Staphylococcal Scalded Skin Syndrome: Potentiation by Immunosuppression in Mice; Toxin-Mediated Exfoliation in a Healthy Adult

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Staphylococcal scalded skin syndrome, associated with exfoliative toxin produced by phage group II \textit{Staphylococcus aureus}, has recently been reported in an adult receiving immunosuppressive therapy. To determine the effect of immunosuppression on the development of the staphylococcal scalded skin syndrome, experimental animals were treated with prednisolone, azathioprine, or a combination of both drugs utilizing the clinical isolate from the adult with scalded skin syndrome. The mean lethal dose and mean exfoliating dose were identical and were 6,000-fold lower in animals receiving both drugs or azathioprine alone. The isolate was not more virulent and did not produce more toxin than other group II phage-type strains. Furthermore, immunosuppressive therapy failed to enhance the susceptibility of experimental animals to a purified preparation of toxin. Finally, purified exfoliative toxin was demonstrated to produce erythema, Nikolsky's sign, bullous formation, and flaking desquamation in a normal human adult. The results demonstrated the enhanced susceptibility of experimental animals receiving immunosuppressive therapy to the development of the staphylococcal scalded skin syndrome. They further showed that human adults are susceptible to the action of exfoliative toxin and suggested that, in the host with compromised defense mechanisms, toxin-producing strains may invade and initiate infection resulting in toxin production and exfoliation.

Strains of \textit{Staphylococcus aureus} belonging to phage group II have been identified as the etiological agent of the scalded skin syndrome in infants and children. The disease is characterized by intra-epidermal separation with bullae formation and exfoliation of the superficial epidermis. This separation occurs when desmosomes connecting adjacent granular cells are split through the layer of intercellular contact (8). The development of an animal model by Melish and Glasgow (9) made possible the identification and subsequent purification of an exfoliative toxin (11) produced by strains of phage group II \textit{S. aureus} which is responsible for the characteristic lesions of the scalded skin syndrome. These observations have now been confirmed and extended by a number of other laboratories (1, 3–5, 10). Until recently, the scalded skin syndrome, in association with \textit{S. aureus}, has been documented only in children, although a disease similar to this syndrome, Lyell's disease, is recognized in adults. Recently Levine and Norden (7) described bullous exfoliating lesions in a 19-year-old man (R.W.) who had an axillary abscess from which a group II phage type \textit{S. aureus} (type 71) was isolated. He had a history of chronic membrano-proliferative glomerulonephritis and was receiving a regimen of immunosuppressive therapy which included prednisone and azathioprine.

The occurrence of the staphylococcal scalded skin syndrome in a patient on immunosuppressive therapy raised the question of the effect of these drugs on the development of the patient's disease. To define the role of the drug therapy in the pathogenesis of this syndrome, we investigated the effect of such therapy on elicitation of the scalded skin syndrome in our mouse model.

**MATERIALS AND METHODS**

\textit{S. aureus} strain RW, kindly supplied by Levine and Norden (7), was isolated from their adult patient with the scalded skin syndrome. Strains of \textit{S. aureus} designated TG, EV, DM, and JA were originally
isolated from patients with scalded skin syndrome in Salt Lake City, Utah, and Rochester, N.Y.

**Production of partially purified exfoliative toxin.** Strain RW or other staphylococci were grown in vivo by implantation of dialysis sacs containing Eagle minimal essential medium with L-glutamine and sodium bicarbonate into the peritoneal cavity of 500-g white rats. High yields of toxin were obtained when the sacs were harvested on day 7 after implantation. The contents of the sacs were centrifuged at 1,000 × g for 8 min and the supernatant fluids were sterilized by filtration through membrane filters (Millipore Corp.). Saturated aqueous (NH₄)₂SO₄ was added to the filtrates to 80% saturation, and the mixtures were stirred and allowed to stand overnight at 4 C. The precipitate that formed contained the exfoliative toxin and was dialyzed to remove (NH₄)₂SO₄ and concentrated by means of Carbowax 20,000 (Fisher Chemical Co.) to one-third of its original volume.

**Calculation of mean lethal dose (LD₅₀) and mean exfoliating dose (ED₅₀) in the various groups.** The probit method described by Miller and Tainter (12) was used to calculate the LD₅₀ and ED₅₀ along with the standard error and the 95% confidence limits.

**Titration of exfoliative activity.** Two- to three-day-old randomized CD-1 mice were injected intracutaneously in the scapular region with 0.05 ml of various dilutions of toxin in buffered saline, five mice per dilution. One unit was defined as the reciprocal of the highest dilution producing a positive Nikolsky sign in the animals 3 h after injection.

