INFECTION AND IMMUNITY, Jan. 1988, p. 135–141
0019-9567/88/010135-07$02.00/0

Vol. 56, No. 1

Neutralization of Toxic Shock Syndrome Toxin-1 by Monoclonal Antibodies In Vitro and In Vivo

PETER F. BONVENTRE,[1]* MICHAEL R. THOMPSON,[1,2] LUIGI E. ADINOLFI,[3] ZOE A. GILLIS,[4] AND JEFFREY PARSONNET[4]

Departments of Microbiology and Molecular Genetics,[1] and Division of Digestive Diseases, Department of Internal Medicine,[2] University of Cincinnati Medical Center, Cincinnati, Ohio 45267; Tropical and Subtropical Disease Clinic, 1st Medical School, University of Napoli, Naples, Italy[3]; and The Channing Laboratory, Department of Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, Massachusetts 02115[4]

Received 7 August 1987/Accepted 2 October 1987

Sixteen monoclonal antibodies (MAbs) directed against toxic shock syndrome toxin-1 (TSST-1) were generated by immunization of mice with purified TSST-1 and subsequent fusion of spleen cells with myeloma cells. Antibody-producing clones, identified by an enzyme-linked immunosorbent assay, were maintained as ascites tumors, and MAbs were purified by protein A chromatography. High-titered clones were further characterized and tested for the ability to neutralize several biological activities of TSST-1. The MAbs, which are of several immunoglobulin subtypes, reacted specifically with purified TSST-1 and TSST-1 present in *Staphylococcus aureus* culture supernatants. Three MAbs neutralized TSST-1-induced mitogenesis in a dose-dependent manner. Three of eight MAbs tested were able to neutralize induction by TSST-1 of interleukin-1 production by human monocytes. One neutralizing MAb, 8-5-7, was tested for the ability to protect rabbits from a constant infusion of TSST-1. Rabbits given the MAb had an attenuated clinical illness and were protected from the hypocalcemia, lipemia, and hepatic and renal insufficiency seen in control rabbits. Six of seven control rabbits died, compared with only one of seven rabbits treated with MAb 8-5-7. These experiments suggest that MAb 8-5-7 is directed against an antigenic determinant critical to the toxicity of TSST-1 and that the MAbs should be useful as probes in structure-function analyses of the TSST-1 molecule.

Toxic shock syndrome (TSS) is a systemic illness caused by *Staphylococcus aureus* and was first defined formally by Todd et al. in 1978 (32). Subsequent studies have established that the syndrome may be the consequence of a variety of localized staphylococcal infections, sometimes but not always accompanying menstruation (9, 25, 26). The systemic manifestations of the illness have been ascribed to one or more toxic products of *S. aureus*, possibly acting in conjunction with endotoxin derived from gram-negative bacteria (27).

A staphylococcal protein known as toxic shock syndrome toxin-1 (TSST-1) (M. S. Bergdoll and P. M. Schlievert, Letter, Lancet ii:691, 1984), originally termed staphylococcal enterotoxin F (2) and pyrogenic exotoxin C (29), has been identified as an important agent of this disease. The secreted protein is synthesized by almost all menstrual and many nonmenstrual strains of *S. aureus* isolated from patients with the clinical manifestations of TSS (2, 3, 6, 13, 29), although it is likely that staphylococcal products other than TSST-1 may also cause the syndrome (8, 13, 20, 28). Nevertheless, the precise role of TSST-1 in the pathogenesis of TSS has been difficult to establish. Antibody directed against TSST-1 is usually absent or minimal in sera of patients with TSS, in contrast to age-matched controls from the population at large (5, 19, 34). The absence of antibody in cases of TSS is circumstantial evidence for a pathogenic role for TSST-1 in this disease.

We designed the present study to achieve several specific objectives and with the ultimate goal of clarifying the mechanisms of action of TSST-1. First, we wanted to develop monoclonal antibodies (MAbs) directed against different regions on the TSST-1 molecule. Next, we wanted to determine whether several immunomodulating activities of TSST-1 could be neutralized in vitro by one or more of the MAbs. Finally, we wanted to determine whether neutralizing antibody could be used to protect rabbits from the toxic effects of TSST-1 in an established animal model. This report describes a total of 16 MAbs, the neutralization by several MAbs of mitogenesis and interleukin-1 (IL-1) induction by TSST-1 in vitro, and the attenuation by one MAb of a TSS-like syndrome caused by administration of TSST-1 to rabbits.

MATERIALS AND METHODS

Production of MAbs. Purified TSST-1 was obtained from the Food and Drug Administration Laboratories, Cincinnati, Ohio. The toxin migrated as a single protein band on sodium dodecyl sulfate (SDS)-polyacrylamide gels (17), with an apparent molecular weight of approximately 22,000. BALB/c mice were immunized by a modification of the method of Stahli et al. (31). Six weeks before fusion of cells, the mice were injected intraperitoneally (i.p.) with 1 ml of a 1:1 mixture of TSST-1 (100 μg), in phosphate-buffered saline (PBS), and Freund incomplete adjuvant. The mice were immunized again 3 weeks before fusion of cells by the same method. Four and two days before the fusion of cells, the mice were injected intravenously with 50 μg of TSST-1 in 200 μl of PBS.

Spleen cells from a single mouse were fused with established P3X63/Ag8.653 myeloma cells, also derived from BALB/c mice. The spleen cells and myeloma cells were mixed at a ratio of 3 to 1. The cell pellet was treated with a 50% polyethylene glycol solution to fuse the cells. The cells were suspended in Dulbecco modified Eagle medium with 20% fetal calf serum and hypoxanthine-aminopterin-thymidine selective medium for fused cells. Cells were plated at

* Corresponding author.

a concentration of 1.5×10^5 myeloma cells per well in 96-well tissue culture plates containing 6×10^3 mouse peritoneal exudate cells as feeder cells.

