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Staphylococcal Scarlet Fever: Role of Pyrogenic Exotoxins

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Staphylococcal pyrogenic exotoxin (PE) types A and B were tested for their role in production of a scarlatiniform rash. The PEs elicited minimal skin reactions after intracutaneous injection into animals not presensitized to the toxins. In contrast, erythematous and edematous rashes were produced after administration of either PE to animals presensitized to homologous toxin. After 3 to 4 days, the erythematous areas showed membranous desquamation. Staphylococcal PEs also enhanced delayed and Arthus hypersensitivity skin reactions developed against unrelated proteins; the reactions subsequently desquamated. In addition, animals previously sensitized to either staphylococcal PE type developed scarlatiniform rashes after challenge with heterologous staphylococcal or any group A streptococcal PE. The data suggest that staphylococcal PEs produce scarlet fever-like rashes comparable to group A streptococcal PEs and that all PE types contain a common core moiety against which delayed hypersensitivity may be developed.

Syndromes closely related to classical group A streptococcal scarlet fever have resulted from infection with *Staphylococcus aureus*. Stevens describes three individuals with staphylococcal scarlet fever, none of whom had a streptococcal infection (20). Other investigators described scarlet fever in patients with staphylococcal osteomyelitis (1), wrist infection (6), or leg abscess (10). In two instances, injection of culture supernatant fluids of the corresponding *S. aureus* strain into Dick-positive persons resulted in scarlatiniform rashes (1, 10).

Previously, it was suggested that group A streptococcal pyrogenic exotoxins (PEs) produce the classical scarlet fever rash by enhancing acquired skin reactivity to streptococcal antigens (15). This included enhancement of hypersensitivity skin reactions developed against the "common core" moiety shared by all three streptococcal PE types (3, 8, 9, 15). Based on these data, it was concluded that erythrogenic toxin represents the enhancement of hypersensitivity reactions by PEs (15).

Recently, two serologically distinct staphylococcal PEs were purified and characterized biochemically and biologically (17; P. M. Schlievert, Biochemistry, in press). These toxins share many important properties with the group A streptococcal PEs (2, 3, 5, 12, 13, 17-19) and may therefore contribute to the development of staphylococcal scarlet fever. In addition, it was proposed that the staphylococcal toxins may contribute to the development of two severe scarlet fever-like syndromes, Kawasaki disease and toxic shock (P. M. Schlievert, M. Chai, M. T. Osterholm, and D. W. Watson, submitted for publication). The basis for the proposal was that all strains of *S. aureus* obtained from patients with either syndrome elaborated PEs, and the PEs have been used in experimental animals to produce many manifestations of the diseases (17; Schlievert, in press).

The purpose of this investigation was to assess the role of staphylococcal PEs in production of the scarlatiniform rash. In addition, PEs from both *S. aureus* and group A streptococci were tested for the presence of the shared common core moiety.

MATERIALS AND METHODS

All reagents and glassware used for preparation of PEs and biological assays were maintained pyrogen free.

Bacteria. Staphylococcal PEs were obtained from the Harrisburg strain of *S. aureus* (17). Group A streptococcal PEs were prepared after culture of strains 594, T18P, and T19P (16). Microorganisms were maintained in the lyophilized state in the presence of whole defibrinated fresh rabbit blood.

Toxin preparation. PEs were prepared after culture of the organisms in a dialyzable beef heart medium (22). PEs were purified by differential precipitation with ethanol and resolubilization in either acetate-buffere saline (8) or distilled water (17), followed by preparative thin-layer isoelectric focusing (14). Toxin concentrations were measured by the microbiuret protein assay (23).

Animals. Darkly pigmented rabbits weighed 2.0 to 2.5 kg.

Assay for pyrogenicity. Assays for the capacity of PEs to produce fever were performed by the 4-h method of Kim and Watson (8, 22). The dose of PEs used in tests for pyrogenicity was 20 MPD-4/kg and represented 20 μg of protein per kg of staphylococcal PE A and 90 μg per kg of type B toxin. (MPD-4 equaled the dose of PE required to produce a 0.5°C

average fever response in five rabbits 4 h after intravenous injection.)

