INFECTION AND IMMUNITY, Dec. 1984, p. 727–732
0019-9567/84/120727-06$02.00/0

Vol. 46, No. 3

Enhanced Susceptibility of Male Rabbits to Infection with a Toxic Shock Strain of *Staphylococcus aureus*†

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Received 2 July 1984/Accepted 4 September 1984

Artifical infection chambers in rabbits were infected with a toxic shock strain of *Staphylococcus aureus* in an attempt to determine the nature of the enhanced virulence of toxic shock strains relative to non-toxic shock strains of staphylococci. The results showed that rabbits immunized with either neutral or acidic proteins were protected from the lethal effects of these infections. Male rabbits were found to be significantly more suspectible to these infections than female rabbits. Castration rendered both sexes equally susceptible to lethal infections. Numerous tissues from all infected rabbits were examined histologically, and most of the pathological findings involved lymphoid tissue. Of special interest was the observation that unprotected male rabbits which died had evidence of lymphoid depletion and that surviving rabbits, both male and female, usually manifested lymphoid hyperplasia. No other pathological response was noted which would characterize these infections, but immunized rabbits had a diminished level of thymic cortex involution that was not different between the sexes.

Earlier studies (19) showed that perforated polyethylene golf balls implanted subcutaneously in rabbits could serve as infection chambers to compare the virulence of *Staphylococcus aureus* strains isolated from toxic shock syndrome (TSS) patients with strains not related to TSS. Using this animal model, we found that 11 of 16 TSS strains produced lethal infections in rabbits and 0 of 5 non-TSS strains caused mortality.

All of the TSS strains and none of the control strains produced a characteristic exoprotein with an isoelectric point of ca. 7.0. This protein was initially designated pyrogenic exotoxin C by Schlievert et al. (18) and staphylococcal enterotoxin F by Bergdoll et al. (1). The confusion regarding the two proteins has apparently been resolved since the two are now considered the same protein (2–4, 15), and there is an agreement to call the substance toxic shock syndrome toxin-1 (TSST-1).

The present study was undertaken to determine which of the numerous extracellular proteins secreted by TSS strains of *S. aureus* could provide immunological protection to rabbits infected with TSS strains. In addition, we wished to determine whether distinctive histopathological or clinicopathological features were associated with these staphylococcal infections. The results presented indicate that the gender of the animal affects its response to an infection by TSS organisms, since males are more susceptible than females.

MATERIALS AND METHODS

Staphylococcal strain. The TSS strain of *S. aureus* used in this study was isolated from a 14-year-old female patient and provided by J. Todd (Children's Hospital of Denver, Denver, Colo.). The culture, designated strain 555, was found to cause mortality in all six rabbits used in an earlier study (19).

Rabbits. New Zealand white rabbits, 12 to 15 weeks old and weighing between 2.5 and 3.0 kg, were anesthetized with ketamine (75 mg/kg) and xylazine (7.5 mg/kg) intramuscularly. A sterile polyethylene chamber (Sportsotron, Inc., Bohemia, N.Y.) was implanted subcutaneously in the lumbar region through a midline dorsal incision. A minimum of 8 weeks was required for the fluid in the chamber to stabilize before the rabbits were infected. Unreliable results are obtained if the chambers have been in place for less than 8 weeks or if the rabbits are sexually immature (less than 4.5 months old).

For studies involving the effect of gender on infection mortality rates, rabbits of both sexes were anesthetized as described above, and surgical procedures were conducted aseptically. Testes were removed from the males, and both ovaries and the uterus were removed from the females. Sham operations were not conducted on controls. Neutering of both sexes was performed 10 to 14 days before infection of the chambers.

Separation of extracellular staphylococcal proteins. *S. aureus* 555 was inoculated into 500 ml of a beef heart dialysate medium prepared by the method of Watson (24). After 18 h of incubation, the cells were removed by centrifugation at 15,000 × *g* for 15 min, and the proteins in the supernatant solutions were precipitated with 4 volumes of absolute ethanol at −20°C. The resulting mixtures were kept at 4°C overnight, and the precipitates were collected by centrifugation at 15,000 × *g* for 20 min at 4°C and then suspended in distilled water. After the preparations were dialyzed against distilled water, water-insoluble material was pelleted by centrifugation at 15,000 × *g* for 20 min at 4°C, and the supernatant solutions were lyophilized.

