Clinical, Microbial, and Biochemical Aspects of the Exfoliative Toxins Causing Staphylococcal Scalded-Skin Syndrome

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INTRODUCTION

Staphylococcal scalded skin syndrome (SSSS) is the term used for a collection of blistering skin diseases induced by the exfoliative (epidermolytic) toxins (ETs) of Staphylococcus aureus. It primarily affects neonates and young children (Fig. 1), although adults with underlying diseases are also susceptible (Fig. 2). The severity of SSSS varies from a few blisters in localized SSSS to severe exfoliation affecting almost the whole body surface in generalized SSSS. Because of the relative rarity of the disease and ease of treatment, SSSS has not received as much attention as it deserves by either clinicians or researchers. Staphylococcal infections are on the increase in all age groups worldwide, and show an increasing resistance to conventional antibiotics; despite the availability of a wide range of antibiotics, these infections still carry a significant morbidity and mortality, particularly among adults. Furthermore, although the condition was described over a century ago, our understanding of it began only 25 years ago, when the toxins were first discovered. Even now, their mechanism of action is still not certain. However, recent exciting data from computer modelling and three-dimensional crystallography of the toxins has provided us with a clearer and more defined approach to understanding the pathologic processes of the disease. Elucidating the mechanism of action of the toxins holds exciting prospects for understanding the normal physiology of the skin, targeting drugs to very specific regions of the skin, and developing antitoxins and toxoids that may soon play a vital role in the treatment and prevention of SSSS. The aim of this review is to provide both the clinician and the researcher with our current understanding—from sources worldwide and spanning more than a century—of the epidemiology, clinical features, diagnosis, differential diagnoses, investigations, treatment and prevention of the disease. The review also discusses the organisms responsible for SSSS, probable mechanisms of action of the ETs, and their potential role in diseases other than SSSS. It is hoped that the information provided will (i) help the clinician to make a correct diagnosis of SSSS by using the available routine investigations and to manage the disease appropriately, (ii) discourage repetitive research by bringing researchers up to date with the current knowledge of the disease, and (iii) encourage collaboration between various research groups working on SSSS around the world.

HISTORICAL PERSPECTIVES

The clinical features of SSSS were first described over a century ago by a German physician, Baron Gottfried Ritter von Rittershain, who observed 297 such cases among young children in a 10-year period while working in a Czechoslovakian foundling asylum; he called it dermatitis exfoliativa neonatorum but was unable to determine its cause (275). The term “Ritter’s disease,” named after its describer, is still used when generalized SSSS occurs in neonates. “Pemphigus neonatorum” describes a milder, self-limiting disease of infants that produces a few blisters (162). S. aureus was first isolated in 1891 from a patient with pemphigus neonatorum by Almquist (7), who called the organism Micrococcus pemphigi neonatorum, and then by Winternitz in 1898 (281).
FIG. 1. Generalized SSSS in a previously well neonate. The neonate (A and B) shows the characteristic well-demarcated erythematous superficial exfoliation with areas of skin sparing. Courtesy of J. Etienne, Faculte de Medecine RTH Laennec, Laboratoire de Bacteriologie, Centre National de Reference Des Toxemies a Staphylocoques, France.
The disease received little attention until 1956, when Lyell described a skin eruption that resembled scalded skin and called it toxic epidermal necrolysis (TEN) (159). He speculated that the condition might be due to a circulating toxin that damages the skin and causes necrosis, although he was not aware that he was describing two distinct, unrelated conditions. In 1967, however, Lyell reviewed 128 cases that fitted his diagnostic criteria of TEN and found that almost one-quarter—all children under 10 years of age—presented predominantly with a staphylococcal infection (160). A link between staphylococcal infection in children and exfoliation was also observed by Lowney et al. (158).