Hybridomas were diluted to an average density of about one colony per well, and wells from seven plates were assayed by a direct, enzyme-linked immunosorbent assay (ELISA). Culture supernatants were added directly to plates sensitized with 0.1 μg of TSST-1 per well. Antibody binding to TSST-1 was detected with affinity-purified goat anti-mouse antibody (heavy and light chains) conjugated with horseradish peroxidase (Kierkegaard and Perry, Gaithersburg, Md.). Approximately half of the culture wells contained antibody that bound specifically to immobilized TSST-1, as judged by specific substrate color development. Wells with significant titers were chosen for a second round of dilution cloning, with cells diluted so that only about 20% of all wells exhibited growth in hypoxanthine-aminopterin-thymidine medium. This was done to insure clonality of the hybridomas. Each of the resulting clones was tested by dot blot assay for immunoglobulin subtype by using mouse immunoglobulin chain-specific antibodies.

Of the antibody-producing hybridoma cell lines obtained, 18 were chosen for additional study based on their immunoglobulin subtypes and differential TSST-1-binding characteristics. Harvested hybridoma cell lines were expanded in culture and then injected i.p. into pristane-primed BALB/c mice. Tumor cells present in ascitic fluid were passaged in additional mice.

Purification and characterization of MAbs. Immunoglobulins from ascitic fluids were purified by protein A chromatography, using a commercially available buffer system (MAPS; Bio-Rad Laboratories, Richmond, Calif.) The purified antibodies were concentrated by ultrafiltration through membranes with a 30,000-molecular-weight cutoff (YM-30; Amicon Corp., Lexington, Mass.) and then dialyzed against PBS. The antibodies were adjusted to a concentration of 1.0 mg/ml in PBS, aliquoted, and stored at −80°C; MAb 8-5-7 prepared for in vivo studies was concentrated to 5 to 7 mg/ml before being frozen. The purification procedure yielded pure immunoglobulin G (IgG) with no apparent contamination by albumin when assayed by SDS-polyacrylamide gel electrophoresis (PAGE).

The antibodies were tested by dilution titration in the ELISA described above. The dilution of each MAb resulting in 50% maximal color development for that MAb was determined. Ascites and purified immunoglobulins were tested for serologic activity against TSST-1 in an electrophoresis immunoblot assay, as previously described (6). Antibodies were incubated with nitrocellulose transfer blots for 6 h at 25°C at a concentration of 1 μg/ml. Each lane contained 0.1 μg of pure TSST-1. All MAbs tested bound to the TSST-1 band obtained by SDS-PAGE and transferred to nitrocellulose membranes. The antibodies were also tested in an immunoblot assay against electrophoresed culture supernatants from both TSST-1-positive and -negative strains of *S. aureus*.

Induction of IL-1. MAbs were tested for their ability to neutralize the induction of IL-1 by purified TSST-1. Induction of IL-1 by TSST-1 and the assay for IL-1 were performed as previously described (20, 22). Briefly, human peripheral blood mononuclear cells were separated from other blood constituents by density gradient centrifugation, washed, suspended in RPMI 1640 medium supplemented with 20% fetal calf serum, and transferred to wells of a 96-well tissue culture plate (10^6 peripheral blood mononuclear cells in 0.2 ml of medium). After incubation for 1 h at 37°C in 5% CO_2, nonadherent cells were removed by aspiration, leaving a layer of adherent monocytes. Concentrations of TSST-1 and MAb were added as indicated in Fig. 2 and 3 to the wells in serum-free medium containing polymyxin B (5 μg/ml) and incubated for 24 h. Monocyte supernatants were centrifuged and assayed in triplicate for IL-1 in a standard lymphocyte-activating-factor assay (14, 15), using thymocytes from C3H/HeJ mice as the target cells. Appropriate concentrations of TSST-1 and MAbs were added directly to the thymocytes to determine their direct proliferative effect. Incorporation of [^{3}H]thymidine by the thymocytes was measured by scintillation spectrometry. The results are expressed as a stimulation index, defined as disintegrations per minute (dpm) induced by monocyte supernatants minus dpm induced by the corresponding controls, divided by dpm for thymocytes incubated in the presence of medium alone.

Mitogenesis assays. Mitogenesis assays were performed as previously described (18). Murine spleen cells (5×10^5 cells in 0.1 ml of RPMI 1640 medium supplemented with 20% fetal calf serum) were incubated in 96-well tissue culture plates at 37°C in 5% CO_2. An equal volume of TSST-1 with or without MAb was added to each well at the concentrations indicated in Fig. 4. All samples were run in quadruplicate. After 72 h, the cells were pulsed with [^{3}H]thymidine overnight, harvested, and collected on glass fiber filters. Incorporation of [^{3}H]thymidine was then determined. Data are expressed as corrected counts per minute (cpm), i.e., the cpm from stimulated cultures minus the mean cpm from control cultures.

Effects of MAb in animal model of TSS. A previously developed rabbit model of TSS (21) was used to determine the effect of one MAb on the toxicity of TSST-1 in vivo. TSST-1 was purified from sac-culture supernatants of *S. aureus* MN8, as previously described (20). The TSST-1 was administered to male New Zealand White rabbits as a constant, subcutaneous infusion by using miniosmotic pumps (Alza Corp., Palo Alto, Calif.). The rabbits were infused with 150 μg of TSST-1 over a period of 7 days, corresponding to a rate of about 0.25 μg/kg per h. Some rabbits also received MAb 8-5-7 by one of two routes, as detailed below. Daily observations were made during the course of the infusion, including weights and rectal temperatures. The rabbits received standard laboratory chow and water ad libitum.

Blood was obtained from the median artery of each rabbit before insertion of the pumps on day 0 and on subsequent days thereafter, as indicated in Fig. 5 and 6. Serum chemistries were determined on a Hitachi Automatic Analyzer 737 (Boehinger Mannheim Biochemicals, Indianapolis, Ind.). The results are expressed as means ± standard errors of the means.

