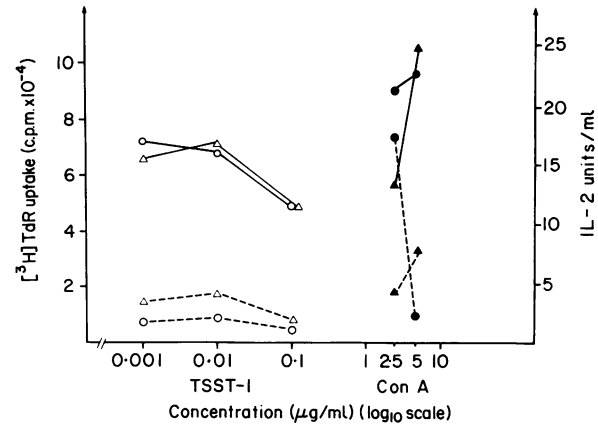

Figure 3. Mitogenicity and IL-2 production by MSL incubated with TSST-1 and Con A at different concentrations after T-cell depletion with 1:500 diluted anti-Thy 1.2 serum in the presence and absence of complement. (○) TSST-1-incubated mitogenicity; (△) IL-2 production with TSST-1; (●) Con A-induced mitogenicity; (▲) IL-2 production with Con A; (- - -) anti-Thy 1.2 and complement; (——) no complement. Each point represents the mean of three separate experiments.

that removal of T lymphocytes with anti-Thy 1.2 serum diluted 1:500 reduced both the direct [^{3}H]TdR uptake and the IL-2 production to 10–20% of the control levels after exposure to 1×10^{-1} to 1×10^{-3} μg/ml of TSST-1. The reduction of both these activities seemed to vary in the same proportions for the different TSST-1 concentrations used. In the case of the Con A-treated cells, the effect of T-cell depletion was found to be dependent on the Con A concentration used for both the [^{3}H]TdR uptake and the IL-2 production. It was also observed that variations of Con A concentration did not result in parallel shifts of [^{3}H]TdR uptake and of IL-2 production when TSST-1 was used at different concentrations. In another experiment 1×10^{-2} μg/ml of TSST-1, or 5 μg/ml of Con A, were used to stimulate MSL which had been pretreated with different dilutions of monoclonal anti-Thy 1.2 serum and complement (Fig. 4). The results are expressed as a percentage of the values obtained with the respective controls, i.e. cells incubated with complement alone. They confirm that, in the case of stimulation with TSST-1, both the [^{3}H]TdR and the IL-2 production were equally inhibited after T-cell depletion. The results also show that a dilution of 1:1000 of anti-Thy 1.2 serum still kept both activities at about 20% of the level found in control cells. However, by using Con A instead of TSST-1 as stimulator, it was observed that [^{3}H]TdR uptake and IL-2 production apparently presented different sensitivities to anti-Thy 1.2-induced T-cell depletion (Fig. 4).

IL-2 production as a function of previous *in vivo* exposure to TSST-1

Although no anti-TSST-1 antibodies had been detected with the ELISA method in the sera of the C57BL/6 mice used throughout these studies, we performed experiments especially designed to study the influence of a hypothetical previous contact of the MSL donors with TSST-1-secreting *S. aureus*.

In a first experiment, two C57BL/6 mice were primed with TSST-1 and their MSL were later exposed *in vitro* to either

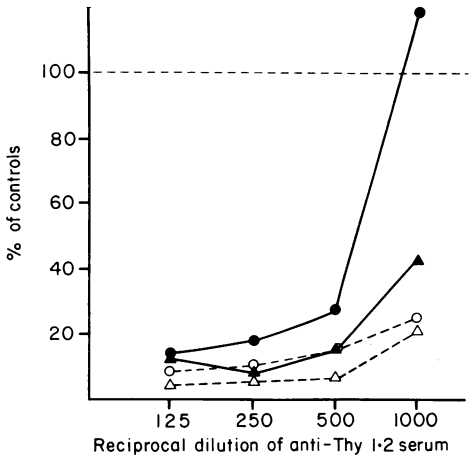

Figure 4. Mitogenicity and IL-2 production by MSL incubated with 10^{-2} μg/ml of TSST-1 or 5 μg/ml of Con A as a function of anti-Thy 1.2 serum dilutions. (○) TSST-1-induced mitogenicity; (△) IL-2 production with TSST-1; (●) Con A-induced mitogenicity; (▲) IL-2 production with Con A; (- - -) anti-Thy 1.2 and complement; (——) no complement. Each point represents the mean of three separate experiments.

Table 2. TSST-1 and Con A-induced IL-2 production by TSST-1 untreated and treated C57BL/6 mice and by untreated germ-free and conventional CDI mice

		IL-2 units/ml (±SD)	
Strain	Status	TSST-1*	Con A†
C57BL/6	Untreated‡	12·7±1·7	19·4±21
	TSST-1-treated No. 1	11·4±1·6	20·0±0·9
	No. 2	14·4±1·0	16·9±1·4
CDI	Germ-free No. 1	15·0±4·2	54·7±8·9
	No. 2	8·6±2·6	24·0±1·5
	No. 3	19·4±2·8	44·2±2·3
	Conventional No. 4	25·8±2·0	56·9±23
	No. 5	11·6±1·2	59·4±7·9
	No. 6	8·4±0·8	60·2±6·8

* 20 hr *in vitro* exposure with 10^{-2} μg/ml TSST-1.
† 20 hr *in vitro* exposure with 5 μg/ml Con A.
‡ Pool of four mice.