It soon became apparent that the exfoliation associated with *S. aureus* infection occurred specifically within the midepidermis whereas exfoliation that was not associated with staphylococcal infection occurred at the dermoepidermal junction (158, 161). As a result, the former condition was termed SSSS and the latter was termed TEN. The etiology and pathogenesis of SSSS are very different from those of TEN, and although they may be clinically difficult to distinguish, correct diagnosis is essential because the treatment for the two conditions is different (236).

By this time, *S. aureus* had also been implicated in bullous impetigo (60, 200), staphylococcal scarlatiniform rash (74, 253), and outbreaks of Ritter's disease (30, 200, 221). While a circulating staphylococcal exotoxin was widely accepted to cause SSSS (158, 229), it was not until the elegant demonstration by Melish and Glasgow that injection of newborn mice with group II staphylococci isolated from patients with localized SSSS such as bullous impetigo or from patients with generalized SSSS resulted in exfoliation that SSSS was appropriately termed (170).

**FIG. 2. Generalized SSSS in a 77-year-old adult with renal impairment. The photograph shows extensive exfoliation of the trunk and arm. Although the condition is rare in adults, the observed lesions are similar to those in neonates. Reprinted from reference 103 with permission of the publisher.**

**CLINICAL FEATURES**

SSSS is primarily a disease of neonates and children (48, 96, 174), and white children may be more prone to SSSS than black children, according to a study of 75 cases published more than 2 decades ago (211). The clinical features of SSSS vary from localized blisters to severe exfoliation affecting over 90% of the entire body surface. In localized forms of SSSS, also known as bullous impetigo, tropical bullous impetigo, measles pemphigoid (162), and bullous varicella (174), characteristic fragile, thin-roofed, flaccid bullae are formed, which rupture easily to release fluid that varies from a thin, cloudy, amber liquid to purulent, opaque, white or yellow pus (174). This is the mildest form of the disease, in which the surrounding skin remains normal and there are no systemic symptoms or signs (100). In neonates, the lesions are found mostly on the perineum, periumbilical area, or both, while in older children, the lesions are found most often on the extremities (174).

In the generalized forms of SSSS, widespread involvement of the entire skin surface can occur but the mucous membranes are usually spared (172, 208). The disease usually follows a localized infection of the upper respiratory tract, inner ear, conjunctiva, or umbilical stump (24), although rare cases of SSSS caused by staphylococci isolated from patients with pneumonia, septic arthritis (174), pyomyositis (284), and maternal breast abscesses (213) have been reported. In adults, in whom isolated cases tend to occur, bacteria are reported to enter via catheterization, abscesses, septic arthritis, infection of arteriovenous shunts, and parenteral infections, although in many cases, no primary localized staphylococcal infection is found (48). In neonates, the age of onset is usually between 3 and 16 days, and only one case of SSSS has been reported to occur within 24 h of birth, where a term infant, delivered by emergency cesarian section to a 20-year-old woman who presented with clinical signs of septicemia, developed SSSS. *S. aureus* was isolated from the baby’s blood and urine (157). Patients with generalized SSSS initially develop fever, malaise, and lethargy, with poor feeding and irritability, followed by a generalized, tender erythematous rash, which generally begins on the head and neck and spreads to the rest of the body within a few days (110, 114, 193). The rash is more marked in flexural creases. Soon thereafter, large, fragile, thinroofed blisters appear, which rapidly
rupture on the slightest pressure (with a positive Nikolsky sign), resulting in large sheets of epidermis sloughing off to leave extensive areas of raw, denuded, varnish-like skin. These patients have poor temperature control, can lose extensive amounts of fluids, and may develop secondary infection; such secondary complications of epidermal loss significantly contribute to mortality in neonates and young children (110, 135). They may also develop primary staphylococcal or secondary sepsis and may present with hypotension, tachycardia, neutropenia, and/or respiratory distress (78, 157).