Preparative electrofocusing was performed in a gel bed of Ultradex (LKB-Produkter, Bromma, Sweden) by using the extended buffer system of Prestidge and Hearn (12). Between 200 and 400 mg of protein, determined by the method of Lowry et al. (10), was added to the gel in an LKB Multiphor apparatus, and focusing was carried out at 17°C and 8 W for 46 h. A paper print was made to detect the focused protein zones within the bed (26). A dry filter paper was placed on top of the gel for 30 to 60 s to allow small

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amounts of protein to be absorbed by the filter paper. The proteins were fixed with 10% trichloroacetic acid and stained with Coomassie brilliant blue R-250. The print was destained with a mixture of methanol, acetic acid, and water (1:1:5 [vol/vol/vol]).

Once the protein bands were located the gel was removed, and the desired protein was eluted with distilled water, dialyzed, and lyophilized. Filter paper wicks containing 80 to 100 μg of protein were applied to the anode end of the polyacrylamide plate gels (pH 3.5 to 9.5; LKB Instruments Inc., Rockville, Md.), and focusing was carried out at 7°C and 8 W constant power for 30 min. The wicks were removed, and focusing was continued for 75 min at 15 W constant power. The channels of the gel were divided into 5-mm sections and placed in 1.5 ml of boiled distilled water to determine the pH gradient. The remainder of the gel was fixed in 3.5% sulfosalicylic acid–11.5% trichloroacetic acid and stained overnight with a 0.5% Coomassie brilliant blue R-250 solution containing 0.5% cupric acetate, 10% acetic acid, and 25% isopropyl alcohol. The gels were destained with 2% ethanol–8% acetic acid.

Immunization of rabbits. The rabbits were immunized with suspensions obtained from the preparative focusing gels described above. Seven groups of seven rabbits each were used for immunization comparisons. The rabbits in group A were each immunized with 10 μg of neutral proteins (pI 6.5 to 7.5) and these in groups B, C, and D were given 20 μg of acidic (pI <6.5), basic (pI >7.5), and total mixed proteins, respectively, at weekly intervals for 5 weeks. The material used in these subcutaneous infections was not eluted from the gels before dialysis and lyophilization because preliminary studies with purified protein indicated that the gel enhances the antibody response. Group E rabbits therefore served as controls and received weekly injections with gel only. The rabbits in the other two groups, F and G, were not immunized, and only group G rabbits were infected along with the other rabbits. Group F rabbits were sacrificed and served as controls for histopathological comparisons.

Infection of rabbits. Actively growing cells of *S. aureus* 555 were harvested from dialyzed beef heart medium, washed twice by centrifugation, and suspended in 0.01 M phosphate-buffered saline (pH 7.2) to give a cell suspension containing 10^9 CFU/ml. The rabbits were infected within 30 min of the time this suspension was prepared by palpating the chamber to locate a perforation, inserting a 21-guage needle into the chamber, and injecting 1 ml of the bacterial suspension.

All rabbits were observed frequently after infection. The number of deaths during each period was recorded, and the temperatures were taken with a rectal thermometer at 24 and 48 h. Each rabbit was necropsied within 12 h of death or sacrificed for necropsy if it survived 72 h. Tissue samples collected for histopathology studies included lung, heart, aorta, liver, gall bladder, duodenum, jejunum, ileum, cecum, colon, kidney, urinary bladder, thymus, spleen, pancreas, skin, and submandibular, axillary, iliocecolic, bronchial, cervical, and popliteal lymph nodes. The tissues were fixed with neutral buffered Formalin and embedded in paraffin, and ca. 5-μm sections were cut. The sections were stained with hematoxylin and eosin (8) and subsequently examined in a blind manner by W.A.C. The pathological responses were scored on the following scale: 1, minimal; 2, slight; 3, moderate; 4, marked; and 5, severe.