RESULTS

Production and characterization of MAbs. Twenty-two MAb-secreting hybridoma cell lines were initially cloned from a single successful fusion. Eighteen of these lines, when injected i.p. into BALB/c mice, formed ascites tumors. Of these, 16 produced abundant ascites whereas 2 consistently yielded dry tumors. Each MAb of the 16 cell lines was purified by affinity chromatography, and five subclasses of immunoglobulin were found in this population. The distribution of these subtypes was as follows: IgG1(κ) (3 MAbs), IgG2a(κ) (10 MAbs), IgG2b(Mλ) (1 MAb), IgG2a(κλ) (1 MAb), and IgG2a(-) (1 MAb). The characteristics of binding

of the individual MAbs to TSST-1 were compared by a direct ELISA. Total color development at low dilutions of purified MAb ranged from low (optical density at 405 nm, <1.0) to extremely high (optical density ≥ 2.0). The dilution titers at which an optical density 50% of maximal was obtained ranged from less than 1:1,000 to greater than 1:2,500,000. This represents a range of protein concentrations of about 1 μg/ml to less than 1 ng/ml.

All 16 MAbs were found to react with denatured TSST-1. The binding of 10 MAbs to pure TSST-1 after SDS-PAGE and electrophoretic transfer to nitrocellulose is shown in Fig. 1. These immunoblots demonstrated that the MAbs reacted with primary sequence determinants. Qualitative differences in binding among the MAbs were apparent from differences in the intensities of staining of the blots. All MAbs also detected TSST-1 in culture supernatants of TSST-1-positive strains of *S. aureus* as a single band in the immunoblots. None of the MAbs reacted specifically with protein bands from supernatants of TSST-1-negative strains.

Neutralization of IL-1 induction by MAb. Ascitic fluids containing eight MAbs were screened for their ability to block induction of IL-1 production by human monocytes in response to TSST-1. These antibodies were chosen on the basis of differences in subtype and endpoint dilution titer. The reactions of the MAbs against TSST-1 were comparable in immunoblot assays. The MAbs were diluted 1:1,000 and added to the monocytes at the same time as the TSST-1 (0.1 μg/ml). Three MAbs, 6-1-1, 6-1-4, and 8-5-7, completely blocked IL-1 production in response to TSST-1, as indicated by low stimulation indices (Fig. 2). Of the remaining MAbs tested, several actually resulted in increased production of IL-1 at the one dilution tested, possibly because of immune-complex formation.

Ascitic fluids containing the three neutralizing MAbs were then coincubated at two dilutions (1:1,000 and 1:10,000) with three concentrations of TSST-1 (1.0, 0.1, and 0.01 μg/ml)

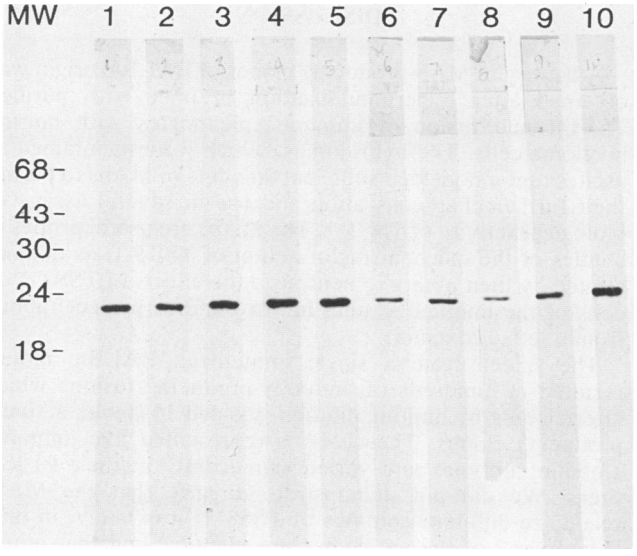

FIG. 1. Immunoblot analysis of anti-TSST-1 MAbs. Ten purified MAbs were tested for reactivity with purified TSST-1 on nitrocellulose transfer blots after SDS-PAGE. The indicated molecular weights (MW) are in thousands. The antibodies tested were 10-9-3 (lane 1), 15-7 (lane 2), 6-1-4 (lane 3), 8-5-7 (lane 4), 6-1-1 (lane 5), 2-3-6-5 (lane 6), 7-2-9 (lane 7), 10-7-1 (lane 8), 7-1-2 (lane 9), and 3-2-1 (lane 10).

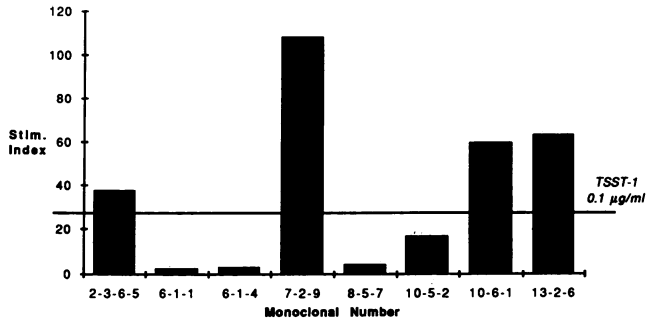

FIG. 2. Effect of MAbs on induction of IL-1 by TSST-1. The stimulation index (stim. index) of TSST-1 (0.1 μg/ml) in the absence of MAb was 27, as indicated. MAbs at a dilution of 1:1,000 yielding stimulation indices less than that of TSST-1 alone were said to be neutralizing.

