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Pharmacology

Induction of nitric oxide synthase activity by toxic shock syndrome toxin 1 in a macrophage–monocyte cell line

(arginine/glucocorticoids/bacterial superantigens)

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ABSTRACT **Toxic shock syndrome toxin 1 (TSST-1) is a M_r 22,000 protein produced by *Staphylococcus aureus*. It is thought to be the cause of toxic shock syndrome. We investigated the hypothesis that TSST-1 induces nitric oxide (NO) synthase and that the NO formed may be involved in the pathogenesis of toxic shock syndrome. We used the murine monocyte–macrophage cell line J744.2 that responds to TSST-1 and also expresses NO synthase activity upon immunological stimulation. J774.2 macrophages stimulated with TSST-1 (10–100 nM) generated nitrite, a breakdown product of NO, and induced concentration-dependent elevations of cGMP in the pig kidney epithelial cell line (LLC-PK$_1$). This latter effect was due to the generation of L-arginine-derived NO for it was (*i*) abolished by oxyhemoglobin (10 μM), a scavenger of NO, or by methylene blue (10 μM), an inhibitor of NO-activated guanylate cyclase; (*ii*) potentiated by superoxide dismutase (100 units/ml), which prolongs the life of NO; (*iii*) inhibited by N^G-monomethyl-L-arginine (0.3 mM), an inhibitor of NO synthase; (*iv*) significantly decreased when L-arginine (0.4 mM) in the medium was replaced by D-arginine (0.4 mM). Moreover, TSST-1 (100 nM) enhanced the activity of cytosolic NO synthase in J774.2 cells. Hydrocortisone (1 μM) but not indomethacin (5 μg/ml) or salicylic acid (5 μg/ml) prevented the generation of NO_2^- and the increases in cGMP levels in LLC-PK$_1$ cells induced by J774.2 cells stimulated with TSST-1. The effects of hydrocortisone were partially reversed by coincubation with RU 486 (1 μM), an antagonist of glucocorticoid receptors. Thus, TSST-1 and perhaps other exotoxins produced by Gram-positive bacteria induce NO synthase and the increased NO formation may contribute to toxic shock syndrome and possibly to changes in the immune responses that accompany infection.**

Toxic shock syndrome is a multisystem disease characterized by hypotension, high fever, erythroderma, desquamation of skin, and dysfunction of three or more organ systems (1). Its occurrence has been closely associated with infection with strains of *Staphylococcus aureus* producing a M_r 22,000 protein (2, 3). This protein, later termed toxic shock syndrome toxin 1 (TSST-1), causes the characteristic symptoms in animal models of this disease (4, 5). TSST-1 is structurally and functionally related to pyrogenic exotoxins, a family of low molecular weight proteins (6) that also includes staphylococcal enterotoxins, a common cause of food poisoning, as well as exotoxins produced by *Streptococcus pyogenes*. Pyrogenic exotoxins have profound effects on the immune system. Because they are potent activators of a subset of T lymphocytes bearing the Vβ receptor in a manner that requires the presence of major histocompatibility complex class II molecules (7), they have been designated microbial superantigens. Moreover, staphylococcal enterotoxins A and C (6) and streptococcal exotoxin A (8) have also been implicated as causes of toxic shock-like states.

Nitric oxide (NO) is generated by mammalian cells from one of the N^G-guanidino nitrogens of L-arginine and this reaction is catalyzed by a NADPH-dependent dioxygenase (9), referred to as NO synthase, which can exist in at least two distinct isoforms. One, a constitutive agonist-triggered and calcium-dependent NO synthase, is mainly present in neuronal cells (10) and vascular endothelial cells (11). In the central and peripheral nervous systems, NO functions as a neurotransmitter (12), whereas, in the cardiovascular system, NO is a potent vasodilator and accounts for the biological activity of agonist-triggered endothelium-derived relaxing factor (13). The other enzyme, a calcium-independent inducible NO synthase, is found in macrophages (14), hepatocytes (15), endothelial cells (16), and smooth muscle cells (17) after activation by bacterial lipopolysaccharide (LPS) or cytokines. NO made by the inducible NO synthase is responsible for killing microbial pathogens and tumor cells by activated macrophages (18–20). NO from induced NO synthase is also involved in the pathogenesis of LPS- or cytokine-induced shock for the inhibitors of NO synthase reverse hypotension (21) and reduced vascular responsiveness to contractile agents induced by treatment with LPS both *in vivo* and *in vitro* (22, 23).

