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## Induction by Toxic-Shock-Syndrome Toxin-1 of a Circulating Tumor Necrosis Factor-Like Substance in Rabbits and of Immunoreactive Tumor Necrosis Factor and Interleukin-1 from Human Mononuclear Cells

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A shock-like syndrome was induced in rabbits by administering toxic-shock-syndrome toxin-1 (TSST-1); tumor necrosis factor (TNF)-like activity was detected in sera of rabbits 3.5 h after injection, as measured by cytotoxic effects on the tumorigenic L929 murine fibroblast cell line. Appearance of this activity in sera coincided with onset of significant shock-related hemodynamic changes. TSST-1 stimulated release of TNF-like material from rabbit mononuclear cells in culture. Human mononuclear cells also secreted a cytotoxic substance shown to be TNF by radioimmunoassay. Maximal TNF secretion was higher in human mononuclear cells stimulated with TSST-1 than in those stimulated with bacterial lipopolysaccharide. Lipopolysaccharide, however, was a more potent inducer of interleukin-1 $\alpha$  and interleukin-1 $\beta$  from the same cells than was TSST-1. Because TNF and interleukin-1 act synergistically during induction of a shock-like state, these results suggest that part of the TSST-1-induced shock is due to production of interleukin-1 and TNF.

Toxic shock syndrome is often a life-threatening disease for humans. The syndrome has been attributed to certain strains of *Staphylococcus aureus* [1, 2] that colonize the vagina or infect wounds. Despite a dramatic and sudden onset of fever, hypotension, and gastrointestinal disturbances, bacteremia has rarely been demonstrated. Therefore the constellation of signs and symptoms of the syndrome is likely to be due to toxemia.

The polypeptide exotoxin toxic-shock-syndrome toxin-1 (TSST-1) causes many of the acute manifestations of toxic shock syndrome, including fever, hypotension, and diarrhea, in animals. The precise mechanism of the toxin's ability to induce the syndrome remains to be elucidated, but TSST-1 is clearly an inducer of the cytokine interleukin-1 (IL-1), and in turn IL-1 causes some, but not all, of the observed clinical and laboratory responses associated with toxic shock syndrome [3, 4].

However, another potent IL-1 inducer, endotoxin or lipopolysaccharide (LPS), also stimulates a different cytokine, tumor necrosis factor (TNF) [5-8]. TNF

is an endogenous pyrogen [9], and TNF shares with IL-1 many biologic functions. Recombinant IL-1 and TNF increase hepatic acute-phase protein synthesis [10], decrease hepatic albumin synthesis [10, 11], induce synovial collagenase and prostaglandin E<sub>2</sub> [12], cause release of procoagulant activity from vascular endothelium [13], and inhibit lipoprotein lipase synthesis [14, 15]. We recently demonstrated that injection of recombinant human IL-1 $\beta$  or recombinant human TNF decreases systemic arterial pressure in the rabbit and that combined administration of IL-1 and TNF is more potent than either cytokine alone in induction of a shock-like state [16].

Here we demonstrate that TSST-1 induces shock in the rabbit, as characterized by decreased mean arterial pressure, systemic vascular resistance, and central venous pressure (CVP). Death occurs subsequent to these hemodynamic changes. We detected a circulating TNF-like activity during the TSST-1-induced shock in rabbits. We also quantified TNF-like activity in vitro after stimulation of rabbit mononuclear cells (MNCs) with TSST-1. Using both the bioassay and a specific RIA, we examined TSST-1-induced production of TNF in vitro from MNCs of human subjects. These data indicate cytokine-mediated hemodynamic changes in toxic shock syndrome.

### Materials and Methods

*Rabbit model for hemodynamic changes.* Female New Zealand white rabbits (weighing ~5 kg)

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were anesthetized with a single injection of 4 mg of xylazine/kg and 10 mg of ketamine/kg. This combination of nonbarbituates has been used in previous studies in models of septic shock where experimentally induced changes in hemodynamic parameters were shown to be unaffected by these anesthetics [16]. Catheters were placed in the left carotid artery (PE 50), the pulmonary artery (no. 3 French Swan-Ganz), and the superior vena cava (PE 50) for continuous recording of arterial pressure and CVP and measurement of cardiac output every 10 min.

After insertion of the catheters, hemodynamic changes were monitored for 60 min to determine baseline levels. During the experiments, blood samples removed from the carotid catheter were replaced by the same volume of saline. The total fluid (saline) administered during the study was 6 mL/kg per h. Cardiac output was measured by the thermodilution method. Systemic vascular resistance was calculated as follows: (mean arterial pressure - CVP)  $\times$  80/cardiac output (in dyne  $\times$  s/cm<sup>5</sup>).

TSST-1 was injected into the superior vena cava as a bolus over a 30-s period. Blood was withdrawn from the carotid artery catheter for white blood cell counts.

At 3-4 h after the injection of TSST-1 at 2 or 5  $\mu$ g/kg, the heart, submandibular gland, sublingual gland, esophagus, stomach, liver, pancreas, jejunum, ileum, colon, rectum, aorta, inferior vena cava, skin, ovary, thymus, and eyes were examined for gross changes. However, gross changes were observed only in the lungs.

Statistical analyses were done by applying one-way analysis of variance, where the post-TSST-1 values of an experimental group at a particular time were compared with the values of the same group immediately before injection. The values of various hemodynamic parameters in five rabbits given saline during the 3.5-h period were not different ( $P < .05$ ) from the values at zero-time.

**TSST-1.** TSST-1 was purchased from Toxin Technology (Madison, Wis). It was homogeneous on SDS-PAGE. TSST-1 at 100 ng/mL dissolved in endotoxin-free, ultrafiltered water contained  $<20$  pg of endotoxin/mL, as measured by the Limulus amoebocyte lysate test (Associates of Cape Cod, Woods Hole, Mass). This Limulus lysate has a sensitivity of 20 pg of *Escherichia coli* LPS/mL (Sigma Chemical Company, St. Louis).