(Fig. 3). TSST-1 in the absence of MAb was stimulatory over the range of concentrations tested. All three MAbs neutralized TSST-1 in a dose-dependent manner, with MAb 8-5-7 marginally the most effective (e.g., complete neutralization of 0.1 μg of TSST-1 per ml at a dilution of 1:1,000). To demonstrate that neutralization was not affected by other components of ascitic fluid, protein A-purified MAb 8-5-7 was coincubated with TSST-1 in a comparable experiment. Stimulation of IL-1 was again blocked at a comparable

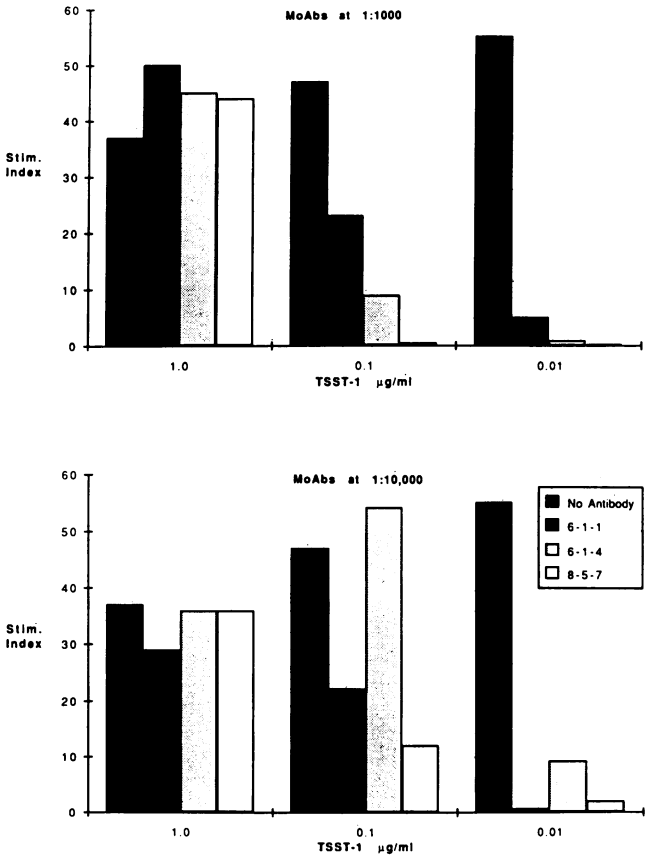

FIG. 3. Neutralization by three MAbs of IL-1 induction by TSST-1. MAbs were coincubated at two dilutions, 1:1,000 (top) and 1:10,000 (bottom), with three concentrations of TSST-1. Stim. index, Stimulation index.

weight ratio of MAb to TSST-1 (data not shown), suggesting that neutralization was a specific consequence of binding of the MAb to an effector site on the TSST-1 molecule.

Neutralization of mitogenesis. Serial dilutions of three MAbs were tested for the ability to neutralize the mitogenic response of spleen cells to TSST-1. The same dilutions of TSST-1 were used to stimulate mitogenesis as were used for induction of IL-1. The results of a representative experiment, using MAb 8-5-7, are shown in Fig. 4. Again, TSST-1 was stimulatory over the range of concentrations tested. MAb 8-5-7 blocked the mitogenic response in a dose-dependent fashion. At the highest concentration of TSST-1 tested (1.0 μg/ml), the mitogenic response was almost completely blunted by a 1:10 dilution of purified MAb 8-5-7; at 0.01 μg of TSST-1 per ml, a 1:1,000 dilution of MAb 8-5-7 was effective. Similar experiments were performed with purified MAbs 6-1-1 and 15-7. MAb 6-1-1 was comparable to 8-5-7 in its ability to block mitogenesis, but MAb 15-7 was considerably less efficient in doing so despite a comparable ELISA dilution titration.

Passive immunization with MAb in an animal model. Based on the proven ability of MAb 8-5-7 to block induction of IL-1 and lymphocyte mitogenesis in vitro, we selected it to attempt passive immunization of rabbits. The constant infusion model of TSS (21) was considered to be appropriate for testing the efficacy of this antibody for three reasons: (i) the toxic effects of TSST-1 can be evaluated without confounding effects of other staphylococcal products; (ii) the osmotic pump delivers small amounts of TSST-1 over a prolonged period of time, as might occur during human TSS, and without the need to restrain the animal; and (iii) a designated amount of TSST-1 is reproducibly delivered to all animals. The infusion results in profound physiologic changes leading to death, with rabbits exhibiting high fever, conjunctival hyperemia, anorexia, cachexia, lethargy, diarrhea, respiratory congestion, and muscle stiffness and irritability. The characteristic abnormalities in blood chemistry and hematology profiles have been described (21).

MAb 8-5-7 was administered to rabbits by one of two routes. In the first experiment, an osmotic pump containing antibody was inserted into the peritoneal cavity 1 day before subcutaneous insertion of the TSST-1-containing pump. Four rabbits were given MAb-containing pumps, and four were given saline pumps and served as untreated controls. The rabbits received about 2 mg of antibody per kg, which was calculated, based on the in vitro data, to be sufficient to neutralize 150 μg of TSST-1. In the second experiment, the same amount of MAb was administered intravenously to three rabbits in two doses, the first just before subcutaneous insertion of the toxin pump and the second 48 h later. Three control rabbits were given saline.

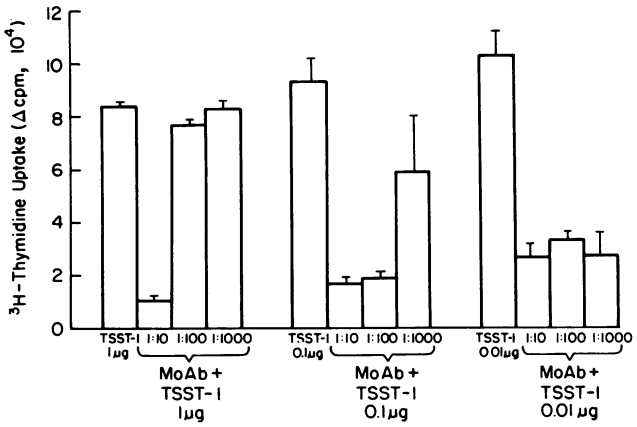

FIG. 4. Neutralization of mitogenesis by MAb 8-5-7. Both TSST-1 and MAb 8-5-7 were added to murine spleen cells at three concentrations.