The hemodynamic and pathological changes observed in patients with toxic shock syndrome or in animal models of the disease are characteristic of hyperdynamic septic shock (24, 25) and resemble changes seen in patients with Gram-negative sepsis or in animal models of LPS-induced shock. TSST-1 and LPS stimulate production of a similar set of cytokines from monocytes. Moreover, TSST-1 potentiates the lethal effects of LPS in animal models (26). Similarities between the biological actions of TSST-1 and LPS prompted us to investigate whether TSST-1 induces NO synthase so that increased generation of NO contributes to the symptoms of toxic shock syndrome.

We used the murine monocyte–macrophage cell line J774.2, which is responsive to TSST-1 (27) and can express NO synthase activity upon immunological stimulation (28). Here we demonstrate that, indeed, TSST-1 induces NO synthase activity in J774.2 macrophages, which suggests the involvement of NO in the pathogenesis of toxic shock syndrome.

MATERIALS AND METHODS

Materials. TSST-1 (batch TT606) and rabbit TSST-1 antiserum (batch TT606; T90-2) were purchased from Toxin Technology (Madison, WI). N^G-Monomethyl-L-arginine ac-

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Abbreviations: TSST-1, toxic shock syndrome toxin 1; LPS, lipopolysaccharide; IL, interleukin.

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etate salt (L-MeArg) was obtained from Calbiochem. RU 486 {(11β,17β)-11-[4-(dimethylamino)phenyl]-17-hydroxy-17-(1-propynyl)estra-4,9-dien-3-one} was a generous gift from Roussel-Uclaf. Indomethacin, salicylic acid (sodium salt), *E. coli* LPS (serotype 0127B8), and all the other reagents were from Sigma.

Cell Culture. J774.2 cells (ECACC 85011428), certified to be mycoplasma-free at the time of purchase, and LLC-PK_1 pig kidney epithelial cells (ATCC CL 101) were obtained from the European Collection of Animal Cell Culture (Salisbury, U.K.). J774.2 macrophages were cultured in six-well plates as a semisuspension in phenol red-free Dulbecco's modified Eagle's medium (DMEM; Flow Laboratories), which contained L-arginine (0.4 mM), L-glutamine (4 mM), and 10% fetal calf serum. Cells were allowed to reach a density of 1–3 $\times 10^6$ cells per ml, and experiments were started by addition of TSST-1. LLC-PK_1 cells were grown in 12-well plates in medium 199 (Flow Laboratories) supplemented with 2 mM L-glutamine and 10% fetal calf serum and were used on the second day after reaching confluence (5×10^5 cells per well).