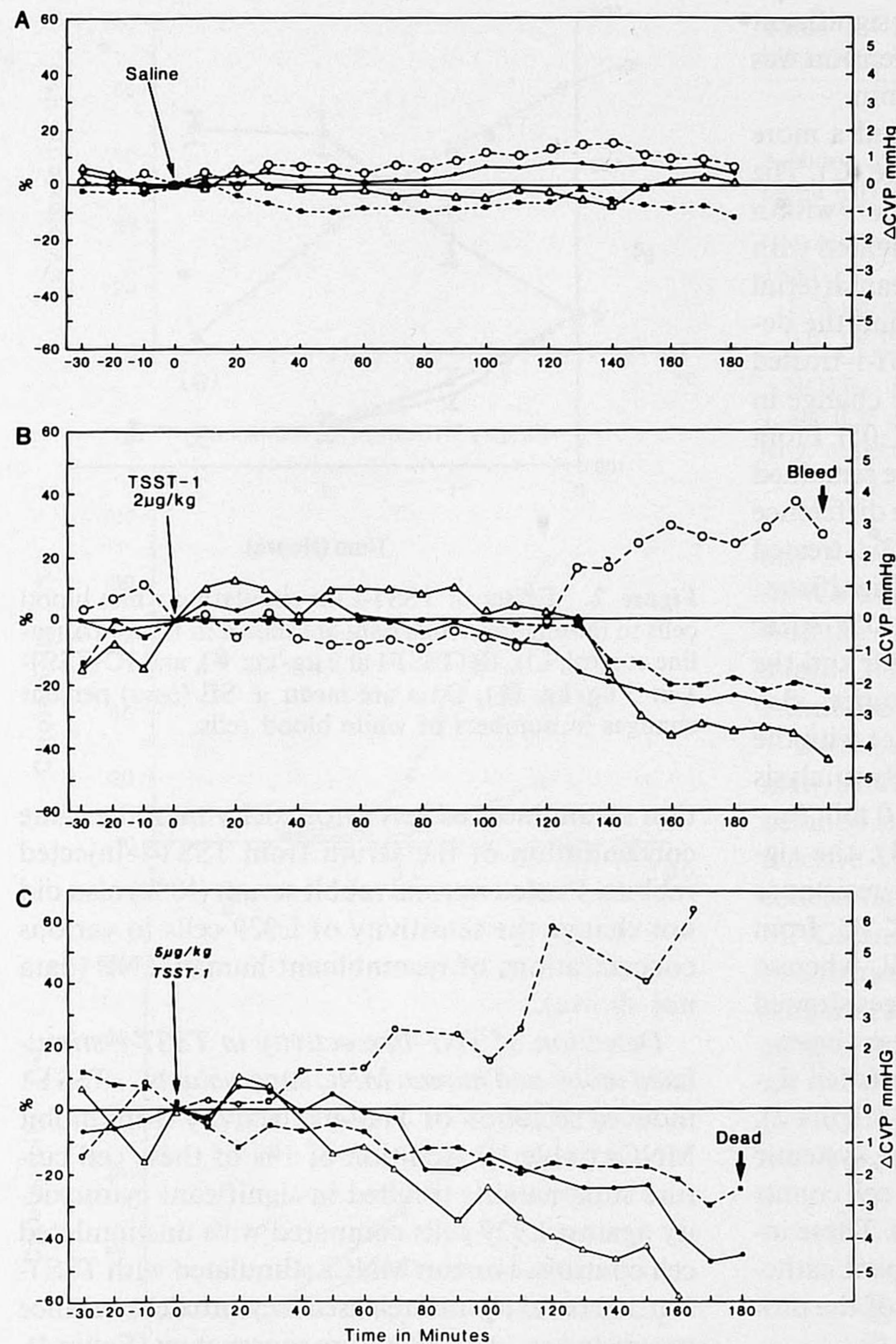
**Bioassay for TNF-like activity.** TNF-like activity was detected by TNF's cytotoxic effect against

the tumorigenic murine fibroblast L929 (ATCC CCL1; American Type Culture Collection, Rockville, Md) [17]. L929 cells were cultured in flat-bottom, 96-well microtiter plates (Becton Dickinson Co., Oxnard, Calif) at a density of  $5.5 \times 10^4$  cells per well in RPMI 1640 culture medium (M. A. Bioproducts, Walkersville, Md) containing 5% fetal calf serum (Hyclone Laboratory, Logan, Utah), 2 mM L-glutamine, 100 U of penicillin/mL, and 100  $\mu$ g of streptomycin/mL. After incubation for 18 h, culture medium was removed, and recombinant human TNF (provided by Genentech, South San Francisco, Calif) as the standard or samples and actinomycin D (2  $\mu$ g per well; Sigma) were added. The cells were cultured for an additional 20 h. The culture medium was removed, and the cells were stained with 0.1% crystal violet in 100% methanol for 20 min. OD<sub>600</sub> was measured with an MR Microelisa Auto Reader<sup>®</sup> (Dynatech, Alexandria, Va). Cytotoxicity was calculated as follows: percent cytotoxicity =  $(1 - [A \text{ of sample}/A \text{ of control}]) \times 100$ .

**Rabbit antisera to human TNF and human IL-1 and specific RIAs.** Immunization of rabbits with recombinant human TNF, recombinant human IL-1 $\alpha$ , and recombinant human IL-1 $\beta$  was accomplished in complete Freund's adjuvant. Monthly boosters were given in Freund's incomplete adjuvant. Methods for RIA for human TNF were recently established by using this antiserum and <sup>125</sup>I-labeled recombinant human TNF [18]. The RIAs for IL-1 $\alpha$  and IL-1 $\beta$  do not detect IL-1 $\beta$  or IL-1 $\alpha$ , respectively. Details concerning the RIAs for IL-1 $\alpha$  and IL-1 $\beta$  have been reported recently [19, 20]. Total (supernatant and cell-associated) TNF, IL-1 $\alpha$ , and IL-1 $\beta$  from human MNCs were quantified by their respective and specific RIA.

**Preparation of rabbit and human MNCs.** Healthy human male volunteers or New Zealand white female rabbits were bled into heparinized (10 U/mL) syringes, and the MNC fraction was prepared by centrifugation over Ficoll-Paque<sup>®</sup> (Pharmacia, Piscataway, NJ; and Winthrop Laboratories, New York). MNCs were suspended at  $5 \times 10^6$  cells/mL in endotoxin-free, ultrafiltered RPMI 1640 medium [21] containing heat-inactivated autologous human or rabbit serum (2%). One milliliter of the MNC suspension was mixed with an equal volume of TSST-1 or LPS at different dilutions in serum-free, ultrafiltered RPMI 1640 medium in 24-well culture plates and then incubated at 37 C in an atmosphere of 95% room air and 5% CO<sub>2</sub> for 24 h. Culture su-





**Figure 1.** TSST-1-induced hemodynamic changes in rabbits after (A) a control bolus injection of 3 mL of 0.9% NaCl into five rabbits, (B) a bolus injection (2  $\mu\text{g}/\text{kg}$ ) of TSST-1 into three rabbits, and (C) a bolus injection (5  $\mu\text{g}/\text{kg}$ ) of TSST-1 into three rabbits. Data are mean percent changes from the zero-time value for mean arterial pressure ( $\bullet$ ), cardiac output ( $\circ$ ), and systemic vascular resistance ( $\Delta$ ) and mean change from the zero-time value for CVP ( $\blacksquare$ ).

pernatants or lysates were prepared. Lysates were obtained by three freeze-and-thaw cycles.

