NOTES

Protection of Rabbits in an Infection Model of Toxic Shock Syndrome (TSS) by a TSS Toxin-1-Specific Monoclonal Antibody†

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An anti-TSST-1-specific monoclonal antibody (MAb 8-5-7) was tested for its protective capacity in a rabbit infection model to toxic shock syndrome (TSS). The challenge strain of Staphylococcus aureus (RN4710), which contained a plasmid encoding TSS toxin-1, was introduced into previously implanted chambers. Purified monoclonal antibody (1.25 mg of immunoglobulin G) administered parenterally 1 day before and 1 day after initiation of infection provided complete protection against the TSS-like syndrome and the mortality which occurred in unprotected rabbits.

The role of toxic shock syndrome toxin-1 (TSST-1) (M. S. Bergoll and P. M. Schlievert, Letter, Lancet ii:691, 1984) in the pathogenesis of TSS is considered to be substantial. The production of TSST-1 by Staphylococcus aureus is commonly associated with TSS strains since greater than 90% of meningally associated isolates produce the toxin (3). Puriﬁed TSST-1 and strains producing TSST-1 elicit symptoms in rabbits resembling human TSS in substantial fashion. The most widely used infection model of TSS uses polyethylene balls subcutaneously implanted in New Zealand rabbits (1, 2, 9, 10). Two months after implantation and stabilization of body ﬂuids within the chamber, the strain of S. aureus to be tested is introduced through one of the holes in the whiffle ball. Those strains producing TSST-1 elicit symptoms characteristic of TSS with multiorgan involvement, and the illness usually terminates fatally (9). Since prior immunization of rabbits with puriﬁed TSST-1 protects rabbits against symptoms and mortality (9), the role of this toxin in precipitating the TSS-like syndrome is unequivocal. In recent years, it has become clear that nonmenstrual TSS may result from focal infections with S. aureus strains which do not produce TSST-1. In these instances, staphylococcal products other than TSST-1 may precipitate the illness by similar or identical pathways (5, 6, 8).

The purpose of our study was to determine whether monoclonal antibody directed against TSST-1 could protect rabbits in an infection model of TSS. The experiments were designed so that products of S. aureus other than TSST-1 would not complicate interpretation of the results. To that end, we obtained from R. Novick (Public Health Service Research Institute, New York, N.Y.) a gene-engineered strain of S. aureus (RN4710) which contains a plasmid encoding TSST-1 (4). The strain containing the tst gene does not produce alpha-hemolysin or staphylococcal enterotoxins (J. Arbuthnott, personal communication). Thus, potential systemic effects of these toxins in the infection model of TSS were eliminated. The plasmid encoding TSST-1 is unstable, and strain RN4710 requires erythromycin in the medium to prevent loss of the plasmid. We cultured the organism in Trypticase soy broth either with or without erythromycin. The latter resulted in the loss of tst and was used as an internal control in the in vivo experiments to be described. This nontoxic variant is described in the text as RN4710-C.

The monoclonal antibody used (MAb 8-5-7) neutralizes TSST-1 induction of interleukin-1 by human monocytes and the mitogenic response of murine lymphocytes to TSST-1 (2a). It also reacts specifically with TSST-1 in enzyme-linked immunosorbent assay and by immunoblot (1a). The monoclonal antibody was puriﬁed by protein A chromatography of BALB/c mouse ascites ﬂuid, and the immunoglobulin was concentrated by ultraﬁltration across 30,000-molecularweight-cutoff membranes and dialyzed against phosphate-buffered saline. After dialysis, the concentration of MAb 8-5-7 was approximately 5 mg/ml.

Male New Zealand White rabbits (12 to 15 weeks old) were anesthetized with ketamine (75 mg/kg) and xylazine (7.5 mg/kg) intramuscularly, and sterile polyethylene chambers were implanted as previously described (10). The chambers remained in place for 2 months before the animals were used. Before challenge with strain RN4710 or RN4710-C, the rabbits were weighed and randomly assigned to groups with code numbers. Rabbits to be infected with RN4710 were given erythromycin (4 mg/kg intravenously) every 24 h starting 1 day before infection. MAb 8-5-7 (approximately 1.25 mg) was given intravenously as bolus injections 24 h before and 24 h after the animals were infected. Each animal was examined daily for 5 days by an unbiased observer for subjective evaluation of symptoms of infection. Mortality in each group was recorded as deaths occurred.

Eight rabbits were challenged with S. aureus RN4710, and three were challenged with the strain cured of the plasmid.

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encoding the *tst* gene (strain RN4710-C) (Table 1). Of the eight rabbits challenged with the TSST-1-producing strain, five were treated with two intravenous injections of MAb 8-5-7 as previously described. The remaining three rabbits were not treated and served as positive controls in the TSS infection model. The three unprotected rabbits succumbed to the infection with RN4710 between 21 and 48 h after challenge. In two of the three animals, symptoms associated with the TSS-like illness in rabbits were observed. Objective evaluation included cold extremities, indicative of hypotension. Other characteristics of the illness in rabbits are fever, conjunctivitis, and labored breathing (10). The third rabbit in the group succumbed within 21 h and was not observed during illness. Passive immunization with MAb 8-5-7 protected all five rabbits from lethal challenge with RN4710. None of the rabbits in this group exhibited any signs of the TSS-like illness, and no mortalities occurred.

We have suggestive evidence in preliminary experiments that MAb 8-5-7 confers incomplete protection against a TSST-1-negative strain of *S. aureus* (D4508) which also causes a TSS-like illness in this animal model (6). The extent of the apparently nonspecific protection by MAb 8-5-7 noted in these preliminary experiments and the identity of staphylococcal exproducts other than TSST-1 responsible for eliciting the TSS-like illness in rabbits remain to be established.

The results of this study support the notion that TSST-1 is responsible for the illness induced by TSS-associated strains of *S. aureus* in this infection model of TSS infection. DeAzavedo et al. (4) also used a strain of *S. aureus* containing the *tst* gene on a plasmid and observed similar characteristic illness after implantation of the TSST-1-producing strain into intrauterine diffusion chambers; loss of the plasmid resulted in loss of virulence. Our results validate these observations in another animal model of TSS and, in addition, show that a monoclonal antibody directed against a critical epitope on the TSST-1 molecule completely ablates the TSS-like illness precipitated in rabbits by infection with *S. aureus* RN4710. Furthermore, this study, using a rabbit infection model of TSS, extends the results obtained with a rabbit constant-toxin-infusion model of TSS (7) showing that symptoms and mortality can be prevented by anti-TSST-1 monoclonal antibody (Bonventre et al., in press). We believe that this infection model of TSS and the use of genetically defined strains of *S. aureus* will provide the means to evaluate prophylactic and therapeutic modalities against TSS.

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**LITERATURE CITED**