Quantification and Toxicity of Group A Streptococcal Pyrogenic Exotoxins in an Animal Model of Toxic Shock Syndrome-Like Illness

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Toxic shock-like syndrome isolates of group A streptococci were evaluated for production of pyrogenic exotoxins (also called SPEs, scarlet fever toxins, and erythrogenic toxins). The isolates were consecutively obtained during 1987 and 1988. Of these isolates, 23 of 26 made SPE type A, 10 of 26 made SPE B, and 8 of 26 made SPE C. SPE A was produced in significantly greater amounts than SPE B and C (3.2 μg/ml of culture fluid compared with 0.7 and 0.6 μg/ml, respectively). SPE A, administered in miniosmotic pumps implanted subcutaneously in rabbits, was significantly more toxic than SPE C; seven of eight rabbits succumbed after challenge with 150 or 300 μg of SPE A, compared with one of six after challenge with SPE C.

Group A streptococci produce three antigenically distinct pyrogenic exotoxins (also called SPEs, scarlet fever toxins, and erythrogenic toxins), designated types A, B, and C (17). These toxins cause scarlet fever and have been implicated in a toxic shock-like syndrome (TSSLS) (6, 17). The biological properties of the toxins include pyrogenicity, enhancement of lethal shock and myocardial damage due to endotoxin or streptolysin O, and induction of non-specific T lymphocyte mitogenicity (2, 9, 13, 15; for a review, see reference 17). This last effect probably contributes to the development of the scarlet fever rash in a susceptible host (13). Many of these properties are shared with staphylococcal toxins associated with toxic shock syndrome (TSS), and SPEs share significant sequence homology with TSS-associated toxins (3, 5, 7).

In 1979, Schlievert et al. (12) determined that toxin types B and C were more commonly made by group A streptococci than toxin type A, thus possibly explaining the concurrent decline in severe scarlet fever cases in recent times. Indeed, no SPE A-producing strains were isolated from 1976 through 1979. Similarly, from 1983 to 1986, we isolated no SPE A-producing strains (6), even from patients with severe streptococcal illness. However, in the recent description of cases of streptococcal TSSLS, SPE A-positive strains were obtained from most of the patients when cultures were available (6; D. L. Stevens, M. H. Tanner, J. Winship, R. Swarts, K. M. Ries, P. M. Schlievert, and E. Kaplan, N. Engl. J. Med., in press). In addition, several other SPE A-producing strains have been submitted to our laboratory from apparently similar TSSLS cases, raising the possibility that there may be a reemergence of SPE A-producing strains.

In this report, consecutively obtained TSSLS-associated strains of group A streptococci have been examined for toxin production and for the ability of the toxins to induce lethality in a rabbit model.

Clinical isolates of group A streptococci were obtained from patients from diverse geographic locations in the United States; the patients were identified by their treating physicians as having TSSLS during 1987 to 1988. In two cases, Staphylococcus aureus was also isolated, but the isolates were negative for TSS toxin 1 and enterotoxins. Group A streptococcal strain T25, cured (T12) (8) was used as a known SPE A producer, and its isogenic pair T25, cured (8) was used as a non-toxin producer. Strain 86-858 was used as a known SPE B producer (4). T18P (18) and 86-104 (kindly provided by Edward Kaplan, University of Minnesota) were used as known SPE C producers. All strains were of low passage and were maintained lyophilized.

Hyperimmune rabbit antibodies against SPEs A, B, and C were prepared (11); these sera did not cross-react with heterologous toxins when tested by Ouchterlony immuno-diffusion. SPEs for immunization were made after culture of streptococci in a dialyzable beef heart medium, followed by ethanol precipitation, resolubilization in acetate-buffered saline, and preparative thin-layer isoelectric focusing (11). SPE A, B, and C concentrations were estimated by serial-dilution Ouchterlony immunodiffusion (14). The lower limit of detectable purified toxin was 6 μg/ml for all SPE types. Strains to be tested for toxin production were grown to stationary phase in beef heart medium (SPEs are maximally produced during late log phase, and no additional toxin is expressed during stationary phase) and then were toxin concentrated 100-fold by ethanol precipitation and resolubilization in water (lower limit of toxin detection, 0.06 μg/ml of original culture fluid). Ethanol treatment was shown to quantitatively precipitate all SPEs. Other potential methods for quantifying toxins, such as enzyme-linked immunosorbent assay, were unreliable because of the presence of large amounts of interfering hyaluronic acid made by many of the isolates. Strains expressing SPE A or C or both were verified as containing the genes speA and speC by Southern hybridization analyses (16): a gene probe for speB is presently unavailable.