Rabbits passively immunized with MAb by either route had a greatly attenuated illness, with greater intake of food, less weight loss, less lethargy, and improved survival. Six of seven control rabbits died during the course of the TSST-1 infusion (mean time until death, 4 days), compared with only one of seven rabbits given MAb ($P = 0.01$ by Fisher's exact test). The single mortality among the MAb-treated rabbits occurred on day 7. Of the various clinical parameters monitored, only fever was not markedly diminished by the administration of antibody. The mean fever curves were difficult to evaluate, however, because some rabbits became hypothermic several days before death. Several rabbits receiving MAb intravenously did, in fact, become afebrile by day 3 of the infusion of toxin, in contrast to control rabbits, which either remained febrile or became hypothermic.

The protective effects of passive immunization were also evident in differences in blood chemistry between treated and untreated rabbits. The intravenous administration of MAb 8-5-7 completely prevented elevations in blood urea nitrogen and serum glutamic-pyruvic transaminase and substantially blunted the elevation of triglycerides and depression of serum calcium seen in control rabbits (Fig. 5). It may be that a higher level of circulating TSST-1 is required to cause hepatic and renal dysfunction than is required to induce fever, hypocalcemia, and hypertriglyceridemia. i.p. administration of MAb was somewhat less protective, possibly because less antibody reached the systemic circulation or because of lot-to-lot variability in the toxin preparations used, but similar trends were evident for each parameter. The combined data from the two experiments, encompassing data for 14 rabbits, are depicted in Fig. 6.

DISCUSSION

A battery of MAb-producing clones of BALB/c origin was obtained after hyperimmunization of mice with purified TSST-1 and fusion of immune splenocytes with murine myeloma cells. The hybridomas, which were maintained as ascites tumors, yielded sufficient amounts of MAbs to permit their further characterization and use in several assays of biological activity of TSST-1. The MAbs are useful probes in studies of the mechanisms of action of TSST-1, as demonstrated by their ability to neutralize the effects of TSST-1 on cells of the immune system in vitro and to protect rabbits from a lethal toxemia.

The spleen from a single immunized BALB/c mouse resulted in hundreds of antibody-producing fusions which after cloning by limiting dilution, yielded 16 stable, actively producing clones. These MAbs represented five immunoglobulin subtypes and varied significantly in their ELISA titers. We did not demonstrate directly that the MAbs recognize different epitopes of TSST-1; it is likely, in fact, that several of the antibodies are identical and that others bind to overlapping epitopes. We suspect strongly that some recognize different epitopes, based on the vigorous response to immunization with TSST-1 and apparent differences in affinity of binding to native TSST-1 in a solid-phase assay. Epitope specificity may be the basis for observed differences in the ability of several MAbs to block mitogenesis and IL-1

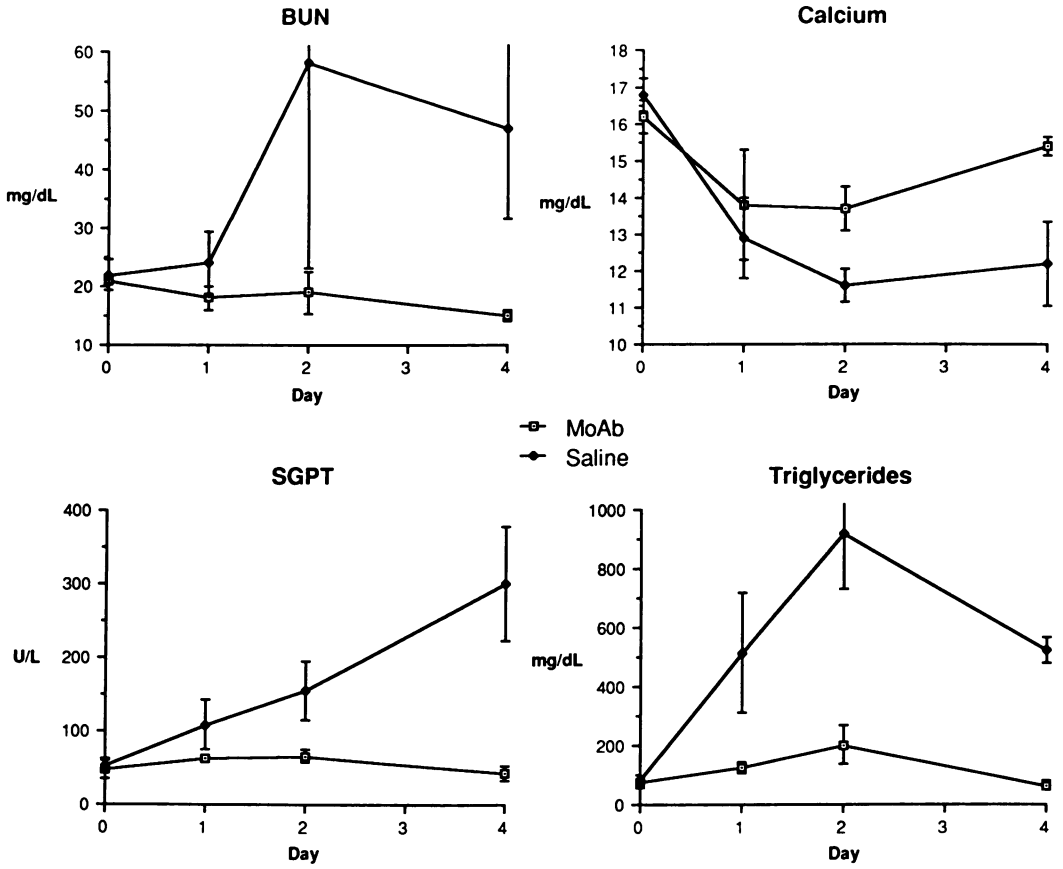

FIG. 5. Effect of MAb 8-5-7 administered by intravenous bolus on serum chemistry changes during constant infusion of TSST-1. The results represent means ± standard errors of the means for three rabbits in each group. BUN, Blood urea nitrogen; SGPT, serum glutamic-pyruvic transaminase.

production. The antibodies could not be distinguished by their ability to bind to denatured toxin since all were reactive in our immunoblots. The fact that they all bound to denatured TSST-1 suggests that primary sequence determinants, rather than tertiary structure determinants, constitute the sites of binding between the MAbs and TSST-1.