Gel diffusion assays. Since TSST-1 has been prominently associated with TSS strains of *S. aureus* (18) and could be involved in virulence, we sought to determine the relationship of antibody to this protein with the responses of the groups of rabbits to their infection. Serum was obtained from each rabbit before its immunization with the various proteins described above and again just before infection. Each serum sample and dilution of the serum was tested for antibody to TSST-1 by using slide gels of agarose (11) with the wells 0.5 cm apart. The antigen wells contained 3.5 μl of TSST-1 at 3.5 mg/ml, and the test wells contained 3.5 μl of serum. The toxin was purified by the procedure of Schlievert et al. (18) and gave a single band in focusing gels. Single bands were also observed after staining with 0.1% Coomassie brilliant blue in a solution containing 10% acetic acid, 45% absolute ethanol, and 45% distilled water. An estimate of the relative titers of antibody to TSST-1 was made from serum dilutions.

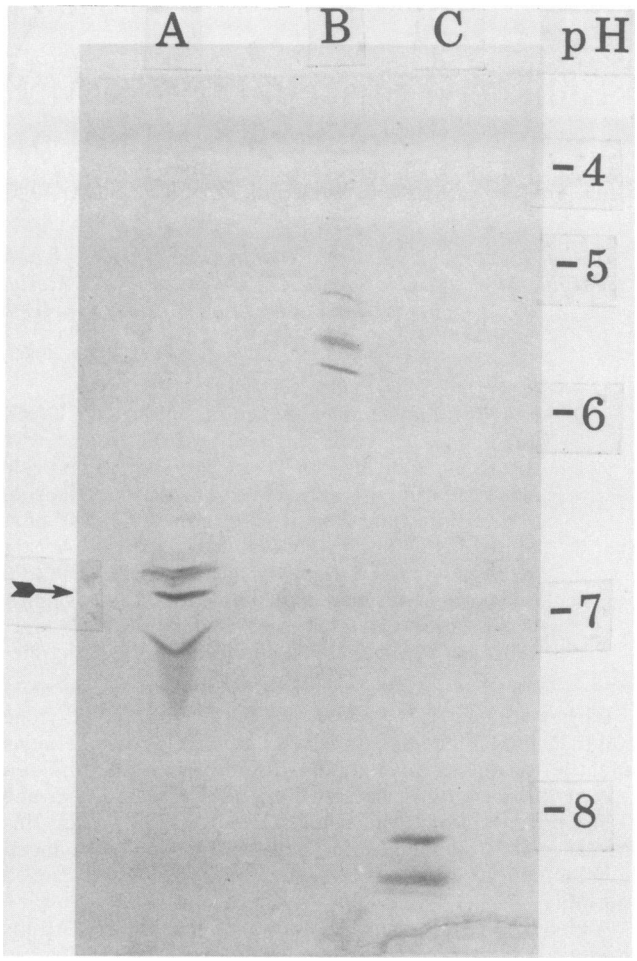

FIG. 1. Isoelectric focusing patterns of protein fractions used to immunize rabbits. Samples of protein (50 to 60 μg) were applied near the anode, and focusing was carried out as described in the text. Lanes A, B, and C correspond to groups A, B, and C described in the text. The arrow indicates the position of TSST-1.

RESULTS

Electrofocusing of staphylococcal proteins. A paper print of the ethanol-precipitable proteins secreted by *S. aureus* was used to reveal the bands on the preparative focusing gel, and the acidic, basic, and neutral proteins were used to immunize the groups of rabbits. An analytical focusing gel showing the protein bands in each fraction is shown in Fig. 1. As is evident from this gel, each of the immunizing preparations was heterogeneous, including the neutral band which con-

TABLE 1. Effect of immunization on survival of rabbits infected with *S. aureus* 555