LLC-PK_1 Bioassay of NO Biosynthesis by J774.2 Macrophages. Preliminary experiments showed that the effects of TSST-1 were not observed before 6 h of incubation of the toxin with J774.2 macrophages and a 24-h incubation time was chosen for further experiments. After incubation in the absence or presence of different concentrations of TSST-1 and/or drugs, adhering cells were detached by using a cell lifter (Costar), suspended by pipetting, transferred to plastic tubes, and centrifuged (200 × *g*; 10 min). The supernatants were stored (−20°C) for future measurements of nitrite. The cell pellets were suspended in 0.6 ml of fetal calf serum-free DMEM and viable cells were counted. The viability of the cells was always >85%, as assessed by trypan blue exclusion. Aliquots (0.5 ml) of suspensions of J774.2 macrophages were added to the monolayers of LLC-PK_1 cells (detector cells) preincubated (30 min; 37°C) with 0.5 ml of DMEM containing 1 mM isobutylmethylxanthine, an inhibitor of phosphodiesterase. After incubation for 10 min in the presence of superoxide dismutase (100 units/ml) supernatants were aspirated, 1 ml of 0.05 M sodium acetate (pH 4.0) was added to each well, and plates were rapidly frozen in liquid nitrogen to stop the reactions and break the cells. After thawing, the contents of wells were transferred to 1.5-ml Eppendorf tubes and centrifuged (10,000 × *g*; 15 min). Protein concentrations were determined in precipitates using a Bio-Rad protein assay after 1 h of incubation (80°C) with 0.5 M NaOH. cGMP levels were measured in supernatants by radioimmunoassay with a specific antiserum provided by H. Strobach (Institute of Pharmacology, University of Düsseldorf, F.R.G.) and ^{125}I-labeled cGMP obtained from Amersham. Results were expressed as pmol of cGMP per μg of protein per 10^6 J774.2 cells. Some experiments were carried out in the absence of superoxide dismutase or in the presence of oxyhemoglobin (10 μM) or methylene blue (10 μM). Oxyhemoglobin was added to the suspension of J774.2 macrophages immediately before coincubation with the detector cells and methylene blue was preincubated with the detector cells for 10 min.

L-Arginine Dependence of NO Generation by TSST-1-Stimulated J774.2 Macrophages. To establish L-arginine dependence of NO production, J774.2 macrophages were incubated with TSST-1 (24 h) and harvested as described above. Then, cells were washed three times with L-arginine-free DMEM, resuspended in DMEM containing L-arginine (0.8 mM) with or without L-MeArg (0.6 mM) or D-arginine (0.8 mM), and incubated (37°C) for 20 min before addition to the detector cells, which were preincubated in L-arginine-free

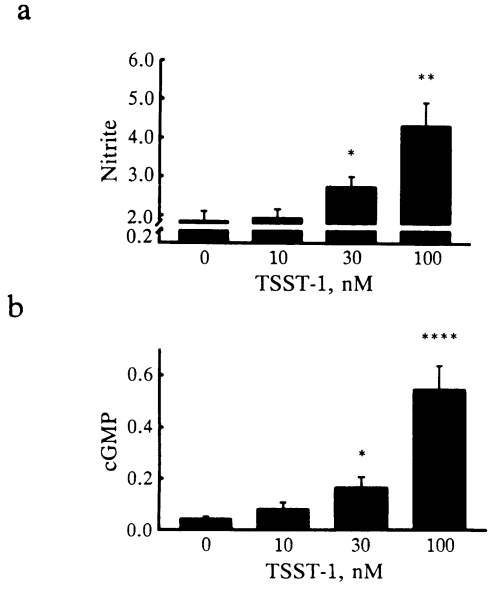

FIG. 1. TSST-1-stimulated J774.2 macrophages generate NO_2^- and increase cGMP levels in LLC-PK_1 cells. (*a*) Concentrations of NO_2^- (nmol per 10^6 cells) in supernatants from J774.2 macrophages incubated (24 h) with increasing concentrations of TSST-1 [$P < 0.001$ by one-way analysis of variance (ANOVA)]. (*b*) Simultaneously, TSST-1-stimulated J774.2 macrophages increased LLC-PK_1 cell cGMP (pmol per μg per 10^6 cells) levels ($P < 0.001$ by one-way ANOVA). Values represent means ± SEM of n = 12–21 measurements from at least six experiments. *, $P < 0.05$; **, $P < 0.01$; ****, $P < 0.0001$ when compared to control.