Staphylococcal scarlet fever, also called scarlatiniform erythroderma/rash, was first described in the 1920s and until recently was generally considered to be a milder or abortive form of SSSS (24, 172, 174, 266), although very little literature exists to confirm this association. Patients usually develop a generalized tender erythroderma with a roughened, sandpaper-like texture, associated with fever. This is followed by thick flakes developing within a few days and the entire skin desquamating over the next week (174). Unlike generalized SSSS, the scarlatiniform eruption is not associated with the formation of bullae or exfoliation and is very difficult to differentiate from other infectious erythrodermal causes such as toxic shock syndrome (TSS) and streptococcal scarlet fever. TSS is a multi-systemic disease, caused by staphylococcal toxic shock syndrome toxin 1 (TSST-1) and other superantigens, that may rapidly progress to death (242). It occurs mainly in menstruating women and is associated with tampon use, although it has been found in nonmenstruating women, postmenopausal women, children, and men (79, 90). Recently, Lina et al. (154) analyzed 60 strains of S. aureus isolated from children with suspected SSSS. They demonstrated that although the strains isolated from patients with localized or generalized exfoliation produced the ETs and caused a positive Nikolsky sign when injected into neonatal mice, only 1 of the 17 strains (6%) isolated from patients with staphylococcal scarlet fever produced ET and the other 16 produced TSST-1, enterotoxins, or both. Therefore, it is likely that most cases of staphylococcal scarlet fever are a clinical manifestation of mild TSS, with the enterotoxins (40) occasionally mimicking this condition. The few cases of staphylococcal scarlet fever caused by the ETs that Lina et al. (154) have reported may be a mild form of SSSS previously described by Melish et al. (172), where a transient positive Nikolsky sign can be demonstrated in most patients. It should also be noted that TSS occasionally coexists with SSSS (42, 269).

**STAPHYLOCOCCUS AUREUS**

*S. aureus* remains one of the most common causes of community- and hospital-acquired localized and systemic infections; indeed, large epidemiological studies, including the National Nosocomial Infections Surveillance in the United States, consistently show that the incidence of *S. aureus* infections has been increasing steadily over the past few decades in all age groups (20, 206), including neonates (122). Despite the recent explosion in antibiotic development, *S. aureus* infections still carry a significantly high morbidity and mortality in children and adults (122, 141, 169). Isolates of *S. aureus* are able to produce a range of more than 30 different extracellular proteins, most of which play a direct role in pathogenicity or enhance virulence (112). *S. aureus* is probably the most frequently implicated organism in bacterial illness, although the mechanisms by which infection is established is not always known (5).

**Carriers of *S. aureus***

Nasal carriage of *S. aureus* occurs in 35% of the normal population (54, 191) but varies according to age (19) and race (176). Carriage rates of *S. aureus* are higher in certain sub-populations such as patients suffering from atopic dermatitis (8, 152), contact dermatitis (190), psoriasis (164), and cutaneous T-cell lymphoma (115). These carriers can be responsible for SSSS outbreaks (108). In neonates, organisms are also common on the skin, eyes, perineum (51), wound sites (194), circumcision wounds (11), and umbilical stumps (282). The hospital remains one of the common sources of *S. aureus*, mainly due to inadequate adherence to infection control practices, such as barrier nursing care, hand-washing, and cleaning stethoscopes between patients (123, 155). About 30% of neonates are colonized by *S. aureus* strains within a week of birth (51, 183, 241), although some studies have shown carriage rates of 60 to 90% of newborn babies discharged from hospitals, particularly in areas where the use of antiseptic umbilical cord care is discouraged and during staphylococcal epidemics (105, 239, 240). In hospitals, neonatal colonization is more likely to originate from nursery attendants than mothers (51, 183, 241), who, in turn, are more likely to be responsible for outbreaks.