We initially determined that several MAbs were able to block production of IL-1 by human monocytes in response to purified TSST-1. A survey of eight MAbs at one concentration revealed that four were able to ablate or reduce the IL-1-inducing ability of TSST-1. The other four did not prevent IL-1 production; indeed, several MAbs actually increased production of IL-1 significantly above the level observed with toxin alone. This phenomenon may have been due to the formation of immune complexes, which in turn activated the monocytes, but more detailed experiments using multiple dilutions of each antibody are needed to determine this with certainty. Since IL-1 may be an important mediator of TSS (16, 20, 22), the identification of MAbs with the capacity to suppress IL-1 production in response to TSST-1 is of potential importance in assessing the pathogenic roles of both TSST-1 and IL-1. Another important biological activity of TSST-1 is its potent mitogenicity for T lymphocytes; on a weight basis, TSST-1 is a more potent mitogen than concanavalin A by several orders of magnitude (7). We found that the three purified MAbs tested neutralized the mitogenic response of murine T cells to TSST-1 in a dose-dependent fashion. MAbs neutralizing stimulation of IL-1 production but not lymphocyte mitogenesis, or vice versa, were not identified. In fact, the two activities may be interdependent, in that mitogenesis depends on the presence of accessory cells (7, 33), and monocytes specifically (T. Chatila, N. Wood, J. Parsonnet, and R. S. Geha, submitted for publication). Nevertheless, TSST-1-binding parameters are different for T cells and monocytes (23), raising the possibility that MAbs exhibiting differential effects on monocyte function and lymphocyte proliferation will be identified.

Ultimately, efforts to address issues of pathogenesis and the potential for immune protection depend on relevant animal models of TSS. In recent years, several interesting and potentially useful rabbit models have been developed. Arko et al. (1) and Scott et al. (30) have described rabbit models which use subcutaneous chambers which are inoculated with viable bacteria. De Azavedo et al. (11) described a rabbit model of TSS featuring an intrauterine diffusion chamber. This group showed that a strain of *S. aureus* which was genetically engineered to express TSST-1 caused a TSS-like illness after implantation of the chamber, whereas the parent strain, lacking the toxin gene, was relatively avirulent. Other investigators have induced sickness and death in rabbits with an intravenous bolus of TSST-1 in relatively large quantities (10, 24). The virtues of the model used in these studies, which employs a subcutaneous osmotic pump to deliver purified TSST-1 over a period of 1 week, are described here and elsewhere (21). The infection models would appear to be useful for comparing the pathogenicity of different strains of *S. aureus*, whereas the toxin

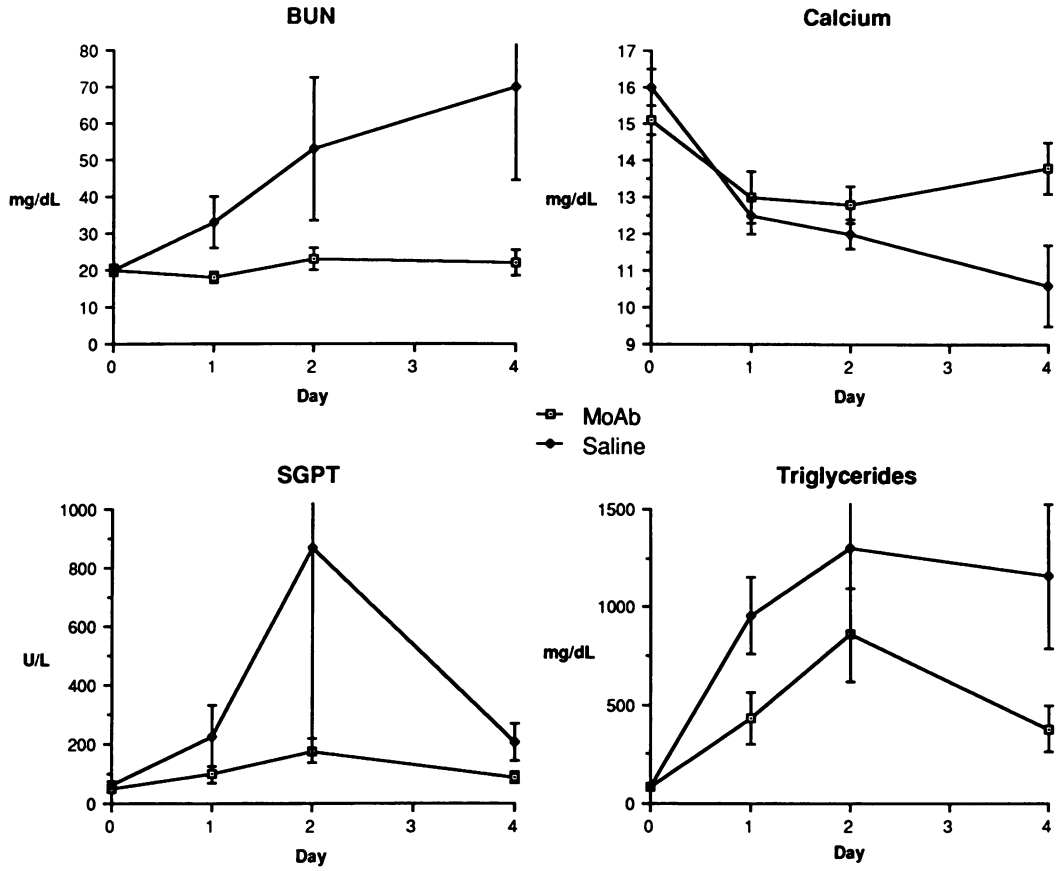

FIG. 6. Combined effects of MAb 8-5-7 administered by intravenous bolus or by i.p. infusion on serum chemistry changes during constant infusion of TSST-1. The results represent means ± standard errors of the means for seven rabbits in each group. BUN, Blood urea nitrogen; SGPT, serum glutamic-pyruvic transaminase.

infusion model may be superior for assessing the roles of specific staphylococcal exoproducts.