**RESULTS**

Experiments were initiated when suckling mice (Charles River Breeding Laboratories, Wilmington, Mass.) were 2 days of age. Experimental animals were randomized in groups receiving the following regimens by the intraperitoneal route for 6 days: (i) 7.5 μg of prednisolone (2.5 mg/kg); (ii) 300 μg of azathioprine (100 mg/kg); (iii) azathioprine plus prednisolone at the same dosage; and (iv) untreated controls. At 8 days of age, groups of 10 to 15 mice from each of the treatment regimens, as well as untreated controls, were challenged with serial dilutions of the RW isolate grown in heart infusion broth. Eighteen to 24 h after injection of the live staphylococci, the epidermis remained wrinkled after light stroking, resulting in the Nikolsky sign characteristic of the scalded skin syndrome. In these animals, the epidermis could easily be reflected back over the unaffected skin surface.

The LD₅₀ and ED₅₀ of the RW strain were calculated for groups of animals receiving the various treatment schedules. The LD₅₀ and ED₅₀ required approximately the same inoculum size, 10^6-8 organisms of the RW strain (Table 1). Although prednisolone significantly lowered the LD₅₀ and ED₅₀ in mice, 10^5-7 to 10^6-5, azathioprine was most effective in enhancing susceptibility to the RW strain. Only 1/6,000 (10^5-7 versus 10^2-5) as many organisms were required to produce exfoliation in azathioprine-treated animals compared with the untreated controls. In no case did an animal die without also exhibiting a positive Nikolsky sign.

As might be expected, the combined effect of prednisolone and azathioprine treatment was equally effective as azathioprine treatment alone in enhancing susceptibility to the scalded skin syndrome. The addition of prednisolone failed to enhance the susceptibility beyond that of azathioprine alone. Comparison of the ED₅₀ for strain RW with another strain routinely used in our laboratory to produce exfoliation in mice (TG) demonstrated that the RW strain was only slightly more virulent than strain TG (ED₅₀, 10^6.34 versus 10^7.19).

These data strongly suggest that immunosuppressive therapy strikingly enhances susceptibility to the scalded skin syndrome, as evidenced by the reduced number of organisms required to produce the disease in experimental animals. The effect observed in this experiment could be explained by: (i) decreased host resistance to the organism, thus permitting a lower inoculum of organisms to multiply and produce the toxin which would result in the formation of bullae, or, alternatively, (ii) increased sensitivity of the host to the action of the exfoliative toxin. To evaluate these possibilities, normal and immunosuppressed animals receiving therapy according to the previous protocol were inoculated with a partially purified preparation of exfoliative toxin. No difference could be detected in the immunosuppressed animals' sensitivity to the toxin. These results indicate that the increased susceptibility observed in the initial experiments was most likely due to

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Strain of Staphylococcus</th>
<th>LD₅₀ and ED₅₀</th>
<th>95% Confidence limits</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>RW</td>
<td>6.34</td>
<td>6.06-6.51</td>
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<tr>
<td>Prednisolone</td>
<td>RW</td>
<td>5.00</td>
<td>4.38-5.25</td>
</tr>
<tr>
<td>Azathioprine</td>
<td>RW</td>
<td>2.57</td>
<td>2.22-2.76</td>
</tr>
<tr>
<td>Prednisolone and azathioprine</td>
<td>RW</td>
<td>2.32</td>
<td>1.88-2.54</td>
</tr>
<tr>
<td>None</td>
<td>TG</td>
<td>7.18</td>
<td>6.59-7.42</td>
</tr>
</tbody>
</table>

* Log to the base 10 of the number of organisms required to produce a 50% kill or exfoliation in CD-1 mice.
decreased host resistance to the organism rather than to alteration of the host response to the exfoliative toxin.

To test the possibility that strain RW may have been able to produce exfoliation in an adult human because it elaborated greater quantities of exfoliative toxin than other strains, a number of clinical isolates of phage group II staphylococci were inoculated into dialysis sacs and implanted into the peritoneal cavity of 500-g albino rats. After 7 days, the sacs were removed and the contents were filter-sterilized after centrifugation to remove organisms. The results of titrations of the exfoliative activity present in the dialysate are summarized in Table 2. Of the seven strains tested, RW, Davis, and TG all yielded highly potent filtrates, whereas the other strains were less potent producers of the toxin under these conditions. Strain DM, although isolated from a child with generalized erythema followed by exfoliation, produced approximately one-tenth of the amount of toxin produced by the other strains examined. These data indicate that, although the RW strain is a high toxin producer, it does not appear to have a significantly greater capacity for toxin production than a number of other strains which were previously isolated from infants or children with the scalded skin syndrome.