TSST-1 or Con A, in parallel with MSL from unprimed C57BL/6 mice. IL-2 production was assayed and the results showed no difference between the two kinds of MSL donors. In a second experiment, non-inbred CDI mice, either germ-free or conventionally reared, were used as non-primed MSL donors. Again, no difference in the TSST-1-induced IL-2 production was observed between these two groups (Table 2).

DISCUSSION

Incubation of HPBL or MSL with TSST-1 resulted in the production of a soluble factor with a strong mitogenic activity on IL-2-sensitive IC. This activity resulted from the interaction between these cells and TSST-1 and not from a TSST-1-induced mitogenicity on IC.

TSST-1 has been recently reported as a potent IL-1 inducer (Ikejima *et al.*, 1984; Parsonnet *et al.*, 1985). In our system, we found that the mitogenic activity of the supernatants resulting from cell–TSST-1 interaction was closely T-cell dependent, suggesting that TSST-1 is also able to stimulate T lymphocytes to produce lymphokine(s). However, IL-2 production could be a consequence of previously produced IL-1 by macrophage-like cells present in our cellular suspensions. The behaviour of the used IC cells strongly suggested that IL-2 was the major component of the lymphokine production by TSST-1-stimulated cells. In fact, the IL-2 activity of supernatants from TSST-1-treated cells on IC proliferation was inhibited by a rat monoclonal antibody specifically directed at the murine IL-receptor. The inhibition is similar to the one observed in the IL-2 activity of supernatants from Con A-stimulated cells.

It has also been shown that, like Con A, TSST-1 has mitogenic properties (Schlievert *et al.*, 1981; Poindexter & Schlievert, 1985). Comparison of responses to Con A on the one hand, and to TSST-1 on the other, in both direct mitogenicity on T cells and IL-2 production, showed that even as little as 1–0·01 ng/ml of TSST-1 were able to stimulate the human as well as the murine lymphocytes. This could be attributed either to a difference in balance between stimulating and inhibiting substances triggered by the two mitogens, or the fact that Con A as well as PHA might be more active mitogens than TSST-1. In either case, at the time selected for the IL-2 measurements, the cells might already have utilized a part of the IL-2 produced by them.

Our results showed that significant amounts of IL-2 production could be obtained over a broad concentration range (1×10^{-1} to 1×10^{-4} μg/ml) of TSST-1-stimulated cells. In contrast, Con A-induced IL-2 production was more concentration-dependent and varied from one experiment to another. PHA-induced IL-2 production proved also to be more concentration-dependent that TSST-1. In parallel experiments (not shown), we confirmed the fact that PHA-induced IL-2 production by HPBL exhibited large variations from one healthy donor to another (de Faucal *et al.*, 1984). The stimulation of the HPBL from several healthy donors with an optimal TSST-1 concentration gave less variable data (20–30 IL-2 units/ml). Another conclusion suggested by this study is that TSST-1 is equally active, as an IL-2 inducer, on T cells of murine and human origin.

The T-cell dependence of both the direct mitogenicity and the IL-2 production was verified on T-cell depleted MSL. The effects of TSST-1 and Con A activities showed different sensitivities to both stimulator concentration and degree of T-cell depletion. This would suggest that TSST-1-sensitive T-cells belong to a subset different from the Con A sensitive T-cells, as has been shown with Con A- and PHA-sensitive T-cell subsets (Stobo, 1972). Another behavioural difference between T-cell subsets can be inferred from the perfect parallelism shown both by IL-2 production and by sensitivity to mitogenic activity after exposure with TSST-1 but not with Con A.

Since TSST-1 is of microbial origin, a statement of its intrinsic mitogenicity and its IL-2-inducing capacity required the demonstration that these properties were not associated with immune reactions against the TSST-1 as antigen. Poindexter & Schlievert (1985) showed that human lymphocytes taken from cord blood did respond to the mitogenic properties of TSST-1 and concluded that pre-exposure to the toxin was

therefore not necessary in observing this activity. In the present work, we showed that murine lymphocytes from germ-free donors were able to produce as much IL-2 as lymphocytes from conventional donors. We also showed that preimmunization of mice with the mitogen, shortly before collection of reactive MSL, did not essentially alter subsequent response to *in vitro* exposure with TSST-1. This permits the conclusion that the observed activity of TSST-1 was due to a non-specific mitogenic effect rather than to its antigenicity. As a non-specific mitogen, TSST-1 seemed to give more reliable results than the currently used lectins such as Con A or PHA and could be used as a non-specific stimulator of lymphoid cells for assessing the immune reactivity in different clinical situations. Current work is now attempting to elucidate further the mechanism of IL-2 production by TSST-1 and to investigate the immunomodulating properties of a detoxified TSST-1.

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