Immunizations. Animals were sensitized to purified protein derivative (PPD) by subcutaneous injection of 1 ml of an equal parts mixture of phosphate-buffered saline (0.005 M sodium phosphate, 0.15 M NaCl, pH 7.0) and complete adjuvant containing heat-killed *Mycobacterium tuberculosis* (Difco Laboratories, Detroit, Mich.). After 2 weeks, animals showed an average skin test response of 24-mm diameter 24 h after intracutaneous challenge with 250 test units of PPD (Mantoux; Connaught Laboratories, Ltd., Willowdale, Ontario, Canada).

Rabbits were sensitized to staphylococcal PEs by subcutaneous injection of 200 μg of toxin emulsified in 1 ml of incomplete adjuvant (Difco Laboratories).

Sensitization of rabbits to bovine serum albumin (BSA) was accomplished by administering 1 mg of protein subcutaneously emulsified in incomplete adjuvant. Rabbits were considered sensitized when small local Arthus reactions were elicited after intracutaneous challenge with 350 μg of BSA.

Skin testing. Rabbits were skin tested with antigen injected intracutaneously, 0.1 ml per animal in phosphate-buffered saline. Test doses were: PEs, 50 μg; PPD, 250 test units; BSA, 350 μg. Student's *t* test analysis of paired or unpaired normally distributed data was used to assess differences between means of control and experimental skin reaction diameters.

RESULTS

Initial studies were done to assess the capacity of staphylococcal PEs to induce the scarlatiniform rash in rabbits without prior sensitization to the toxins (Table 1). Slight skin reactions were obtained which were pink and somewhat edematous, whether the challenge toxin was staphylococcal PE type A or B. The weak reactivity may reflect prior infection with *S. aureus*.

Animals which were presensitized to either staphylococcal PE type showed significant erythematous and edematous skin reactions after intracutaneous challenge with homologous toxin (Table 1). The reaction diameters obtained in these animals were different from those obtained in nonsensitized animals at the $P < 0.001$ level of significance. The skin reactions in the sensitized animals were comparable in appearance to the enhanced reactivity obtained in a previous study in which group A streptococcal PEs were used as sensitizing and challenge toxins (15). At 3 to 4 days after challenge with the staphylococcal toxins, membranous desquamation around the challenge sites was observed.

When the rabbits were resensitized with staphylococcal PEs and then challenged again with homologous toxin, the average skin reaction of some of the animals was diminished (Table 2). This reduction in skin reactivity in these rabbits corresponded to the development of immunity to the pyrogenicity of the PEs (Fig. 1). The remaining animals again showed significant skin reactivity (Table 2) and also were not immune to the pyrogenicity of the challenge PE (Fig. 1).

Staphylococcal PEs were tested for capacity to enhance hypersensitivity skin reactions developed against unrelated proteins in rabbits. Animals presensitized to PPD showed significant enhanced skin reactivity on one flank when challenged with PPD plus PE (Table 3), compared to the other flank which received PPD alone. Exotoxin alone given to rabbits presensitized to PPD elicited skin reactions comparable to those shown in Table 1 and were subtracted from values obtained after challenge with PPD plus PE.

Staphylococcal PEs also enhanced Arthus skin reactions to BSA (Table 4). Animals actively immunized against BSA showed significantly enhanced skin reactivity when challenged on one flank with BSA plus PEs compared to challenge with BSA alone. Exotoxins alone ad-

TABLE 1. *Skin test reactivity of rabbits with and without prior sensitization to PEs*

PE type		No. of rabbits tested	Skin reaction diam (mm) ± SE[c] after 24 h
Sensitizing[a]	Challenge[b]		
—[d]	Staphylococcal A	5	5 ± 4
Staphylococcal A	Staphylococcal A	5	54 ± 4
—	Staphylococcal B	5	7 ± 6
Staphylococcal B	Staphylococcal B	5	43 ± 3

[a] Animals were presensitized with staphylococcal PEs (200 μg) emulsified in incomplete adjuvant and given subcutaneously 2 weeks before challenge with homologous PE.

[b] Dose of challenge PE, 50 μg administered intracutaneously in 0.1 ml of phosphate-buffered saline.

[c] SE, Standard error.

[d] —, None.