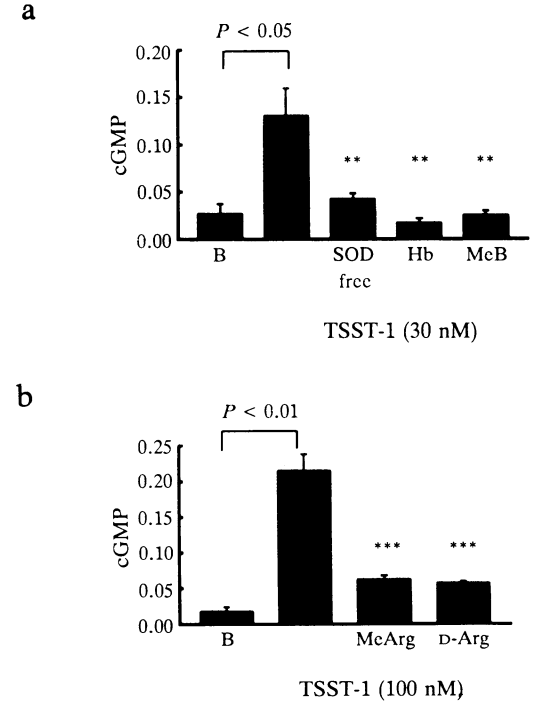

FIG. 2. TSST-1 induces biosynthesis of NO by J774.2 macrophages, which is dependent on L-arginine. (*a*) Inhibition of increases in LLC-PK_1 cell cGMP (pmol per μg per 10^6 cells) levels induced by J774.2 macrophages stimulated for 24 h with TSST-1 (30 nM) by oxyhemoglobin (Hb; 10 μM), methylene blue (MeB; 10 μM), or when incubations were carried out in the absence of superoxide dismutase (SOD free). (*b*) Moreover, increases of cGMP in LLC-PK_1 cells induced by J774.2 macrophages stimulated with TSST-1 (100 nM) were inhibited by MeArg (0.3 mM) or when L-arginine (0.4 mM) in the medium was replaced with D-arginine (0.4 mM). Values represent means ± SEM of n = 8 (*a*) or n = 4 (*b*) measurements from four experiments. **, $P < 0.01$; ***, $P < 0.001$ when compared to TSST-1 alone. B, basal.

medium. During the assay, the final concentrations were 0.4 mM for L-arginine, 0.4 mM for D-arginine, and 0.3 mM for L-MeArg. All the other conditions and measurements were conducted as described above.

Preparation of the Cytosolic Fraction from J774.2 Macrophages. J774.2 macrophages (1–2 × 10^6 cells per ml), cultured in stirrer bottles (125 or 250 ml) were incubated in the presence or absence of TSST-1 (30 or 100 nM) for 24 h, harvested by centrifugation, washed twice with Ca^{2+}-free, Mg^{2+}-free Dulbecco's phosphate-buffered saline (PBS), and resuspended in 4 ml of Hepes buffer (0.1 M; pH 7.4) containing dithiothreitol (0.1 M), phenylmethylsulfonyl fluoride (25 μg/ml), aprotinin (5 μg/ml), chymostatin (1 μg/ml), pepstatin A (5 μg/ml), soybean trypsin inhibitor (10 μg/ml), and leupeptin (10 μg/ml). Then, the cells were lysed by five cycles of freezing in liquid nitrogen and thawing and the cell homogenate was centrifuged (100,000 × g; 1 h). The supernatant (cytosol) was used for measurement of NO synthase activity.

Determination of NO Synthase Activity. Aliquots of the cytosolic protein (100–300 μg) were diluted in Ca^{2+}-free and Mg^{2+}-free PBS (pH 7.4), containing NADPH (2 mM), dithiothreitol (0.1 mM), superoxide dismutase (100 units/ml), and L-arginine (10 or 100 μM) and incubated for 10 min with monolayers of the detector cells preincubated (30 min) in PBS containing isobutylmethylxanthine (1 mM). Incubations were terminated and cGMP levels were measured as described above. The results were expressed as pmol of cGMP per μg of protein per μg of enzyme protein.