**Data analysis.** Data obtained on production of the three cytokines by MNCs from several human volunteers were analyzed using the Scheffé *F* test. This test compares variance between two groups; for example, the mean IL-1 $\beta$  production induced by a given stimulant, such as TSST-1, in several volunteers compared with the production induced by LPS.

**Results**

**TSST-1 induction of hypotension.** Control rabbits injected with saline remained hemodynamically

stable for 180 min: Mean  $\pm$  SD arterial pressure for five rabbits was  $66 \pm 3.0$  mm Hg 30 min before injection,  $63.8 \pm 3.3$  mm Hg at 0 min (immediately after saline injection),  $64.6 \pm 3.6$  mm Hg at 60 min,  $69 \pm 4.2$  mm Hg at 120 min, and  $67 \pm 4.6$  mm Hg at 180 min (figure 1A). Other hemodynamic parameters (cardiac output, systemic vascular resistance, and CVP) were not significantly changed (by analysis of variance) throughout the experimental period. However, a bolus injection of TSST-1 at 2  $\mu\text{g}/\text{kg}$  induced considerable change in hemodynamic parameters (figure 1B). Mean arterial pressure began to fall within 140 min and continuously declined to  $42.3 \pm 12.7$  mm Hg at 210 min (28.4%). The decrease



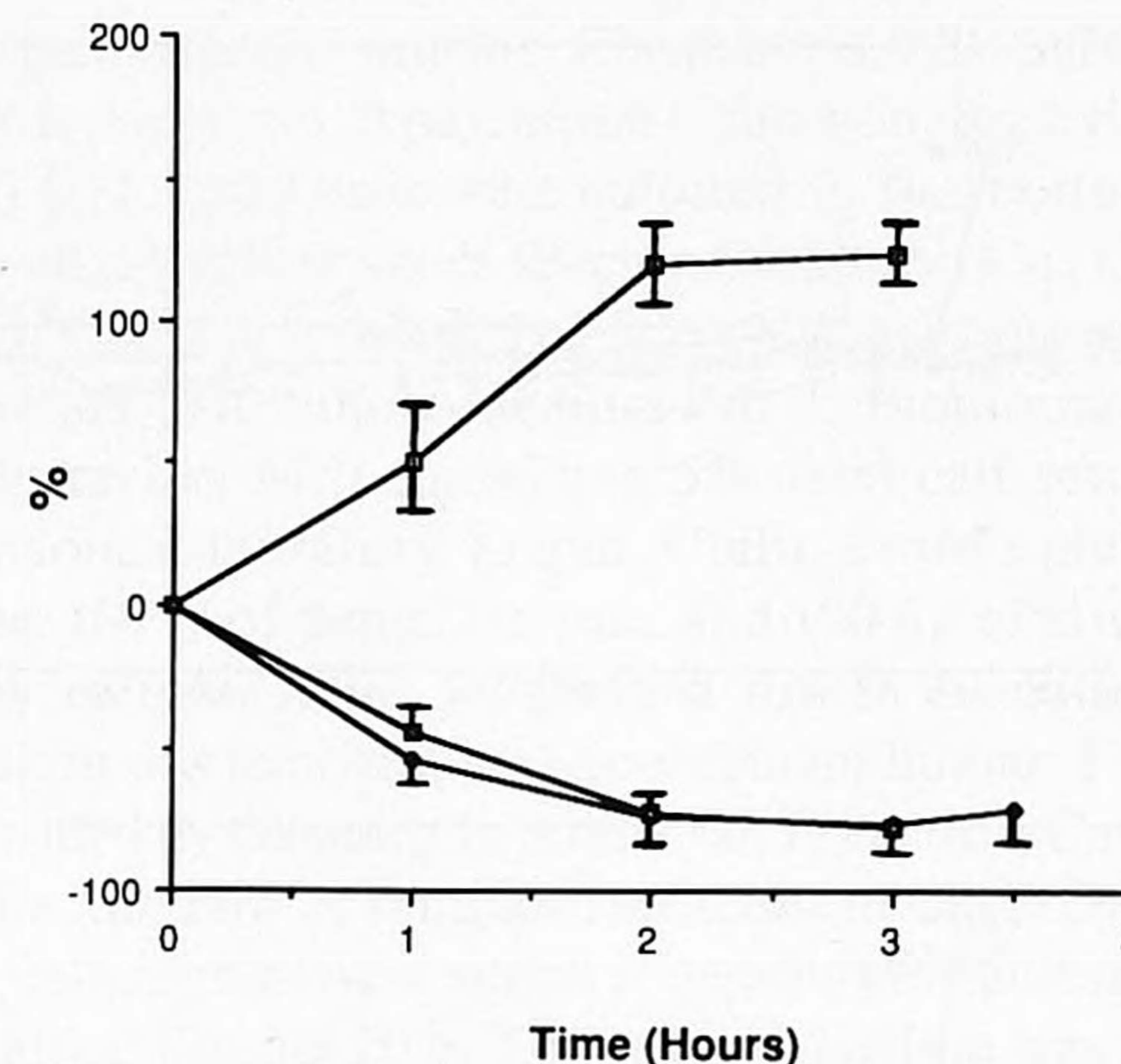
in mean arterial pressure was statistically significant at 190 min ( $P < .05$ ). However, the hypotension was not statistically significant before 190 min.

Injection of 5  $\mu\text{g}$  of TSST-1/kg induced a more pronounced hypotension in rabbits (figure 1C). The fall in mean arterial pressure began earlier (within 80 min) in these rabbits than in rabbits treated with 2  $\mu\text{g}$  of TSST-1/kg. At 140 min, the mean arterial pressure reached  $43.0 \pm 10.7$  mm Hg, and the decrease in mean arterial pressure in the TSST-1-treated group was significantly greater than the change in the control group injected with saline ( $P < .05$ ). From 140 to 180 min, the mean arterial pressure remained between 43 and 45 mm Hg. However, the difference in mean arterial pressure between the TSST-1-treated group and the control group was more significant at 180 min ( $P < .01$ ) than at 140 min ( $P < .05$ ). After 180 min, blood pressure progressively fell, and the rabbits died. Systemic vascular resistance also showed a dramatic decrease, in accordance with the marked fall in mean arterial pressure. By analysis of variance, compared with the value at 0 min (immediately after the injection of TSST-1), the significance of the fall in systemic vascular resistance was as follows: from 110 to 120 min,  $P < .05$ ; from 130 to 210 min,  $P < .01$ . CVP also fell, whereas cardiac output increased, and these changes showed similar levels of significance during these times.