TABLE 2. *Skin reactivity of rabbits presensitized twice to PEs and challenged with homologous toxin*

PE type[a]	No. of rabbits tested	Skin reaction diam (mm) ± SE after 24 h
Staphylococcal A	2	57 ± 2
Staphylococcal A	3	19 ± 4
Staphylococcal B	1	45
Staphylococcal B	4	17 ± 3

[a] Dose, 50 μg administered intracutaneously in 0.1 ml of phosphate-buffered saline. Animals were presensitized with staphylococcal exotoxins (200 μg/injection), emulsified in incomplete adjuvant and given subcutaneously 2 and 4 weeks before challenge with homologous PE.

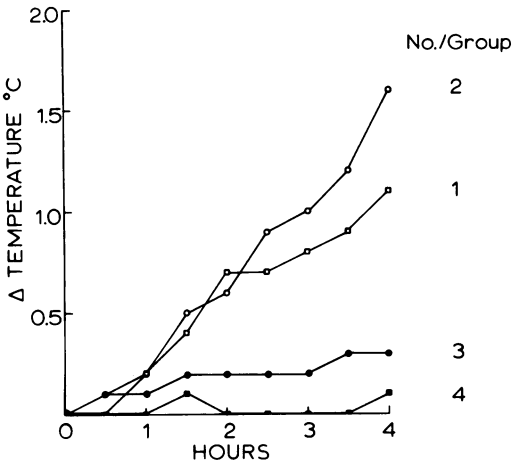

FIG. 1. *Pyrogenicity of staphylococcal PEs in rabbits preimmunized against homologous toxin. Intravenous challenge doses of PEs were: A (○, ●), 20 μg/kg; B (□, ■), 90 μg/kg. Number of rabbits in each group is indicated on the right side of the figure.*

TABLE 3. *Skin reactivity of rabbits to PPD enhanced by PEs*

Skin test material	No. of rabbits tested	Skin reaction diam (mm) ± SE after 24 h	Significance (*P*)[a]
PPD (250 TU)[b]	5	15 ± 3	$P < 0.001$
PPD (250 TU) + staphylococcal A[c]	5	48 ± 3[d]	
PPD (250 TU)	5	17 ± 4	$P < 0.001$
PPD (250 TU) + staphylococcal B[c]	5	42 ± 2[d]	

[a] *P* value was obtained using paired *t* test analysis of reaction diameters.
[b] TU, Test units.
[c] PE dose, 50 μg.
[d] Skin reactivity values obtained when PE was administered to normal rabbits were subtracted from values shown.

ministered to rabbits immunized against BSA produced minimal skin reactions, and again the values were subtracted from those obtained after challenge with BSA plus PE.

Previously, group A streptococcal PEs were shown to contain a common core moiety which was shared between toxin types (3, 8, 9, 15). Delayed hypersensitivity that was developed against the common core of one PE type conferred delayed hypersensitivity on the other PE types, and enhancement of skin reactivity to the common core resulted in the typical scarlatiniform rash (15). Staphylococcal PEs were tested for the presence of the common core related to that of the group A streptococcal PEs (Table 5). Rabbits previously sensitized to either staphylococcal PE type showed significant erythematous and edematous skin reactions after challenge with heterologous staphylococcal or group A streptococcal PEs. After 3 to 4 days, membranous desquamation of the reaction sites was observed. The data indicate that delayed hypersensitivity raised against one PE type conferred hypersensitivity on all other PEs thus far identified.

DISCUSSION

The development of the scarlatiniform rash was previously shown to result from enhancement of acquired hypersensitivity skin reactions by PEs of group A streptococci (8, 9, 15). Thus,

TABLE 4. *Skin reactivity of rabbits to BSA enhanced by PEs*

Skin test material	No. of rabbits tested	Skin reaction diam (mm) ± SE after 24 h
BSA[a]	5	24 ± 3
BSA[a] + staphylococcal A[b]	5	46 ± 3[c]
BSA[a] + staphylococcal B[b]	5	40 ± 4[c]

[a] BSA concentration, 350 μg.
[b] PE dose, 50 μg.
[c] Skin reactivity values obtained when PE was administered to normal rabbits were subtracted from values shown.