Determination of NO_2^-. NO_2^- concentrations in the supernatants from TSST-1-stimulated cells were measured by the Griess reaction (29). Briefly, supernatants (600 μl) were mixed with 300 μl of 1% sulfanilamide/5% H_3PO_4 and 300 μl of 0.1% naphthylethylenediamine dihydrochloride and incubated for 10 min to form a chromophore. Absorbance was read at 547 nm, and NO_2^- dissolved in the culture medium was used as a standard.

Statistical Analysis. All values in the figures and text are expressed as means ± SEM of n observations. A one-way analysis of variance followed by a Bonferroni test or a two-tailed Student's t test was used, where appropriate, to assess the statistical significance of results. A P value of <0.05 was considered statistically significant.

RESULTS

TSST-1-Stimulated J774.2 Macrophages Produce NO_2^- and Increase cGMP Levels in LLC-PK_1 Cells. TSST-1 (10–100 nM) increased in a concentration-dependent manner the generation of NO_2^- by J774.2 macrophages (Fig. 1*a*). Nonstimulated J774.2 macrophages (1.7 ± 0.4 × 10^6 cells per ml) produced a 2.3 ± 0.2-fold (n = 5; P < 0.05) increase in the levels of cGMP in LLC-PKC_1 cells (detector cells). The increases in cGMP in the detector cells were further augmented when J774.2 macrophages were stimulated with increasing concentrations of TSST-1 (10–100 nM) (Fig. 1*b*). TSST-1 did not affect the proliferation or viability of J774.2 macrophages (data not shown) and the effects of TSST-1 were not due to contamination by endotoxin, which was <0.3 × 10^{-3} international unit per μg of TSST-1 as assessed by the limulus test (E-Toxate; Sigma). Furthermore, preincubation (37°C; 30 min) of TSST-1 with a specific TSST-1 antiserum (1:30 dilution) significantly inhibited the increases in cGMP levels in the detector cells (85.5% ± 1.6% inhibition; n = 6; P < 0.001) and completely abolished the accumulation of NO_2^- in the medium (100% inhibition; n = 5; P < 0.001) of J774.2 macrophages stimulated with TSST-1 (100 nM).

TSST-1 Induces Biosynthesis of NO by J774.2 Macrophages in an L-Arginine-Dependent Manner. Increases in cGMP levels in the detector cells induced by J774.2 macrophages incubated with TSST-1 (30 nM; 24 h) were inhibited by oxyhemoglobin (10 μM) or methylene blue (10 μM). Moreover, the increases were also abrogated when superoxide dismutase (100 units/ml), normally present during incubations, was omitted from the incubation medium (Fig. 2*a*). Thus, TSST-1-stimulated J774.2 macrophages generate NO or a closely related substance. Furthermore, the increases in cGMP levels in the detector cells induced by J774.2 macrophages incubated with TSST-1 (100 nM) depended on the presence of L-arginine. They were prevented when L-arginine (0.4 mM) in the medium of J774.2 macrophages was replaced by D-arginine (0.4 mM) or in the presence of MeArg (0.3 mM), an inhibitor of L-arginine-dependent NO synthase (Fig. 2*b*). These findings all strongly suggest that TSST-1 induces NO synthase in J774.2 macrophages.

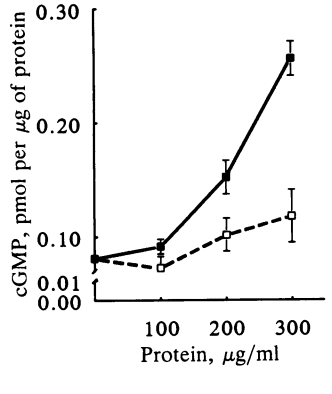

FIG. 3. TSST-1 induces cytosolic NO synthase activity in J774.2 macrophages. NO synthase activity was assayed as described. Cytosol preparations (100–300 μg of protein) from TSST-1 (100 nM)-stimulated (■; P < 0.0001 by one-way analysis of variance) but not control J774.2 macrophages (□) increased cGMP levels in LLC-PK_1 cells. Values represent means ± SEM of triplicate determinations. Similar results were obtained in two other experiments.