During this time, white blood cell counts fell significantly in the TSST-1-injected rabbits (figure 2), similar to the fall in mean arterial pressure, systemic vascular resistance, or CVP. White blood cell counts of rabbits receiving saline rose with time. These increases may be caused by the preexperiment catheter placements or other stressful aspects of the procedure.

On gross observation, the lungs from control rabbits receiving saline appeared pink, with occasional areas of subpleural blood (1.5–2 mm in diameter). In contrast, the lungs of rabbits receiving TSST-1 at 5.0  $\mu\text{g}/\text{kg}$  showed diffuse surface hemorrhage and a liver-like appearance and consistency on inspection and palpation. In rabbits injected with 2  $\mu\text{g}$  of TSST-1/kg, the lungs appeared less affected in color, and marked hemorrhages were not observed.

**Detection of TNF-like activity in serum of rabbits injected with TSST-1.** Rabbit serum collected 3.5 h after injection of 2  $\mu\text{g}$  of TSST-1/kg was cytotoxic for L929 cells (figure 3). The cytotoxicity we observed was not due to the high concentration of rabbit serum in the assay, because control preinjec-



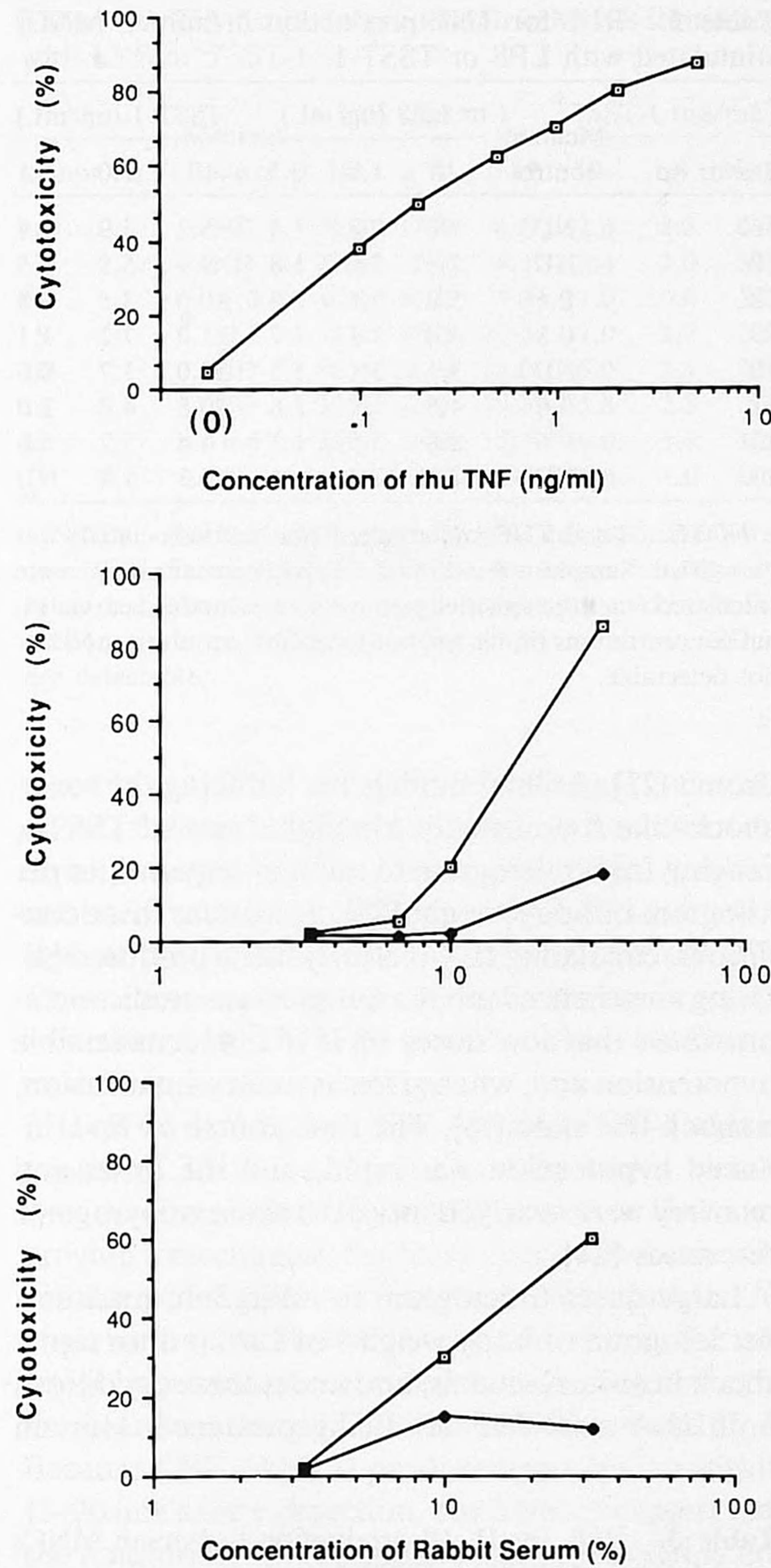
**Figure 2.** Effect of TSST-1 on circulating white blood cells in rabbits for which data are shown in figure 1A (saline control;  $\square$ ), 1B (TSST-1 at 2  $\mu\text{g}/\text{kg}$ ;  $\blacklozenge$ ), and 1C (TSST-1 at 5  $\mu\text{g}/\text{kg}$ ;  $\blacksquare$ ). Data are mean  $\pm$  SE (bars) percent changes in numbers of white blood cells.

tion serum induced less cytotoxicity than the same concentration of the serum from TSST-1-injected rabbits. Pooled normal rabbit serum (10%) also did not change the sensitivity of L929 cells to various concentrations of recombinant human TNF (data not shown).