TABLE 5. *Skin test cross-reactivity of PEs from S. aureus and group A streptococci*

Sensitizing PE[a]	Challenge PE[b]	No. of rabbits tested	Skin reaction diam (mm) ± SE after 24 h
—[c]	Staphylococcal B	5	6 ± 3
Staphylococcal A	Staphylococcal B	5	39 ± 5
—	Streptococcal A	5	0
Staphylococcal A	Streptococcal A	5	46 ± 3
—	Streptococcal B	5	0
Staphylococcal A	Streptococcal B	5	37 ± 4
—	Streptococcal C	5	0
Staphylococcal A	Streptococcal C	5	42 ± 2
—	Staphylococcal A	5	4 ± 5
Staphylococcal B	Staphylococcal A	5	40 ± 3
—	Streptococcal A	5	0
Staphylococcal B	Streptococcal A	5	36 ± 3
—	Streptococcal B	5	0
Staphylococcal B	Streptococcal B	5	38 ± 1
—	Streptococcal C	5	0
Staphylococcal B	Streptococcal C	5	37 ± 3

[a] Animals were presensitized with PEs (200 μg) emulsified in incomplete adjuvant and given subcutaneously 2 weeks before challenge with heterologous PEs.
[b] Dose, 50 μg administered intracutaneously in 0.1 ml of phosphate-buffered saline.
[c] —, None.

erythrogenic toxin and a positive Dick reaction were defined in terms of two factors: (i) PE in the absence of neutralizing antibody, and (ii) pre-existing delayed or Arthus hypersensitivity developed against a streptococcal antigen which was coadministered with PE. Further, PEs were shown to contain a shared common core moiety, against which delayed hypersensitivity can be developed, as well as serologically specific primary toxic components, which are responsible for enhancing skin reactivity (8, 9, 15). Therefore, once delayed hypersensitivity was developed against one PE type, the scarlet fever rash could be produced by any streptococcal PE type without further sensitization.

In this study, data were presented which indicate that staphylococcal PEs also produce erythematous and edematous rashes by the enhancement of acquired hypersensitivity. This property is consistent with the PEs' being responsible for the clinical signs of staphylococcal scarlet fever and supports a recent proposal that the staphylococcal PEs may contribute to the development of Kawasaki disease and toxic shock syndrome (Schlievert et al., submitted for publication), both of which must be distinguished from classical streptococcal scarlet fever (4, 7, 11, 21). Also, data were presented which demonstrated that all PEs, whether streptococcal or staphylococcal, shared the common core component of the toxins. This observation is significant since it suggests that scarlet fever or related diseases may be produced up to five times, twice by *S. aureus* and three times by group A streptococci, and that prior sensitization with one PE type is sufficient for development of the scarlatiniform rash after the host encounters either the homologous or any heterologous PE. The shared common core of all PE types also is consistent with reports that Dick-positive individuals show positive skin reactions when challenged with staphylococcal filtrates (1, 10). It is noteworthy that few individuals who develop scarlet fever would be expected to have five episodes, each the result of a different toxin type, because people may develop immunity to more than one toxin during an episode since, in many instances, group A streptococci and *S. aureus* strains elaborate multiple toxin types (16; Schlievert et al., submitted for publication).

It is interesting that not all of the rabbits tested in this study developed antibody capable of neutralizing PEs and concomitantly retained significant skin reactivity. Data obtained previously (not shown) indicated that many rabbits were unable to develop antibody capable of neutralizing PE type A even after immunization with toxin every other week for 8 injections and then monthly for a total of 7 months. None of the animals survived longer than the 7-month period. The underlying cause of lack of specific responsiveness is not known, but the phenomenon may explain why approximately 25% of patients with toxic shock syndrome have recurrent episodes (4) and continue to manifest the scarlatiniform rash. Studies are presently under way to assess immune responsiveness in individuals with one, two to three, or numerous episodes of toxic shock syndrome.