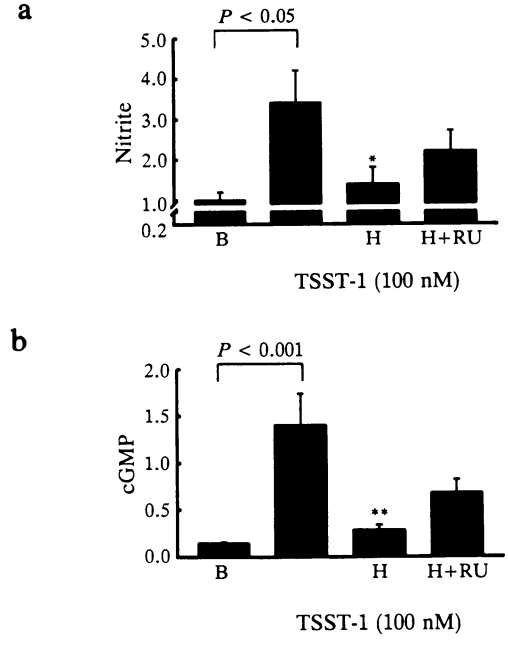

FIG. 4. Glucocorticoids inhibit induction of NO synthase activity by TSST-1. Inhibition by hydrocortisone (H; 1 μM) of NO_2^- (nmol per 10^6 cells) generation (*a*) and increases in LLC-PK_1 cell cGMP (pmol per μg per 10^6 cells) levels (*b*) induced by J774.2 macrophages stimulated (24 h) with TSST-1 (100 nM). Effect of hydrocortisone was partially reversed by RU 486 (RU; 1 μM), a glucocorticoid receptor antagonist. Values represent means ± SEM of n = 15–18 measurements. *, P < 0.05; **, P < 0.01 when compared to TSST-1 alone. B, basal.

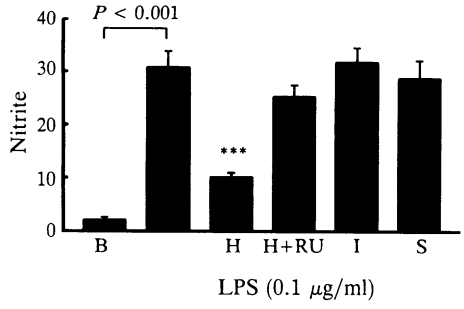

FIG. 5. Effect of hydrocortisone, indomethacin, and salicylic acid on generation of NO_2^- by J774.2 macrophages stimulated with LPS. Inhibition by hydrocortisone (H; 1 μM), but not indomethacin (I; 5 μg/ml) or salicylic acid (S; 5 μg/ml), of the generation of NO_2^- (nmol per 10^6 cells) by J774.2 macrophages stimulated with LPS (0.1 μg/ml). Effect of hydrocortisone was reversed by RU 486 (RU; 1 μM), a glucocorticoid receptor antagonist. Values represent means ± SEM of 11 or 12 measurements. $P < 0.001$ when compared to LPS alone. B, basal.

TSST-1 Induces Cytosolic NO Synthase Activity. To further substantiate this conclusion, cytosolic fractions from TSST-1-stimulated and control J744.2 macrophages were prepared. Increasing amounts of cytosolic protein (100–300 μg of protein) were incubated in the presence of L-arginine (10 or 100 μM) and the formation of NO was determined by monitoring the NO-related increases in intracellular cGMP in the detector cells for 10 min. Only the cytosols from TSST-1-stimulated J774.2 macrophages (Fig. 3) caused a protein-dependent increase in the level of cGMP.