We considered it essential to test the MAbs in vivo as a corollary to the biological assays performed in vitro. Protection by MAb specific for TSST-1 in an appropriate animal model would support the hypothesis that stimulation of immune cells by this staphylococcal product is related to its toxic effects in vivo. In two blind experiments, rabbits were protected from the toxic effects of TSST-1 by purified MAb 8-5-7, which was administered either by constant i.p. infusion or intravenous bolus. Various clinical abnormalities were attenuated, as were the extreme changes in blood chemistry, and death of rabbits was prevented. These results indicate that this particular antibody is directed against a determinant on the TSST-1 molecule that stimulates cells of the immune system in vitro and mediates systemic toxicity in vivo. It is not possible, on the basis of these experiments, to isolate the effects of MAb on the various parameters monitored in the animal model. For example, it may be that more than a single determinant is capable of causing fever, for which reason even passively immunized rabbits became febrile during the infusion of toxin. Alternatively, the dose of MAb used in these experiments may have been inadequate to suppress fever, or immune complexes formed in vivo may have induced IL-1 or other endogenous pyrogens. Similarly, the hypocalcemic effect of TSST-1 may be unrelated to the antigenic determinant responsible for the induction of IL-1 in vitro. The complexity of the possible interactions is obvious. Further experiments will be necessary in which MAbs binding to different determinants on the TSST-1 molecule and possibly showing different activities in vitro can be tested.

The pathogenesis of TSS remains conjectural. IL-1 produced in large quantities in response to TSST-1 (16, 22) or to other staphylococcal products (20) may precipitate a cascade of events culminating in TSS. In addition to being an endogenous pyrogen, IL-1 has diverse immunologic and nonimmunologic effects on a variety of host tissues (12). Certain features of TSS suggest a central role for this monokine, including high fever, neutrophilia, changes in plasma divalent cation concentrations, and in many instances, evidence of skeletal muscle proteolysis. However, IL-1 is unlikely to be the sole endogenous mediator of TSS. Other candidates include tumor necrosis factor, a monokine with profound pharmacologic properties which was recently shown to mediate endotoxin lethality (4), and metabolites of arachidonic acid, prostaglandins, and leukotrienes. Endotoxin from gram-negative bacteria is believed by some investigators to contribute to the pathogenesis of TSS, a hypothesis neither supported nor refuted by the present experiments. TSST-1 itself may have specific effects on host tissues that do not require mediation by endogenous proteins (although such tissues have not been identified). Finally, there may be a contribution by other as yet unidentified staphylococcal products. In any event, a panel of MAbs with different specificities should prove to be valuable in assessing these and other unresolved issues related to the pathogenesis of TSS.

ACKNOWLEDGMENTS

This work was supported by grants from Tambrands, Inc., and the Proctor and Gamble Co.

We acknowledge the valuable contributions of Sharon Dingle and Kenneth Tonne in obtaining and screening immunoreactivity of the monoclonal antibodies.

