Effect of Toxic Shock Syndrome Toxin 1 on Chicken Embryos

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Staphylococcus aureus strains associated with toxic shock syndrome produce toxic shock syndrome toxin 1 (TSST1). This toxin has a variety of biological effects, including enhanced lethality in rabbits in the presence of sublethal amounts of lipopolysaccharide (LPS). Because chicken embryos are highly susceptible to LPS, the synergistic effect of TSST1 and LPS was examined in this system. Although TSST1 per se had no effect on chicken embryos, it potentiated the lethal effect of LPS. The 50% lethal dose of LPS was greatly reduced in the presence of up to 10 µg of TSST1 per ml. However, at high doses of TSST1 (>100 µg/ml), no enhanced lethality was observed. The lowest dose of TSST1 tested which potentiated lethality was 10 ng/ml.

Staphylococcal toxic shock syndrome (TSS) is an acute multisystem disease characterized by fever, rash, vomiting, diarrhea, and clinical shock. Most Staphylococcus aureus strains associated with TSS produce a 23,000-dalton exoprotein with a pl of 7.3. Although this protein has been referred to as PEC (18), SEF (2), TSF (5), TSSE (11), TSA (15), or TST (13), it was recently decided to call it TSST1 toxin 1 (TSST1) (1a).

TSST1 is mitogenic for human lymphocytes (18) and stimulates the release of interleukin 1 from human monocytes (10). It enhances capillary permeability and elicits a pyrogenic response in rabbits (5, 18). It is also lethal in rabbits, and the effect is potentiated by gram-negative lipopolysaccharide (LPS) (5, 16, 18). It has no effect on other laboratory animals or on tissue culture cells (5).

The effect on rabbits is unpredictable for a variety of reasons. There is an age difference (5) and a strain difference (6); R. Stone, G. Johnson, and H. Pickrum, Abstr. Ann. Meet. Am. Soc. Microbiol. 1984, B53, p. 26) in susceptibility to TSST1. Because of this we felt that rabbits were not a good model for routine biological assays. We therefore sought other systems in which TSST1 may have an effect. In view of the synergism between TSST1 and LPS, we chose chicken embryos because they are highly susceptible to LPS (19). In this paper, we show that although TSST1 per se has no effect on chicken embryos, it is lethal in the presence of sublethal amounts of LPS. The chicken embryo assay could, therefore, be used as a quick and reproducible bioassay for TSST1.

MATERIALS AND METHODS

Purification of TSST1. A TSS strain, TC158 (kindly provided by M. de Saxe, Public Health Laboratory Service, Colindale, England) was grown aerobically in yeast difusate medium (3) with shaking for 40 h at 37°C. The culture supernatant was concentrated 10- to 20-fold by precipitation with 70% saturated ammonium sulfate and purified by preparative isoelectric focusing in two stages (1). Fractions showing a single band in sodium dodecyl sulfate-polyacrylamide gel electrophoresis (12) were pooled. Pooled fractions were dialyzed extensively against distilled water, lyophilized in 1-ml volumes, and stored at −20°C. Protein concentration was measured by the method of Bradford (4) with a dye concentrate (Bio-Rad Laboratories, Richmond, Calif.) and with bovine gamma globulin as a standard; the optical density was read after 15 min at room temperature. Contamination of TSST1 with LPS was assessed by the Limulus amoebocyte lysate (E-toxate; Sigma Chemical Co., St. Louis, Mo.) gelation assay; purified TSST1 contained <0.5 ng of LPS per mg of TSST1.

Chicken embryo assays. Fertile hens eggs were set in a humidified, self-turning incubator maintained at 37°C. After 11 days of incubation, they were inoculated through a blood vessel, usually the chorioallantoic vein, by the method of Finkelstein (8). Dilutions of TSST1 and LPS from Escherichia coli (E. coli O111 LPS; Mallinckrodt, St. Louis, Mo.) were made in 0.85% (wt/vol) pyrogen-free saline (Wellcome Laboratories, Beckenham, Kent, United Kingdom); 100-µl volumes of TSST1. LPS, or mixtures of TSST1 and LPS were injected into groups of 8 to 10 eggs per dose. Eggs were examined for death 24 h after the injection.

Antiserum to TSST1. New Zealand White rabbits (6 months old) were injected intradermally in multiple sites on their backs with 20 µg of TSST1 in Freund incomplete adjuvant. After 2 weeks, the dose was increased to 30 µg, followed by two subsequent doses of 60 µg each at 2-week intervals. Rabbits were bled after 7 weeks, and immunoglobulins were purified by ammonium sulfate precipitation (9).