Effects of Hydrocortisone, Indomethacin, and Salicylic Acid. Hydrocortisone (1 μM) significantly inhibited the generation of NO_2^- and increases in cGMP levels in the detector cells induced by J774.2 macrophages stimulated for 24 h with TSST-1 (100 nM) (Fig. 4). The inhibitory effect of hydrocortisone was partially reversed in the presence of RU 486 (1 μM), an antagonist of glucocorticoid receptors. In the same experiments, indomethacin (5 μg/ml) or salicylic acid (5 μg/ml) did not prevent the effects of TSST-1 ($n = 15$; $P > 0.05$; data not shown). Hydrocortisone, indomethacin, salicylic acid, or RU 486 did not affect the proliferation or viability of J774.2 macrophages (data not shown).

Effects of Hydrocortisone, Indomethacin, and Salicylic Acid on Generation of NO_2^- by J774.2 Macrophages Stimulated with Bacterial LPS. LPS (0.1 μg/ml) incubated for 24 h with J774.2 macrophages stimulated the generation of NO_2^- by these cells (Fig. 5). This effect was significantly inhibited by hydrocortisone (1 μM) but not indomethacin (5 μg/ml) or salicylic acid (5 μg/ml). The inhibitory effect of hydrocortisone was prevented by RU 486 (1 μM) (Fig. 5).

DISCUSSION

Our results demonstrate the induction by TSST-1, in a concentration-dependent manner, of an L-arginine-dependent NO synthase in the murine monocyte–macrophage cell line J774.2. We used increases of cGMP levels in LLC-PK_1 pig kidney epithelial cells as a NO detector, for these cells contain high concentrations of NO-activated soluble guanylate cyclase. NO formed by the induced NO synthase was responsible for the increases in cGMP levels in the detector cells, for these increases were prevented by (*i*) oxyhemoglobin, which scavenges NO by reacting with it to form NO_3^- and methemoglobin (30), (*ii*) methylene blue, an inhibitor of NO-dependent activation of guanylate cyclase (31), and (*iii*) omission from the incubation medium of superoxide dismutase, thereby allowing superoxide anions to inactivate NO (32). Moreover, TSST-1 also increased the generation by J774.2 macrophages of NO_2^-, a breakdown product of NO in water (14, 19). The generation of NO by TSST-1-stimulated J774.2 macrophages needed extracellular L-arginine for it was prevented by replacement of L-arginine in the medium with D-arginine. Furthermore, NO generation was inhibited by MeArg, an inhibitor of L-arginine-dependent macrophage NO synthase (33). Treatment of J774.2 macrophages with TSST-1 caused the induction of a Ca^{2+}-independent NO synthase activity in the cytosol from stimulated cells, further supporting our conclusion that TSST-1 induces NO synthase in these cells.

The macrophage and monocyte have become a focal point of investigation into the pathogenic mechanisms of toxic shock syndrome because TSST-1 induces the production of interleukin 1 (IL-1) (34, 35) and tumor necrosis factor α (35, 36) *in vitro* as well as *in vivo*. Moreover, in the presence of cells bearing major histocompatibility complex class II antigens, TSST-1 activates T lymphocytes to produce interferon γ (37, 38), lymphotoxin (37), and IL-2 (39). Our results demonstrate that, in addition to the generation of cytokines, monocytic cells stimulated with TSST-1 express increased activity of NO synthase. Whether this is a direct effect of TSST-1 or secondary to the induction of IL-1 or tumor necrosis factor α remains to be established. However, it is important to note that the induction of NO synthase activity does not require the presence of T cells.