Group	Immunization material	Mean anti-TSST-1 titer ± SE (range)[a]	Total no. of deaths at 24–48 h/no. of rabbits	
			Males	Females
A	Neutral proteins (pI 6.5–7.5)	18 ± 4 (8–32)	0/3	0.4
B	Acidic proteins (pI <6.5)	3 ± 0.5 (1–4)	0/3	0/4
C	Basic proteins (pI >7.5)	0	3/5	0/2
D	All proteins	2 ± 0.2 (1–2)	0.5	0/2
E	Gel only	0	2/3	0/4
F	None (not infected)	0		
G	None (infected)	0	2/3	0/4

[a] The mean anti-TSST-1 was estimated from dilutions of serum from animals in each group as described in the text.

tains TSST-1. ^{125}I-labeled protein A was used in subsequent Western blot analysis of the neutral proteins (16); this analysis showed the major protein in the neutral band (indicated by an arrow in Fig. 1) to be TSST-1 (data not shown).

Effect of immunization and gender on rabbit survival. These immunization studies were designed to provide a preliminary indication of which proteins produced by TSS strains of *S. aureus* could afford protection against subsequent infection. Our approach did not presuppose TSST-1 to be "the toxin", but its close association with TSS strains prompted an examination of the sera of the infected rabbits for antibody to this marker protein. All rabbits in group A, B, and D developed antibody to purified TSST-1 as determined from double diffusion gels (Table 1). Since none of the rabbits had detectable antibody to this protein in their preimmune serum, it was surprising to find that the rabbits in group B all mounted a detectable titer to TSST-1. At this point it is not known whether TSST-1 was an undetectable contaminant of our acidic protein preparation (Fig. 1) or whether there could be an immunologically related protein that has an acidic isoelectric point.

Table 1 also summarizes the mortality observed in this experiment. Although there was no previous selection of the rabbits on the basis of gender, the sex of each rabbit was noted at necropsy. All of the rabbits that died during the first 48 h after inoculation into the subcutaneous chambers were male. None of the female rabbits died during this period. Furthermore, none of the male rabbits which had detectable levels of antibody to TSST-1 died. In comparing rabbits in which antibody to TSST-1 was not detected (groups C, E, and F), the difference in susceptibility of males and females to the lethal effects of *S. aureus* 555 infections was significant ($P = 0.0028$) by Fisher's exact test.

This observation was pursued by using additional groups of male and female rabbits and comparing their susceptibility to *S. aureus* 555 infections with that of rabbits which had been surgically sterilized 2 weeks before being infected (Table 2). Neutered rabbits of both sexes were comparably susceptible. The effect of sterilization on male rabbits was greater; the number of deaths was diminished and the observed mortality rate was not as rapid.

Morbidity of infected animals. All of the rabbits infected with *S. aureus* 555 became ill during the first 12 to 24 h after their inoculation. The rectal temperatures of protected (group A) and unprotected (groups E and G) rabbits were significantly higher than those of the control rabbits (group F) (Table 3). However, the temperatures of the protected rabbits were significantly lower ($P < 0.01$, Student's t test) than those of the unprotected rabbits. Moreover, the temperatures of the male rabbits were higher than those of the female rabbits 24 h after infection. These differences in rectal temperature with respect to gender and immunization status were not observed 48 h after infection, when the temperatures of the rabbits in all three groups were between 1 and 2°C higher than those of uninfected rabbits (data not shown).

TABLE 2. Effect of castration on rabbits infected with *S. aureus* 555

Group	No. of deaths on day[a]:				Total no. of deaths/ no. of rabbits
	1	2	3	5	
Males	11	3	0	0	14/19
Females	2	0	0	1	3/23
Castrated males	1	3	1	0	5/15
Castrated females	2	1	1	2	6/15

[a] All rabbits were infected with 10^9 CFU of *S. aureus* 555. Deaths were recorded at daily intervals, and so rabbits dying in less than 24 h were listed under day 1, those dying in 24 to 48 h as day 2, etc. Castrated males and females were sterilized 2 weeks before their infection.

In addition to the difference in rectal temperature at 24 h, the rabbits with detectable antibody titers to TSST-1 appeared to be more alert and lacked the obvious signs of illness, such as nasal discharge, diarrhea, conjunctival hyperemia, and general listlessness, that were observed in the unprotected animals.