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LITERATURE CITED

1. **Aranow, H., Jr., and W. B. Wood, Jr.** 1942. Staphylococcal infection simulating scarlet fever. J. Am. Med. Assoc. **119**:1491-1495.
2. **Barsumian, E. L., C. M. Cunningham, P. M. Schlievert, and D. W. Watson.** 1978. Heterogeneity of group A streptococcal pyrogenic exotoxin type B. Infect. Immun. **20**:512-518.
3. **Barsumian, E. L., P. M. Schlievert, and D. W. Watson.** 1978. Nonspecific and specific immunological mitogenicity by group A streptococcal pyrogenic exotoxins. Infect. Immun. **22**:681-688.
4. **Center for Disease Control.** 1980. Toxic-shock syndrome—United States. Morbid. Mortal. Weekly Rep. **29**:229-230.
5. **Cunningham, C. M., and D. W. Watson.** 1978. Suppression of the antibody response by group A streptococcal pyrogenic exotoxin and characterization of the cells involved. Infect. Immun. **19**:470-476.
6. **Feldman, C. A.** 1962. Staphylococcal scarlet fever. N. Engl. J. Med. **267**:877-878.
7. **Kawasaki, R., F. Kosaki, S. Okawa, I. Shigematsu, and H. Yanagawa.** 1974. A new infantile acute febrile mucocutaneous lymph node syndrome (MLNS) prevailing in Japan. Pediatrics **54**:271-276.
8. **Kim, Y. B., and D. W. Watson.** 1970. A purified group A streptococcal pyrogenic exotoxin. Physicochemical and biological properties including the enhancement of susceptibility of endotoxin lethal shock. J. Exp. Med. **131**:611-628.
9. **Kim, Y. B., and D. W. Watson.** 1972. Streptococcal exotoxins: biological and pathological properties, p. 33-50. *In* L. W. Wannamaker and J. M. Matsen (ed.), Streptococci and streptococcal diseases. Academic Press Inc., New York.
10. **McCloskey, R. V.** 1973. Scarlet fever and necrotizing fasciitis caused by coagulase-positive hemolytic *Staphylococcus aureus*, phage type 85. Ann. Intern. Med. **78**: 85-87.
11. **Melish, M. E., R. M. Hicks, and E. J. Larson.** 1976. Mucocutaneous lymph node syndrome in the United States. Am. J. Dis. Child. **130**:599-607.
12. **Nauciel, C.** 1973. Mitogenic activity of purified streptococcal erythrogenic toxin on lymphocytes. Ann. Immunol. (Paris) **124C**:383-390.
13. **Schlievert, P. M.** 1980. Activation of murine T-suppressor lymphocytes by group A streptococcal and staphylococcal pyrogenic exotoxins. Infect. Immun. **28**:876-880.
14. **Schlievert, P. M., K. M. Bettin, and D. W. Watson.**

1977. Purification and characterization of group A streptococcal pyrogenic exotoxin type C. Infect. Immun. **16:** 673–679.

15. **Schlievert, P. M., K. M. Bettin, and D. W. Watson.** 1979. Reinterpretation of the Dick test: role of group A streptococcal pyrogenic exotoxin. Infect. Immun. **26:** 467–472.
16. **Schlievert, P. M., K. M. Bettin, and D. W. Watson.** 1979. Production of pyrogenic exotoxin by groups of streptococci: association with group A. J. Infect. Dis. **140:**676–681.
17. **Schlievert, P. M., D. J. Schoettle, and P. W. Watson.** 1979. Purification and physicochemical and biological characterization of a staphylococcal pyrogenic exotoxin. Infect. Immun. **23:**609–617.
18. **Schlievert, P. M., D. J. Schoettle, and D. W. Watson.** 1979. Nonspecific T lymphocyte mitogenesis by pyrogenic exotoxins from group A streptococci and *Staphylococcus aureus*. Infect. Immun. **25:**1075–1077.
19. **Schwab, J. H., D. W. Watson, and W. J. Cromartie.** 1955. Further studies of group A streptococcal factors with lethal and cardiotoxic properties. J. Infect. Dis. **96:** 14–18.
20. **Stevens, F. A.** 1927. Occurrence of *Staphylococcus aureus* infection with scarlatiniform rash. J. Am. Med. Assoc. **88:**1957.
21. **Todd, J., M. Fishaut, F. Kapral, and T. Welch.** 1978. Toxic-shock syndrome associated with phage-group I staphylococci. Lancet **ii:**1116–1118.
22. **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.
23. **Zamenhof, S.** 1957. Preparation and assay of deoxyribonucleic acid from animal tissue. Methods Enzymol. **3:** 696–704.