**Detection of TNF-like activity in TSST-1-stimulated rabbit and human MNC supernatants.** TSST-1 induced secretion of TNF-like activity from rabbit MNCs (table 1). Addition of 1% of these cell culture supernatants resulted in significant cytotoxicity against L929 cells compared with unstimulated cell controls. Human MNCs stimulated with TSST-1 at 100 or 10 ng/mL released a cytotoxic substance or substances into the culture supernatant (figure 4). Addition of rabbit antiserum to human TNF markedly reduced the cytotoxicity of the MNC supernatants, although 20%–30% of the activity remained unneutralized. Carryover of TSST-1 from the first MNC culture to the second culture (L929) had no effect on the cytotoxicity against L929 cells because culture medium containing TSST-1 without MNCs did not induce cytotoxicity. LPS at a concentration of 100 or 10 ng/mL induced amounts of TNF-like activity similar to that induced by TSST-1.

**Detection of human TNF, IL-1 $\alpha$ , and IL-1 $\beta$  by RIA in TSST-1-stimulated MNCs.** TSST-1 induced TNF production in MNC cultures from five human male donors in a dose-dependent manner as measured by RIA (figure 5). Variability in the amount





**Figure 3.** Cytotoxic activity in sera from rabbits obtained 3.5 h after injection of 2  $\mu$ g of TSST-1/kg ( $\square$ ; see figure 1B). L929 cells were incubated overnight with dilutions of the serum in the presence of actinomycin D. As a positive control, recombinant human (*rhu*) TNF was used; cytotoxicity with this factor is shown (*top*). As a negative control, serum from the same rabbit, taken before injection of TSST-1, was tested ( $\blacklozenge$ ). Two different experiments are shown (*middle* and *bottom*).

of TNF (cell associated plus extracellular) produced by these donors was considerable. In donor 2, 2500 pg of TNF/mL was induced by 10 ng of TSST-1/mL, whereas only 450 pg of TNF/mL was detected in do-

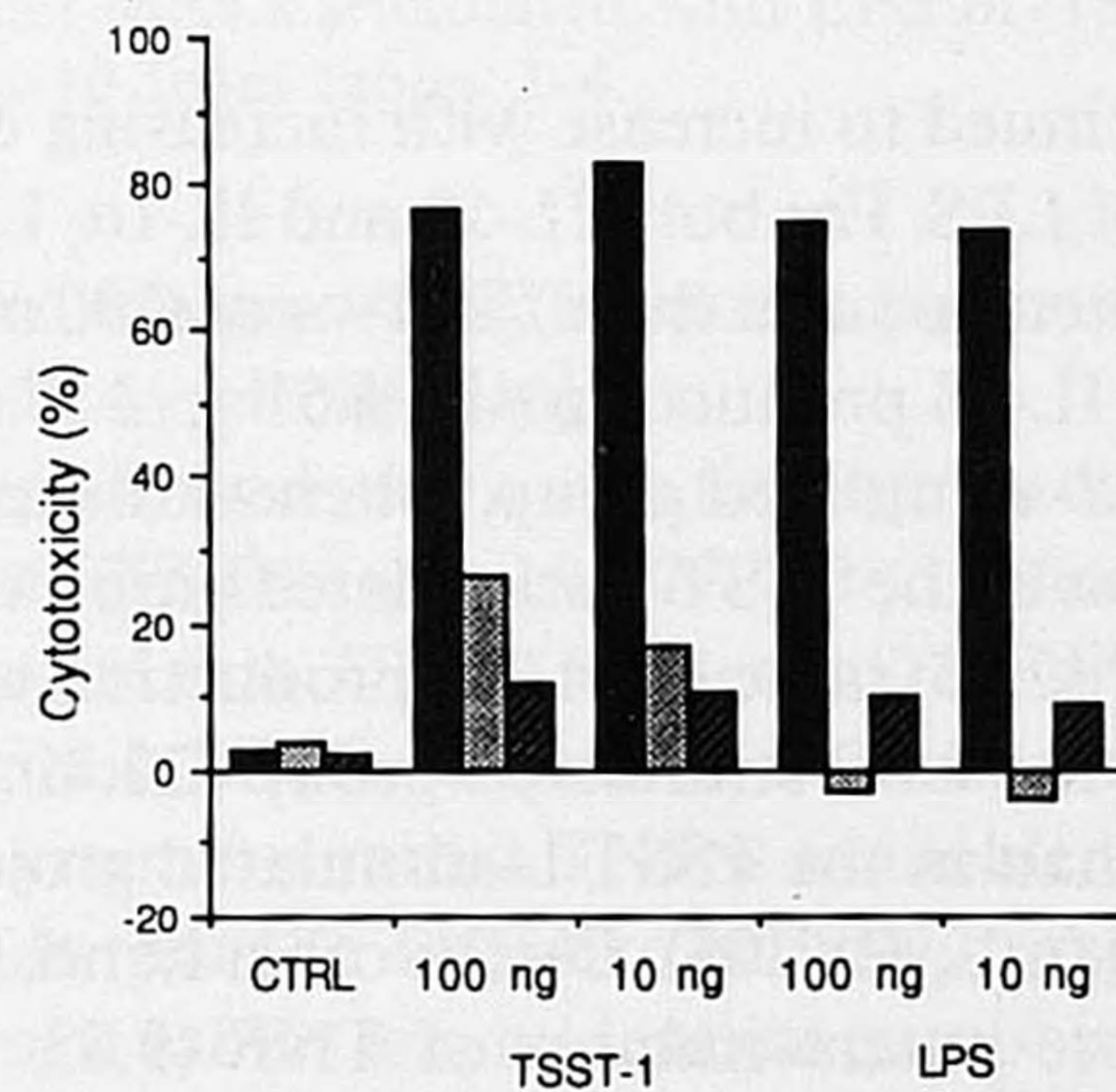
**Table 1.** Percent cytotoxicity of supernatants from rabbit MNCs stimulated with TSST-1.

Addition to rabbit MNCs	Dilution of MNC culture supernatants in L929 cell culture (%)			
	50	25	10	1
Control (no addition)	0	0	2.2	0.1
TSST-1				
1.25 $\mu$ g/mL	83.2	88.1	76.1	39.1
0.63 $\mu$ g/mL	84.2	90.6	81.1	33.6

NOTE. Recombinant human TNF at concentrations of 2, 1, and 0.25 ng/mL induced 77.2%, 41.4%, and 23.6% cytotoxicity, respectively, for L929 cells.