Pathological findings. Each rabbit which died of its infection during the first 48 h was noted to have red, swollen lymph nodes and a red thymus, and the liver of each rabbit was mottled white to red. White striations were frequently seen on various lobes of the liver. Other rabbits in the unprotected groups (C, E, and G) which did not die also had swollen lymph nodes and most had the hepatic striations, but the red thymus was not noted in these rabbits.

Rabbits with an antibody titer to TSST-1 differed from the others (groups C, E, and G) in that the frequency and extent of lymph node swelling was greatly reduced and only one of these rabbits had liver striations. In all of the infected rabbits, however, the fibrous tissue surrounding the implanted whiffle ball was red and thickened.

Histopathological findings. Tissue samples from all of the organs listed above were compared in an attempt to charac-

TABLE 3. Rectal temperatures in infected rabbits

Group	Sex (no. of rabbits)	Mean temperature (± SE) at 24 h
A	Female (4)	104.3 ± 0.2
	Male (2)	105.3
E	Female (4)	105.8 ± 0.3
	Male (3)	106.3 ± 0.5
G	Female (4)	105.6 ± 0.3
	Male (1)	106.5
F	Female (2)	102.7 ± 0.1
	Male (4)	102.6 ± 0.1

TABLE 4. Relative thymic involution in protected and unprotected groups of rabbits

Group	Avg extent of thymic involution in[a]:	
	Males	Females
A, B, and D (immunized)	2.6 (n = 7 + 3)[b]	2.3 (n = 6 + 2)
C, E, and G (nonimmunized)	3.5 (n = 10 + 0)	4.0 (n = 9 + 0)

[a] Animals were scored on the 1 to 5 severity scale mentioned in the text.
[b] n, Number of animals in which thymic involution was noted plus the number with no evidence of thymus involvement.

terize the response of the rabbits in each group to their infection. Histopathological changes were noted in the liver, lymph nodes, and thymus. The livers of all infected animals showed a periportal lymphoid infiltrate. However, the severity or extent of this response was about the same in each group. Moreover, this infiltration was not lessened by immunization with any staphylococcal product (and therefore cannot be correlated with mortality).

Tingible body macrophages, which are indicative of the turnover of lymphoid tissues, were observed in the lymph nodes of all animals. However, they were most prominent in rabbits in groups E and G which were not immunized with staphylococcal proteins. Among the immunized rabbits, the lowest frequency of these macrophages was noted in group C, which was immunized with basic proteins alone, and in group D, which was immunized with all of the extracellular proteins. Thus, a basic protein may increase the number of these macrophages in lymph nodes.

A third organ affected in most rabbits was the thymus. The most obvious microscopic effect was an involution of the thymic cortex. In contrast to the liver infiltrate and the stainable body macrophages in the various lymph nodes, the relative extent or severity of thymic involution appeared to be inversely related to the anti-TSST-1 titer developed by each group. Thus, in group A, in which the highest titer to the marker protein was observed, the thymus tissue was indistinguishable from that of uninfected, control rabbits.

Despite this apparent relation of thymic involution to antibody to TSST-1 antigen, there was no such correlation with susceptibility to the lethal effects of *S. aureus* 555 infections. This is evident from Table 4, in which the severity of thymic involution is compared in male and female animals in protected and unprotected groups (i.e., those with and without anti-TSST-1 levels). As shown in Table 4, animals with a detectable level of antibody to TSST-1 had a less severe thymic involution than did unprotected rabbits, but there was no significant difference between male and female rabbits. Thus, the thymus does not represent a single indicator of susceptibility to mortality from infection with this TSS strain of *S. aureus*.

Lymph node changes in infected rabbits. A histological examination of the lymph nodes revealed two distinctive changes in addition to the increased level of stainable body macrophages. Lymphoid depletion was evident in the lymph nodes of all the animals that died from their infection (Fig. 2), and the reactive hyperplasmia was present in all but one of the infected animals that survived; however, some nodes in a given rabbit showed evidence of hyperplasmia and others did not. The ratio of affected to unaffected nodes in rabbits of both sexes was determined, and an estimate of the relative extent of hyperplasia in male and female rabbits was made. The results indicate that, in general, female rabbits had a higher percentage of nodes with evidence of hyperplasmia (59 versus 33%) and the average extent of this condition was slightly greater in female rabbits (1.7 versus 1.4). However, these differences were not great since the rabbits in each group had only a minimal to slight (1 to 2) level of lymph node hyperplasia, and 40 to 60% of the nodes in each infected group had evidence of reactive hyperplasia.