American Dutch belted rabbits, weighing 1 to 2 kg, were purchased from Birchwood Farms, Grantsburg, Wis. The rabbits were shaved on one flank and then anesthetized with xylazine (20 mg/kg of body weight, intramuscular) and ketamine (25 mg/kg, intramuscular). Alzet miniosmotic pumps (Alza, Palo Alto, Calif.) were filled with 0.15, 150, and 300 μg of SPE A or C, as determined by the Bradford dye-binding protein assay (Bio-Rad Laboratories, Richmond, Calif.), in 0.2 ml of phosphate-buffered saline and prepared according to the instructions of the manufacturer.
TABLE 1. Production of SPEs by group A streptococci isolated from TSS-like illnesses in 1987 and 1988

<table>
<thead>
<tr>
<th>SPE type</th>
<th>No. of positive strains (total no. tested) (avg µg of toxin/ml of culture)</th>
<th>P compared with SPE A production</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>23/26 (3.2)</td>
<td>&lt;0.002</td>
</tr>
<tr>
<td>B</td>
<td>10/26 (0.7)</td>
<td>&lt;0.002</td>
</tr>
<tr>
<td>C</td>
<td>8/26 (0.6)</td>
<td>&lt;0.004</td>
</tr>
</tbody>
</table>

* Some strains expressed multiple toxin types. Thirteen strains made A only, three made C only, five made A and B together, and five made all three. 
* Determined at stationary phase.

The pumps, which are designed to exchange a constant amount of volume over 7 days, were inserted into small (1-cm) incision sites on the flanks of the rabbits as described previously in studies of TSS toxin 1 (10), in which that toxin induced TSS-like signs. After their surgical wounds were sutured, the rabbits were monitored daily for up to 9 days in the following categories: weight loss, rectal temperature change, stool and urine change, redness of conjunctivae and ears, and death. Baseline weight and rectal temperature for each rabbit were taken 1 day prior to pump implantation. The range of weight change from baseline and the maximal temperature rise above baseline during the experimental period were recorded.

Student’s t test analysis of unpaired, normally distributed data was used to determine differences between means of groups.

Twenty-six streptococcal strains from apparent TSS LS in 1987 and 1988 were tested for SPE production (Table 1). Of these, 23 were SPE A producers, with an average concentration of 3.2 µg of toxin per ml of culture as determined by quantitative Ouchterlony immunodiffusion (range, 0.1 to 7.7 µg/ml of culture). Ten isolates were SPE B producers, making an average of 0.2 µg/ml of culture (range, 0.06 to 1.9 µg/ml), and only eight expressed SPE C at an average of 0.6 µg/ml (range, 0.06 to 1.9 µg/ml). Thus, SPE A was made in significantly greater amounts than B or C (P < 0.002 and P < 0.004, respectively). Of the 26 strains, 13 made SPE A only, 3 made C only, 5 made A and B together, and 5 made all three SPEs. There was no significant correlation between expression of one or more than one toxin and the amount of toxin produced. Also, statistically significant differences between SPE B and SPE C concentrations were not seen.

In addition to greater production of SPE A versus B or C by TSSLS-associated streptococcal strains, it was also possible that SPE A was intrinsically more toxic to animals than SPE B or C. Implantation of miniosmotic pumps containing either SPE A or SPE C showed the effects of toxin type and various toxin concentrations on rabbits (Table 2); SPE B was not tested because in previous studies this toxin was 10-fold less active than either A or C (1). None of the control, non-toxin-treated rabbits (group 1) died or showed signs of illness; instead, they gained an average of 52 g and showed only an average of 0.4°C maximal temperature rise above baseline during the experimental period. Groups 2 (SPE A) and 5 (SPE C), both given 15 µg/ml, also did not lose any rabbits and had only small average temperature increases over baseline (0.6°C for group 2 and 1.0°C for group 5). However, all of the rabbits in both groups showed similar signs of weight loss (average of 60 g for group 2 and 35 g for group 5); diarrhea; clear, dark-yellow urine; and reddening of eyes and ears.

Implants with higher toxin concentrations (150 and 300 µg of toxin) induced increased responses in rabbits from groups 3 and 4 (SPE A) and groups 6 and 7 (SPE C). All of the rabbits in SPE A groups 3 (150 µg of toxin) and 4 (300 µg) exhibited signs of illness as described above along with the most severe weight loss and fever response, and seven of eight animals succumbed. The average maximal temperature rise above baseline were 1.5°C for group 3 and 2.0°C for group 4. Both groups lost over 100 g of weight during the experimental period.

In contrast to SPE A (groups 3 and 4), SPE C was not highly lethal (groups 6 and 7) to rabbits. All rabbits in SPE C groups 6 and 7 showed signs of illness and lost nearly 100 g of weight during the experimental periods, but only one of four in group 6 and none from group 7 died. Also, the average maximal temperature rise above baseline during the entire experimental period was only 1.2°C for both groups, compared with 1.5 and 2.0 for SPE A-challenged animals. We conclude that SPE A production is highly associated with TSSLS and that the severe illness produced by these strains may be the result of both greater toxin production by SPE A-positive strains compared with those not making SPE A and the greater toxicity of SPE A relative to other SPEs.

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