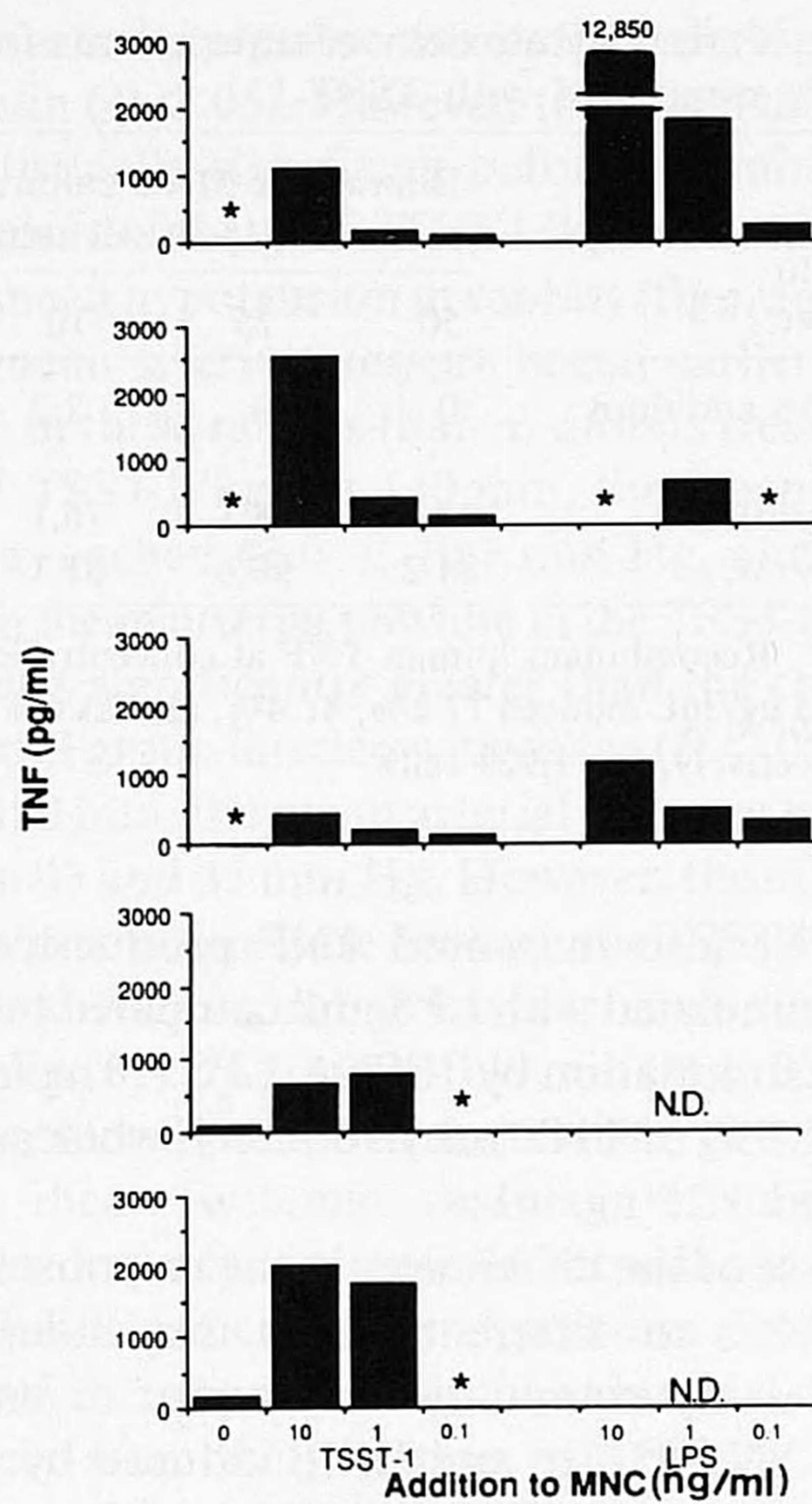
nor 3. We also measured TNF production from MNCs stimulated with LPS and compared this value with the stimulation by TSST-1. LPS (10 ng/mL) induced 12.8 ng of TNF/mL in donor 1, whereas TSST-1 induced 1.25 ng/mL.

Because of the differences in the responses of human MNCs to different toxins, we undertook a detailed study examining the amount of immunoreactive TNF, IL-1 $\alpha$ , and IL-1 $\beta$  induced by various concentrations of TSST-1 or LPS in the same donor's MNCs and as measured for each cytokine by RIA. Production of TNF (table 2) and IL-1 $\beta$  (table 3) was maximal when cells were stimulated by either 1 or 10 ng of LPS/mL. IL-1 $\alpha$  production (table 4), how-



**Figure 4.** Bioassay for TNF in culture supernatant of human MNCs treated with TSST-1. Culture supernatant (20%) was assayed for cytotoxicity to L929 cells with ( $\square$ ) or without ( $\blacksquare$ ) 1% rabbit antiserum to human TNF. Assays for TNF were also done in cultures without MNCs ( $\square$ ). Concentrations are in ng/mL. Concentrations of recombinant human TNF of 1 and 0.1 ng/mL induced 100% and 42% cytotoxicity, respectively. The results were obtained from nine wells and had  $\sim$ 10% variation. Results were similar in two other experiments. CTRL = control.





**Figure 5.** Concentrations of total (supernatant plus cell-associated) TNF in human MNCs from donors 1-5 (from top to bottom) stimulated with TSST-1 or LPS. Samples were quantified by RIA for TNF. An asterisk indicates that the level of TNF was below the detection limit (80 pg/mL). N.D. = not done.

ever, continued to increase with increasing concentrations of LPS. For both IL-1 $\beta$  and IL-1 $\alpha$ , LPS was a more potent inducer than TSST-1 was (100 ng/mL). Maximal IL-1 $\beta$  production was  $6.51 \pm 3.17$  ng/mL in the LPS-stimulated group, whereas the maximal production in the TSST-1-stimulated group was  $2.91 \pm 1.20$  ( $P < .05$ ; table 3). IL-1 $\alpha$  production was also higher in the LPS-stimulated group ( $22.41 \pm 9.09$  ng/mL) than in the TSST-1-stimulated group ( $7.15 \pm 4.35$  ng/mL;  $P < .05$ ). On the other hand, TSST-1 was a more potent inducer of TNF ( $9.83 \pm 4.83$  ng/mL) than was LPS ( $4.31 \pm 1.75$  ng/mL;  $P < .05$ ). Figure 6 shows each individual's maximal response to each toxin. TSST-1 clearly is, for most individuals studied, a more potent inducer of TNF than is LPS.