We suggest that NO plays a role in the pathogenesis of toxic shock syndrome and that it mediates some of the biological effects of TSST-1; indeed, the properties of NO make it a very likely candidate. NO is a potent vasodilator, so that its increased generation will lead to erythroderma and hypotension, the hallmarks of toxic shock syndrome. Moreover, the cardiovascular and pathological effects of TSST-1 and LPS are strikingly similar and NO is involved in the pathogenesis of endotoxic shock. Bacterial LPS or cytokines induce Ca^{2+}-independent NO synthase in numerous cell types, including J774.2 cells (40), macrophages (14), endothelial cells (16), hepatocytes (15), and vascular smooth muscle cells (17). Treatment of rats with LPS results in the increased activity of Ca^{2+}-independent NO synthase in the liver and lungs (41). Furthermore, LPS-induced hypotension (21) and reduced vascular responsiveness to contractile agents can be reversed by inhibitors of NO formation *in vitro* and *in vivo* (22, 23). L-Arginine-derived NO mediates the killing by activated macrophages of tumor cells and various intracellular and extracellular microorganisms (18–20). NO is also involved in the tissue injury caused by deposition of immune complexes *in vivo* (42). Binding of NO to iron–sulfur centers and the inhibition of important enzymes such as aconitase, complex I and complex II of the mitochondrial respiratory chain, or ribonucleotide reductase has been proposed as a molecular mechanism of the cytotoxic action of NO (43, 44). Moreover, NO inhibits protein synthesis in hepatocytes (45). Thus, the increased generation of NO by macrophages and perhaps other cell types might contribute to the multiple organ failure seen in patients with toxic shock syndrome.

Induction of NO synthase in macrophages serves as a regulatory (suppressive) mechanism in macrophage–lymphocyte interactions. Thus, alloantigen-induced activation of rat splenocytes is potentiated by MeArg (46) and NO production is required for murine peritoneal macrophages to suppress the proliferation of T cells stimulated with various mitogens (47, 48). Moreover, SIN-1, a NO donor that spontaneously releases NO at physiological pH (49), inhibits production of IL-2 by antigen-specific T-lymphocyte hybridomas stimulated with antigen (50). Therefore, it is tempting to speculate that NO may also be involved as a mediator of the immunosuppressive effects of staphylococcal superantigens such as suppression of humoral (51) and cellular immune responses (52).

Glucocorticoids have been used in treatment of toxic shock syndrome (53). They prevent TSST-1-induced mortality and morbidity in the rabbit model of toxic shock syndrome (5). Furthermore, methylprednisolone, but not aspirin or indomethacin, inhibits TSST-1-induced enhancement of susceptibility to the lethal endotoxin shock in the rabbit (54). In agreement with clinical and *in vivo* data, our results demonstrate the inhibition by hydrocortisone, but not by indomethacin or salicylic acid, of TSST-1 or LPS-mediated induction of NO synthase. Thus, beneficial effects of glucocorticoids in toxic shock syndrome may be due to the prevention of induction of NO synthase. LPS was clearly a more potent inducer of NO synthase in J774.2 macrophages than TSST-1. This is consistent with significant susceptibility of mice to LPS and their relative resistance to the effects of TSST-1 (55).

Glucocorticoids regulate expression and activity of many cytokine-inducible enzymes, including NO synthase (56), cyclooxygenase (57), and phospholipase A_2 (58). Cyclooxygenase activity is reduced by salicylic acid through inhibition of mRNA expression (59). The lack of effect of salicylic acid on TSST-1- and LPS-induced generation of NO by J774.2 macrophages suggests that, unlike glucocorticoids, salicylic acid regulates the expression of cytokine-inducible enzymes by a mechanism that is specific for cyclooxygenase but not NO synthase.

We conclude that TSST-1 and, perhaps, other exotoxins produced by Gram-positive bacteria induce NO synthase activity and suggest that the increased generation of NO may be involved in the pathogenesis of toxic shock syndrome and may mediate some of the biological actions of pyrogenic exotoxins.

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