DISCUSSION

Because the neutral proteins described by Schlievert et al. (17, 18) and Bergdoll et al. (1) appeared to be convincingly associated with staphylococcal strains isolated from TSS patients, we sought to pursue the suggestion that the protein we call TSST-1 is more than a marker for TSS strains. The rabbit model we described earlier (19) was used because it permitted a controlled, localized infection site and because we found the system to allow a differentiation between TSS and non-TSS strains with respect to virulence.

Since the rabbits were immunized with heterogeneous protein preparations, it is not possible to conclude that proteins other than the neutral TSST-1 are not responsible for the spectrum of symptoms shown by infected rabbits. It can only be concluded that rabbits which developed detectable antibody to purified TSST-1 did not die of their infection and their morbidity was significantly lessened, but another, unknown toxin could have coincidentially elicited the protection we observed, either alone or in some combined activity. Since highly purified TSST-1 is now available in reasonable quantities, the extent to which specifically immu-

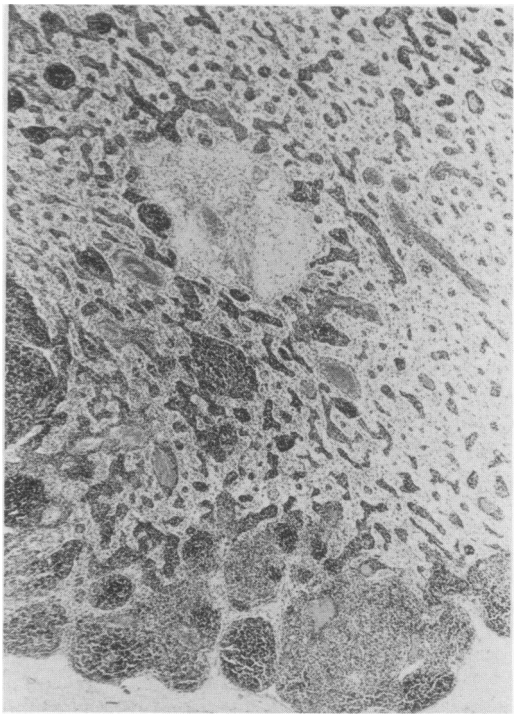

FIG. 2. Axillary lymph node from a rabbit that died after infection with *S. aureus* 555. Note the marked lymphoid depletion. The rabbit has been injected with gel only (group E). Hematoxylin and eosin strain, ×40.

nized animals manifest the effects of infection in this animal system can be determined.

In this regard, it is necessary to emphasize that acidic proteins, which presumably did not include TSST-1, prompted an antibody response to this protein and afforded protection to the rabbits in group B. Additional studies are required to determine whether the acidic proteins from focusing gels are contaminated with TSST-1 or whether there is an antigenically cross-reacting relative of TSST-1 in this fraction of the gel. The latter alternative is attractive since it raises the possibility that a major toxicity could reside in a protein larger than the 20,000 to 24,000 molecular weight reported for pyrogenic exotoxin C and staphylococcal enterotoxin F (1, 18). Studies are under way to resolve this question, but it is interesting to note that Smith et al. (22) recently reported what they describe as a variant of TSS in which the organism isolated from the patient produced a minor band in focusing gels which may correspond to TSST-1 and a unique, major protein band which migrated with the acidic proteins. Since the patient in this instance was a 16-year-old boy, the authors speculated that the acidic protein could be significant in the expression of the disease in males.