### Discussion

The rabbit has been used widely as an animal model to elucidate the pathogenesis of toxic shock syn-

**Table 2.** RIA for TNF production in human MNCs stimulated with LPS or TSST-1.

Donor no.	Medium control	LPS (ng/mL)			TSST-1 (ng/mL)		
		10	1.0	0.1	10	1.0	0.1
6	ND	7.6	7.8	1.6	5.2	4.9	1.8
7	ND	2.8	3.0	1.8	9.9	5.9	0.5
8	0.4	5.2	5.3	1.8	10.0	3.5	0.8
9	0.8	4.4	3.1	1.7	11.0	7.2	1.1
10	ND	3.7	3.1	1.5	20.0	1.7	0.6
11	0.6	4.5	5.0	2.6	10.8	4.0	1.0
12	0.6	2.8	3.0	1.7	4.4	3.7	0.6
13	ND	2.0	2.3	1.1	7.3	1.4	ND

NOTE. Total TNF (supernatant plus cell associated) was quantified. Samples were diluted 1:3, and concentrations were calculated from the sensitive portion of the standard curve. Final concentrations (in ng/mL) of total TNF are shown. ND = not detectable.

drome [22]. Animal models for inducing the toxic-shock-like state usually use high doses of TSST-1, ranging from microgram to milligram quantities per kilogram of body weight [23], and under these conditions, circulating IL-1 is clearly being produced [3]. Using anesthetized rabbits our previous studies demonstrated that low doses of IL-1 induce reversible hypotension and, when given as a constant infusion, a shock-like state [16]. The time course of IL-1-induced hypotension was rapid, and the onset and recovery were nearly identical to those of pyrogenic responses [24].

Large doses (microgram to milligram quantities per kilogram of body weight) of LPS induce septic shock in animal models, and under these conditions both IL-1 and TNF are being produced. Human

**Table 3.** RIA for IL-1 $\beta$  production in human MNCs stimulated with LPS or TSST-1.

Donor no.	Medium control	LPS (ng/mL)			TSST-1 (ng/mL)		
		10	1.0	0.1	10	1.0	0.1
6	ND	2.5	10.1	2.6	2.3	2.2	0.6
7	0.5	4.4	2.8	1.6	4.1	1.3	0.5
8	0.7	11.2	9.0	2.2	5.0	2.4	ND
9	ND	6.9	6.9	2.2	3.0	1.6	0.6
10	0.5	6.4	7.6	2.6	3.4	1.4	0.7
11	0.6	4.4	6.4	3.6	2.1	1.1	0.8
12	0.6	3.6	3.1	1.7	2.0	1.1	0.6
13	ND	1.9	1.9	1.1	1.4	0.6	0.5

NOTE. Total IL-1 $\beta$  (supernatant plus cell associated) was quantified. Samples were diluted 1:3, and concentrations were calculated from the sensitive portion of the standard curve. Final concentrations (in ng/mL) of total IL-1 $\beta$  are shown. ND = not detectable.



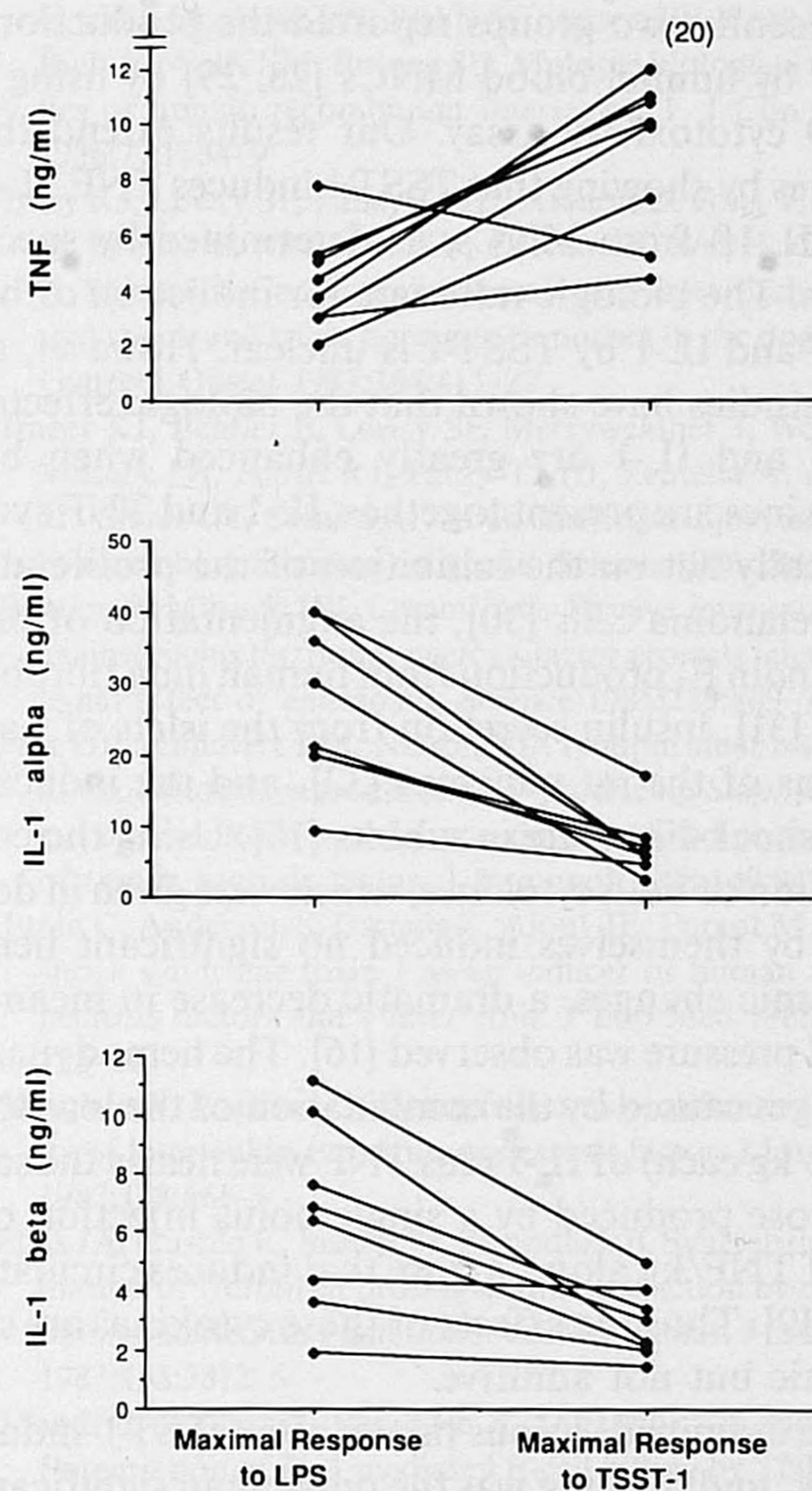
**Table 4.** RIA for IL-1 $\alpha$  production in MNCs stimulated with LPS or TSST-1.