LITERATURE CITED

1. **Arko, R. J., J. K. Rasheed, C. V. Broome, F. W. Chandler, and A. L. Paris.** 1984. A rabbit model of toxic shock syndrome: clinicopathological features. J. Infect. **8**:205–211.
2. **Bergdoll, M. S., B. A. Crass, R. F. Reiser, R. N. Robbins, and J. P. Davis.** 1981. A new staphylococcal enterotoxin, enterotoxin F, associated with toxic shock syndrome *Staphylococcus aureus* isolates. Lancet **i**:1017–1021.
3. **Bergdoll, M. S., B. A. Crass, R. F. Reiser, R. N. Robbins, A. C.-M. Lee, P. J. Chesney, J. P. Davis, J. M. Vergeront, and P. J. Wand.** 1982. An enterotoxin-like protein in *Staphylococcus aureus* strains from patients with toxic shock syndrome. Ann. Intern. Med. **96**:969–971.
4. **Beutler, B., I. W. Milsark, and A. C. Cerami.** 1985. Passive immunization against cachectin/tumor necrosis factor protects mice from lethal effect of endotoxin. Science **229**:869–871.
5. **Bonventre, P. F., C. Linnemann, L. S. Weckbach, J. L. Staneck, C. R. Buncher, E. Vigdorth, H. Ritz, D. Archer, and B. Smith.** 1984. Antibody responses to toxic-shock-syndrome (TSS) toxin by patients with TSS and by healthy staphylococcal carriers. J. Infect. Dis. **150**:662–666.
6. **Bonventre, P. F., L. Weckbach, J. Staneck, P. M. Schleivert, and M. Thompson.** 1983. Production of staphylococcal enterotoxin F and pyrogenic exotoxin C by *Staphylococcus aureus* isolates from toxic shock syndrome-associated sources. Infect. Immun. **40**:1023–1029.
7. **Calvano, S. E., F. W. Quimby, A. C. Antonacci, R. F. Reiser, M. S. Bergdoll, and P. Dineen.** 1984. Analysis of the mitogenic effects of toxic shock toxin on human peripheral blood mononuclear cells in vitro. Clin. Immunol. Immunopathol. **33**:99–110.
8. **Crass, B. A., and M. S. Bergdoll.** 1986. Involvement of staphylococcal enterotoxins in nonmenstrual toxic shock syndrome. J. Clin. Microbiol. **23**:1138–1139.
9. **Davis, J. P., P. J. Chesney, P. J. Wand, M. LaVenture, and the Investigation and Laboratory Team.** 1980. Toxic-shock syndrome: epidemiologic features, recurrence, risk factors, and prevention. N. Engl. J. Med. **303**:1429–1435.
10. **de Azavedo, J. C. S., and J. P. Arbuthnott.** 1984. Toxicity of staphylococcal toxic shock syndrome toxin 1 in rabbits. Infect. Immun. **46**:314–317.
11. **de Azavedo, J. C. S., T. J. Foster, P. J. Hartigan, J. P. Arbuthnott, M. O'Reilly, B. N. Kreiswirth, and R. P. Novick.** 1985. Expression of the cloned toxic shock syndrome toxin 1 gene (*tst*) in vivo with a rabbit uterine model. Infect. Immun. **50**:304–309.
12. **Dinarello, C. A.** 1984. Interleukin-1. Rev. Infect. Dis. **6**:51–95.
13. **Garbe, P. L., R. J. Arko, A. L. Reingold, L. M. Graves, P. S. Hayes, A. W. Hightower, F. W. Chandler, and C. V. Broome.** 1985. *Staphylococcus aureus* isolates from patients with nonmenstrual toxic shock syndrome. J. Am. Med. Assoc. **253**:2538–2542.
14. **Gery, I., R. K. Gershon, and B. H. Waksman.** 1972. Potentiation of the T-lymphocyte response to mitogens. I. The responding cell. J. Exp. Med. **136**:128–142.
15. **Gery, I., and B. H. Waksman.** 1972. Potentiation of the T-lymphocyte response to mitogens. II. The cellular source of potentiating mediator(s). J. Exp. Med. **136**:143–166.
16. **Ikejima, T., C. A. Dinarello, D. M. Gill, and S. M. Wolff.** 1984. Induction of human interleukin-1 by a product of *Staphylococcus aureus* associated with toxic shock syndrome. J. Clin. Invest. **73**:1312–1320.
17. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227**:680–685.
18. **Nickol, A. D., and P. F. Bonventre.** 1985. Visceral leishmaniasis in congenic mice of susceptible and resistant phenotypes: immunosuppresion by adherent spleen cells. Infect. Immun. **50**: 160–168.
19. **Notermans, S., W. J. Van Leeuwen, J. Dufrenne, and P. D. Tips.** 1983. Serum antibodies to enterotoxins produced by *Staphylococcus aureus* with special reference to enterotoxin F and toxic shock syndrome. J. Clin. Microbiol. **18**:1055–1060.
20. **Parsonnet, J., Z. A. Gillis, and G. B. Pier.** 1986. Induction of interleukin-1 by strains of *Staphylococcus aureus* from patients with nonmenstrual toxic shock syndrome. J. Infect. Dis. **154**:54–63.
21. **Parsonnet, J., Z. A. Gillis, A. G. Richter, and G. B. Pier.** 1987. A rabbit model of toxic shock syndrome that uses a constant, subcutaneous infusion of toxic shock syndrome toxin 1. Infect. Immun. **55**:1070–1076.
22. **Parsonnet, J., R. K. Hickman, D. D. Eardley, and G. B. Pier.** 1985. Induction of human interleukin-1 by toxic shock syndrome toxin-1. J. Infect. Dis. **151**:514–522.
23. **Poindexter, N. J., and P. M. Schlievert.** 1987. Binding of toxic-shock-syndrome toxin-1 to human peripheral blood mononuclear cells. J. Infect. Dis. **156**:122–129.
24. **Reeves, M. W., R. J. Arko, F. W. Chandler, and N. B. Bridges.** 1986. Affinity purification of staphylococcal toxic shock syndrome toxin 1 and its pathologic effects in rabbits. Infect. Immun. **51**:431–439.
25. **Reingold, A. L.** 1984. Epidemiology of toxic-shock syndrome, US 1980–1984. CDC surveillance summary. Morbid. Mortal. Weekly Rep. **33**:19SS–22SS.
26. **Reingold, A. L., N. T. Hargrett, B. B. Dan, K. N. Shands, B. Y. Strickland, and C. V. Broome.** 1982. Nonmenstrual toxic shock syndrome: a review of 130 cases. Ann. Intern. Med. **96**:871–874.
27. **Schlievert, P. M.** 1982. Enhancement of host susceptibility to lethal endotoxin shock by staphylococcal pyrogenic exotoxin type C. Infect. Immun. **36**:123–128.
28. **Schlievert, P. M.** 1986. Staphylococcal enterotoxin B and toxic-shock syndrome toxin-1 are significantly associated with nonmenstrual TSS. Lancet **i**:1149–1150.
29. **Schlievert, P. M., K. N. Shands, B. B. Dan, G. P. Schmid, and R. D. Nishimuri.** 1981. Identification and characterization of an exotoxin from *Staphylococcus aureus* associated with toxic shock syndrome. J. Infect. Dis. **143**:509–516.
30. **Scott, D. F., J. M. Kling, J. J. Kirkland, and G. K. Best.** 1983. Characterization of *Staphylococcus aureus* isolates from patients with toxic shock syndrome, using polyethylene infection chambers in rabbits. Infect. Immun. **39**:383–387.
31. **Stahli, C., T. Staehelin, and V. Miggiano.** 1983. Spleen cell analysis and optimal immunization for high-frequency production of specific hybridomas. Methods Enzymol. **92**:26–36.
32. **Todd, J., M. Fishaut, F. Kapral, and T. Welch.** 1978. Toxic shock syndrome associated with phage-group-I staphylococci. Lancet **ii**:1116–1118.
33. **Uchiyama, T., Y. Kamagata, M. Wakai, M. Yoshioka, H. Fujikawa, and H. Igarashi.** 1986. Study of the biological activities of toxic shock syndrome toxin-1. I. Proliferative response and interleukin-2 production by T cells stimulated with the toxin. Microbiol. Immunol. **30**:469–483.
34. **Vergeront, J. M., S. J. Stolz, B. A. Crass, D. B. Nelson, J. B. Davis, and M. S. Bergdoll.** 1983. Prevalence of serum antibody to staphylococcal enterotoxin F among Wisconsin residents: implications for toxic-shock syndrome. J. Infect. Dis. **148**:692–698.