In view of the fact that mice developed resistance with age, the possibility existed that the older human is also less susceptible to the action of exfoliative toxin. During the course of these experiments, one of the authors (S. A.) elected to test the capacity of the toxin to produce exfoliation in the human adult by inoculating his own forearm with 100 suckling mouse exfoliating units of purified electrofocused exfoliative toxin (11). The injection site began to itch immediately after inoculation by the intradermal route. Within 1 h, a positive Nikolsky sign could be elicited. By 24 h, a bullous lesion surrounded by an erythematous and edematous area approximately 10 cm in diameter was evident at the injection site. The fluid from the bulla contained inflammatory cells, but no organisms were present. The fluid harvested from this lesion was inoculated into 2-day-old mice to determine whether exfoliative toxin could be demonstrated. No exfoliation was observed in these animals. If toxin was present, it was not in sufficient quantity to be detectable by the current biological assay. The toxin appeared to spread via the lymphatic channels, and red streaks were visible up the arm. By 72 h after inoculation, the lesion had reached maximal size and, by day 4, had begun to heal. Thick, dry, seborrhea-like flakes were observed over the involved area. Healing occurred without scarring. The sequence of the exfoliative reaction is illustrated in Fig. 1 to 3.

These data indicate that human adults are susceptible to the action of the exfoliative toxin produced by group II phage-type staphylococci.

**DISCUSSION**

The development of the scalded skin syndrome associated with group II phage-type *S. aureus* is apparently a relatively rare occurrence in adults. Zak et al. (13) and Bailey and co-workers (2) described cases of scalded skin syndrome in adults in 1964 and 1965, but drug reactions were postulated as the cause of the lesions. The data presented in this report clearly indicate that the adult human is susceptible to the action of the exfoliative toxin. The patient, R. W., reported by Levine and Norden (7) was receiving immunosuppressive therapy. Although the patient developed circulating anti-

**Table 2. Titters of exfoliative toxin of various phage group II staphylococcal isolates**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Units of exfoliative toxin per ml of crude toxin*</th>
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<tbody>
<tr>
<td>RW</td>
<td>20,000</td>
</tr>
<tr>
<td>Davis</td>
<td>20,000</td>
</tr>
<tr>
<td>TG</td>
<td>20,000</td>
</tr>
<tr>
<td>EV</td>
<td>12,000</td>
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<td>MJ</td>
<td>10,000</td>
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<tr>
<td>TA</td>
<td>16,000</td>
</tr>
<tr>
<td>DM</td>
<td>2,000</td>
</tr>
</tbody>
</table>

*Figures were obtained by ascertaining the highest dilution of toxin producing exfoliation in 50% of CD-1 mice and multiplying dilution by 20 to convert to units per milliliter.

**Fig. 1. Appearance of the injection site in an adult human 1 h after intradermal administration of 100 units (mouse) of purified exfoliative toxin. Lesion was erythematous, edematous, and pruritic.**
This concept is further supported by the evidence that the RW strain produced similar quantities of toxin as a number of other strains isolated from infants or children with scalded skin syndrome. The determination of the number of organisms of the RW strain required to produce exfoliation in normal animals suggests that the strain was not more virulent, at least in the newborn mice, than other clinical isolates from infants and children.

The age limitation in the animal model has also been shown to be variable. Although the exfoliative activity could only be demonstrated by Melish and Glasgow (9) in mice less than 6 days of age, Kapral and Miller (6) have recently shown that adult hairless mice develop exfoliation on inoculation with preparations of the exfoliative toxin. Finally, the lack of effect of suppressive therapy on the susceptibility of experimental animals to a partially purified preparation of toxin strongly indicates that the increased susceptibility manifested by the immunosuppressed animals was not a result of increased sensitivity to the action of the toxin.

In summary, the experimental data from our animal studies suggest that immunosuppressive therapy increases susceptibility not by altering the effect of the toxin on the site of action, but by decreasing host resistance permitting a Tox+ strain of phage group II S. aureus to initiate a focus of infection and produce sufficient toxin to result in formation of bullae. It seems unlikely that this represents a unique effect on the pathogenesis of this disease; rather, it seems to reflect a generally compromised level of host resistance.

Although the evidence in humans is based on the single observation of the susceptibility to the exfoliative toxin of an adult male (without underlying disease and on no medications), it clearly demonstrates the capability of the exfoliative toxin produced by staphylococci to cause exfoliation in human adults. These data further suggest that the rare occurrence of the staphylococcal scalded skin syndrome in adults may be related to the presence of neutralizing antibodies against the exfoliative toxin in a majority of the adult population. Studies are currently in progress to test this hypothesis.

ACKNOWLEDGMENT

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LITERATURE CITED