Donor no.	Medium control	LPS (ng/mL)			TSST-1 (ng/mL)		
		10	1.0	0.1	10	1.0	0.1
6	ND	30.0	17.4	6.5	2.4	2.2	0.7
7	ND	20.0	8.4	4.3	8.4	2.0	ND
8	1.0	40.0	13.0	7.0	17.0	10.0	3.8
9	0.5	8.8	20.0	20.0	7.0	2.7	0.5
10	ND	40.0	30.0	20.0	6.0	3.4	0.8
11	ND	36.0	22.0	16.0	5.8	2.8	0.8
12	0.6	21.0	14.0	5.3	6.0	3.0	1.6
13	ND	9.2	7.6	4.4	4.6	1.0	0.6

NOTE. Total IL- $\alpha$  (supernatant plus cell associated) was quantified. Samples were diluted 1:3, and concentrations were calculated from the sensitive portion of the standard curve. Final concentrations (in ng/mL) of total TNF are shown. ND = not detectable.

recombinant TNF at a dose of  $\geq 10$   $\mu\text{g}/\text{kg}$  also induces profound hypotension (30% fall in mean arterial pressure) in dogs [25] and rats [26]. A rabbit antibody to murine TNF protected BALB/c mice against the lethal effects of *E. coli* endotoxin [27]. Thus there is a growing concept that septic shock is mediated by TNF and that IL-1 also contributes to the septic-shock syndrome.

Here we demonstrate that purified TSST-1 causes release of TNF-like activity into the rabbit bloodstream. Hence circulating TNF as well as IL-1 may provide a mechanism for toxic shock syndrome. Of considerable importance to the present study is the observation that in TSST-1-induced shock, the onset of hypotension coincided with the time for synthesis and release of TNF-like substances and IL-1. Because TNF and IL-1 produce hypotension within 15–20 min after iv injection, our findings suggest that the amount of TSST-1 we used in our model is not by itself exerting a direct effect in blood pressure, but rather that the induction of mediators, such as IL-1 and TNF, is first required. Although the half-life of iv-injected recombinant human TNF in the rabbit is 10 min (authors' unpublished data) in the rabbit model we used, TSST-1 probably induces TNF and TNF-like activity for several hours. We previously demonstrated [3] that iv injection of filtrate from bacteria producing TSST-1 caused a sustained fever in rabbits. Plasma from TSST-1-injected rabbits that were bled during the peak of the fever produced, in recipient rabbits, a brief monophasic fever typical of endogenous pyrogen [3]. The combination of IL-1 and TNF has been shown to induce a shock-like state, as indicated by changes in several



**Figure 6.** Comparison of maximal production of the cytokines TNF (*top*), IL-1 $\alpha$  (*middle*), and IL-1 $\beta$  (*bottom*) in human MNCs stimulated with LPS or TSST-1. Data are derived from tables 2–4.

hemodynamic parameters and a pulmonary-capillary-leak syndrome [16].

We confirmed our *in vivo* findings by demonstrating that TSST-1 induces TNF production *in vitro*. We showed that TSST-1 induces a TNF-like activity from rabbit MNCs. However, because no immunoassay system for rabbit TNF is available, we cannot conclude that the TNF-like activity in rabbit MNC cultures or rabbit sera is identical to rabbit TNF. Our rabbit antiserum to human TNF does not neutralize rabbit TNF. Supernatants from human MNCs incubated with TSST-1 were partially neutralized by 1% antiserum to human TNF in cytotoxicity assays. Concentrations of these supernatants ranging from 10% to 25% showed a similar percentage of unneutralized cytotoxicity (20%–30%). This observation raises the possibility that TSST-1 induces both TNF and a substance that possesses TNF-like activity but that is antigenically distinct.



Recently two groups reported the production of TNF by human blood MNCs [28, 29] by using the L929 cytotoxicity assay. Our results extend these studies by showing that TSST-1 induces TNF, IL-1 $\alpha$ , and IL-1 $\beta$  from MNCs, as determined by specific RIAs. The biologic rationale for induction of both TNF and IL-1 by TSST-1 is unclear. However, several studies have shown that the biologic effects of TNF and IL-1 are greatly enhanced when both cytokines are present together. IL-1 and TNF synergistically act on the inhibition of the proliferation of melanoma cells [30], the augmentation of prostaglandin E<sub>2</sub> production from human lung fibroblast lines [31], insulin secretion from the islets of Langerhans of the rat pancreas [32], and the induction of a shock-like state in rabbits [16]. Using the combination of both cytokines, which were given in doses that by themselves induced no significant hemodynamic changes, a dramatic decrease in mean arterial pressure was observed [16]. The hemodynamic changes caused by the combination of the low doses (1  $\mu$ g/kg each) of IL-1 plus TNF were nearly the same as those produced by a single bolus injection of 5  $\mu$ g of TNF/kg alone, a dose that induces circulating IL-1 [9]. Thus the effects of these cytokines are synergistic but not additive.

We examined various tissues after TSST-1-induced shock, and the lung was the only organ significantly affected. Hemorrhage and hepatization were observed. In various studies the combination of TNF and IL-1 also induced hemorrhage and hepatization and, in addition, massive accumulation of proteinaceous fluid and cells in the alveolar spaces [16]. These observations suggest that TSST-1-induced IL-1 and TNF may act together in disrupting the pulmonary vascular endothelium. After TSST-1 injection we also observed a fall in the peripheral white blood cell count, an occurrence that has been reported in humans receiving TNF [33] as well as in rabbits receiving TNF/IL-1 [16].

Studies on production of immunoreactive IL-1 $\alpha$ , IL-1 $\beta$ , and TNF in human MNCs induced by either LPS or TSST-1 may shed some light on toxic shock syndrome. Because the three major cytokines were quantified by RIA, doubts cannot be raised about interfering substances in bioassays for these cytokines. Our results indicate that TSST-1 is a more potent inducer of TNF than is LPS in MNCs from eight of 11 donors tested for this comparison. On the other hand, IL-1 (both  $\alpha$  and  $\beta$ ) was induced to a greater degree by LPS than by TSST-1 in these same MNCs.

These results suggest that the severity of the clinical response to TSST-1 may depend in part on the amount of TNF the individual produces when challenged with TSST-1. Further studies will be necessary to correlate, if possible, the susceptibility to toxic shock syndrome with the amount of each cytokine (IL-1 and TNF) produced in response to TSST-1.

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