RESULTS

TSST1 per se at doses of up to 100 µg/ml had no apparent effect on chicken embryos. The mean 50% lethal dose (LD50) of LPS was 0.45 µg/ml (95% confidence limits, 0.29 to 0.61); doses of 0.1 µg/ml or less had no effect. However, when TSST1 and sublethal doses of LPS were injected together, there was a marked potentiation of lethality (Table 1). The LD50 of LPS decreased to 0.05 µg/ml when a 1-µg/ml dose of TSST1 was also administered, and a similar enhancement was observed with 10 µg of TSST1 per ml.

Surprisingly, a 100-µg/ml dose of TSST1 did not potentiate the lethal effect. This unexpected phenomenon occurred at all the sublethal LPS concentrations used (Table 1) and was confirmed with a wider range of TSST1 doses and a single dose of LPS (Table 2). Doses of TSST1 from 0.01 to 50 µg/ml potentiated the lethal effect, but mortality decreased with higher doses, and a 250-µg/ml dose was nontoxic. The synergistic effect was neutralized when TSST1 was preincubated with anti-TSST1 serum for 30 min before being mixed with LPS (Table 3).
TABLE 1. Enhanced susceptibility of chicken embryos to LPS in the presence of TSST1

<table>
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<tr>
<th>TSST1 (µg/ml)</th>
<th>No. of embryos killed/no. challenged with indicated dose of LPS (µg/ml)*</th>
<th>0</th>
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<th>0.05</th>
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</table>

* The LD₅₀ of LPS was 0.45 µg/ml.

**DISCUSSION**

Although TSST1 per se had no effect on chicken embryos, it is clear that it potentiates the lethality of LPS, because the LD₅₀ of LPS was reduced ca. 10-fold by TSST1 concentrations of up to 50 µg/ml. The molecular basis of this synergism is unknown. LPS causes extensive perivascular hemorrhaging in chicken embryos (19), and death is preceded by severe hypoglycemia (8). Whether TSST1 and LPS in combination induce other lesions remains to be seen.

As has been suggested in the rabbit model (16, 17), TSST1 may destroy or inhibit certain cells of the reticuloendothelial system, such as Kupffer cells, which detoxify LPS, thus reducing the lethal dose. If this is the case, it is difficult to explain why the synergistic effect on chicken embryos decreased at TSST1 levels of >50 µg/ml. A similar trend was noted in rabbits more than 2 years old; all rabbits (11 of 11) died when challenged with 20 µg of TSST1, but fewer rabbits (12 of 20) died at doses of 50 to 200 µg of TSST1 (P < 0.05 as determined by the Fisher exact test) (5). Although TSST1 per se is lethal in rabbits (5), it is likely that death occurs through a synergistic effect with endogenous circulating LPS, because rabbits pretreated with polymyxin B, which neutralizes the effect of LPS, were not susceptible to TSST1 (6). It is possible that TSST1 may bind to LPS, thus enhancing the way in which the latter is "presented" to target cells; in the presence of excess TSST1, the binding sites of LPS for target cells may be masked.

In this study, the lowest dose of TSST1 (in combination with 0.1 µg of LPS per ml) which killed just under 50% of the embryos was 10 ng/ml. The chicken embryo assay therefore provides a reasonably sensitive bioassay for TSST1. Biological assays for TSST1 have been based on its pyrogenic or lethal effect on rabbits (5, 16, 18), and it was recently suggested that a bioassay for TSST1 should be based on its ability to release interleukin 1 from monocytes (10). Assays with rabbits are subject to a great deal of variation. In the pyrogen assay, individual rabbits often react differently to the same pyrogenic dose (14) and can become tolerant. The LD₅₀ of TSST1 differs among laboratories, possibly reflecting host differences in endogenous circulating LPS, because lethality is enhanced in the presence of LPS. Also, because susceptibility in rabbits is age dependent and strain dependent, bioassays with rabbits are difficult to standardize. The release of interleukin 1 from human monocytes provides a highly sensitive and suitable assay, but it should be noted that a variety of agents, including S. epidermidis, staphylococcal and streptococcal exotoxins, and staphylococcal protein antigens, also stimulate the release of interleukin 1 in vitro (7).

The chicken embryo assay is simple to execute, has an easily recognizable endpoint, and is not subject to variations arising from differences in immune response and endogenous bacterial flora which occur in rabbits. Therefore, the chicken embryo assay could prove to be a highly suitable bioassay for TSST1 and could also be a suitable model for investigating the mode of action of TSST1.

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**LITERATURE CITED**