Essentially all of the histopathology noted in this study relates to an effect on lymphoid tissue. Some of the findings are very similar to those observed in human autopsy cases. These include the lymphoid infiltrate or triaditis in the liver (9) and the lymphocyte depletion in lymph nodes (9, 23). Other reactions, such as tingible body macrophages and thymic involution, do not directly correspond to human lesions but do suggest that rabbits experience significant lymphoid changes as occur in humans. In the other major lymphoid organ, the spleen, the only pathology noted was a slight to moderate congestion that occurred in male and female rabbits to a comparable extent (data not shown).

It is interesting to note that Schlievert (17) found that pyrogenic exotoxin C alone injected intravenously into American Dutch belted rabbits caused them to develop fevers, but also promoted an enhanced susceptibility to subsequent injections of endotoxin. The other manifestations of PEC all involved the immune system and included the stimulation of T-lymphocytes, suppression of immunoglobulin M synthesis, and enhanced hypersensitivity. How this relates to our finding that rabbits with an anti-TSST-1 titer have a lessened degree of thymic involution is not yet known. However, as mentioned previously, the thymic changes did not correspond quantitatively to death of the rabbits and could be affected by the different times at which the protected and unprotected animals were necropsied. Thus, a specific, reliable reaction which can be attributed to mortality was not seen. The only histopathological difference noted at necropsy was lymphocyte depletion in rabbits that died, compared with a minimal to slight lymphoid hyperplasia in one that survived. These reactions, however, can occur in a variety of infections and disease processes (9).

Perhaps the most surprising aspect of this study was the finding that male rabbits are more susceptible to lethal infections with this TSS strain than female rabbits and that neutered animals are equally susceptible. This observation was made only when all rabbits were necropsied for histopathology purposes and was not considered during our initial study (19), in which the virulence of TSS and non-TSS strains was compared. However, since we used rabbits of both sex randomly in that original set of experiments, our initial conclusion that some TSS strains are more virulent than others and, in fact, some do not cause any mortality, could perhaps be modified if the sex of each rabbit was known. We have not yet reexamined the virulence of all of our TSS strains to determine whether virulence differences exist despite a common ability to produce extracellular TSST-1. However, we have infected additional rabbits with *S. aureus* strains from non-TSS infections (TSST-1 negative) and confirmed our original observation that such infections are not lethal in either male or female rabbits (data not shown).

Our observation that male rabbits are more susceptible to mortality by the TSS organism tested might seem incongruous since most of the public notoriety concerned menstrually associated TSS cases. However, nonmenstrual TSS is becoming increasingly more widely recognized (6, 13, 14, 21, 23, 25), and the possibility that hormones could be a factor in TSS has been postulated for several years (20). It has been reported, for example, that women taking birth control pills have a reduced incidence of TSS relative to their proportion of the population (5, 20). In addition, of course, a woman's estrogen level is lowest during menses (7). Studies are therefore currently under way to examine the effects of androgens and estrogens on infected rabbits and on the production of exoproducts by staphylococcal strains.

ACKNOWLEDGMENTS

This work was supported by a grant from the Procter and Gamble Co.

We greatly appreciate the valuable discussions with and periodic suggestions by P. Ifland, J. Widder, G. Petty, and G. Johnson. Valuable assistance with the animal necropsies was provided by B. Wyder, D. Barnett, and S. Spicker. We are especially grateful for the continued assistance of J. Pollard with the rabbit experiments.

LITERATURE CITED

1. **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.
2. **Bonventre, P. F., L. Weckbach, J. Staneck, P. M. Schlievert, 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.
3. **Carlson, E.** 1983. Effect of strain of *Staphylococcus aureus* on synergism with *Candida albicans* resulting in mouse mortality and morbidity. Infect. Immun. **42**:285–292.
4. **Cohen, M. L., L. M. Graves, P. S. Hayes, R. J. Gibson, J. K. Rasheed, and J. C. Feeley.** 1983. Toxic shock syndrome: modification and comparison of methods for detecting marker proteins in *Staphylococcus aureus*. J. Clin. Microbiol. **18**:372–375.
5. **Davis, J. P., P. J. Chesney, P. J. Wand, and M. LaVenture.** 1980. Toxic shock syndrome: epidemiological features, recurrence, risk factors, and prevention. N. Engl. J. Med. **303**: 1429–1435.
6. **Dmytryshyn, J. R., M. J. Gribble, and B. O. Kassen.** 1983. Chemical face peel complicated by toxic shock syndrome. Arch. Otolaryngol. **109**:170–171.
7. **Eastwood, E. B.** 1970. Estrogens and progestins, p. 1538–1565. *In* L. S. Goodman and A. Gilman (ed.), The pharmacological basis of therapeutics. Macmillan Publishing Co., New York.
8. **Humason, G. L.** 1972. Animal tissue techniques, p. 148–169. W. H. Freeman & Co., San Francisco.
9. **Larkin, S. M., D. N. Williams, M. T. Osterholm, R. W. Tofte, and Z. Posalaky.** 1982. Toxic shock syndrome: clinical, laboratory, and pathologic findings in nine fatal cases. Ann. Intern. Med. **96**(part 2):858–864.
10. **Lowry, O. H., N. J. Rosebrough, A. L. Farr, and A. J. Randall.** 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193**:265–275.

11. **Ouchterlony, O.** 1962. Diffusion-in-gel methods for immunological analysis. Prog. Allergy **6**:30–54.
12. **Prestidge, R. L., and M. T. W. Hearn.** 1979. Preparative flatbed electrofocusing in granulated gels with natural pH gradients from simple buffers. Anal. Biochem. **97**:95–102.
13. **Reingold, A. L.** 1983. Nonmenstrual toxic shock syndrome: the growing picture. J. Am. Med. Assoc. **249**:932.
14. **Reingold, A. L., B. B. Dan, K. N. Shands, and C. V. Broome.** 1982. Toxic-shock syndrome not associated with menstruation. Lancet **i**:1–4.
15. **Reiser, R. F., R. N. Robbins, G. P. Khoe, and M. S. Bergdoll.** 1983. Purification and some physicochemical properties of toxic-shock toxin. Biochem. J. **22**:3907–3912.
16. **Renart, J., J. Reiser, and G. R. Stark.** 1979. Transfer of proteins from gels to diazobenzyloxymethol paper and detection with antisera. A method for studying antibody specificity and antigen structure. Biochemistry **72**:3116–3120.
17. **Schlievert, P. M.** 1982. Enhancement of host susceptibility to lethal endotoxin shock by staphylococcal pyrogenic exotoxin type C. Infect. Immun. **36**:123–128.
18. **Schlievert, P. M., K. N. Shands, B. B. Dan, G. P. Schmid, and R. D. Nishimura.** 1982. Identification and characterization of an exotoxin from *Staphylococcus aureus* associated with toxic-shock syndrome. J. Infect. Dis. **143**:509–516.
19. **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.
20. **Shelton, J. D., and J. E. Higgins.** 1981. Contraception and toxic-shock syndrome: a reanalysis. Contraception **24**:631–634.
21. **Silver, M. A., and G. L. Simon.** 1981. Toxic shock syndrome in a male postoperative patient. J. Trauma **21**:650–651.
22. **Smith, J. H., F. Krull, G. H. Cohen, A. L. Truant, R. Goldblum, A. Haque, and C.T. Ladoulis.** 1983. A variant of toxic shock syndrome. Arch. Pathol. Lab. Med. **107**:351–357.
23. **Thomas, S. W., I. M. Baird, and R. D. Frazier.** 1982. Toxic shock syndrome following submucous resection and rhinoplasty. J. Am. Med. Assoc. **247**:2402–2403.
24. **Watson, D. W.** 1960. Host-parasite factors in group A streptococcal infections. Pyrogenic and other effects of immunologic distinct exotoxins related to scarlet fever toxins. J. Exp. Med. **111**:255–284.
25. **Wiest, R. H.** 1981. Toxic shock syndrome in a middle-aged male. J. Fam. Pract. **13**:929–932.
26. **Winter, A., H. Perlmutter, and H. Davis.** 1980. Preparative flat-bed electrofocusing in granulated gel with the LKB 2117 Multiphor. LKB application note 198 (modified). LKB-Produkter, Bromma, Sweden.