A study published more than two decades ago showed that ventilation shafts and other inanimate objects such as stethoscope bells, ophthalmoscope and otoscope handles, laundry carts, and nursery magazines in hospitals may also harbour ET-producing strains and were responsible for prolonged epidemics of staphylococcal infections in neonatal nurseries (123). More recently, a Nigerian study revealed that healthy animals may also be carriers of ET-producing *S. aureus* strains, with 3.9% of animal *S. aureus* strains producing ETs (4). However, their importance as reservoirs of SSSS is not known.

**Outbreaks of SSSS**

Occasional isolated cases of SSSS can occur after organisms colonize the infant from the maternal vaginal flora acquired during delivery (157). There has been one recent reported case of a breast-fed infant developing SSSS from *S. aureus* that possibly originated from the mother’s breast abscess (213). However, more commonly, as a consequence of cross-infection, SSSS infections tend to occur in outbreak clusters (29, 51, 52). During the 1980s, several papers were published showing that the use of antiseptics such as chlorhexidine on neonatal umbilical stumps delayed the time to cord separation (26, 142, 227, 272). Because of the resulting increase in community midwives’ workload due to the delay in cord separation, as well as rare isolated cases of hexachlorophane toxicity causing spongiform myelinopathy after percutaneous absorption (10, 237), hospitals have gradually stopped using antiseptic umbilical cord care. This move has occurred despite previous studies demonstrating that the use of antiseptics for cord care is essential for preventing staphylococcal infection and cross-infection in hospitals (47, 244).

As a consequence of stopping antiseptic cord care, many clinicians have observed an increase in the rate of staphylococcal colonization and infections among neonates (6, 251, 276). Furthermore, several studies have demonstrated that re-implementation of antiseptic cord care resulted in a decrease in neonatal staphylococcal colonization and infections (168, 239, 240, 246). Therefore, many clinicians are very concerned that if hospitals do not start re-implementing the antiseptic cord care policy, the number of neonatal staphylococcal infections is likely to increase.

Over the decades, many outbreaks have been reported, often with dozens of babies affected over several months and...
usually due to several asymptomatic carriers of ET-producing S. aureus strains (49, 51, 52, 55, 95). Nursery-associated outbreaks usually manifest themselves during the first month, after the baby has been discharged from hospital (29, 174). Along with the recent pressure to increase day surgery and encourage early discharges from hospitals, outbreaks such as those associated with SSSS are being dissipated into the community, where infections are often treated by the primary health care team—an observation made almost two decades ago (174). As a result, the hospital carrier of the offending organism, usually nursery attendants in close contact with the infants, such as midwives and pediatricians (29, 51, 52, 55, 95), will continue to spread the infection over months until the outbreak is acknowledged and the carrier is removed from work. Recently, for example, four babies aged 6 to 8 days were found to have blistering rashes characteristic of SSSS during a 6-week period. An inquiry and retrospective case finding revealed that 36 babies had been infected with an ET-producing strain of S. aureus. Swabs from suspect health care personnel revealed the source, a pediatrician who was suspended from duty until shown to be free of staphylococcal colonization (29).

**Exfoliative Toxin-Producing S. aureus Strains**

Most S. aureus strains giving rise to SSSS in Europe and the United States belong to phage group II, particularly types 71 and 55/71 (50, 170, 199), which account for 7 to 25% of human S. aureus strains isolated in the United Kingdom (54). In Japan, most strains causing SSSS belong to phage groups other than II (132, 186, 230). The only study published on the ETs in Africa revealed that almost 60% of toxin-producing staphylococcal strains were nontypeable, with phage group II strains accounting for only 18% (4). It should be noted that only 31 to 40% of phage group II S. aureus strains produce ETs (85, 128, 279).

Unfortunately, due to the lack of a suitable and standardized in vitro assay, there is very little literature available on the epidemiology of ET-producing S. aureus strains. One prospective study of 944 S. aureus isolates from 577 dermatological patients of all age groups revealed a 5.1% carriage rate of ET-producing strains, although only 32% of these patients presented with any symptoms of SSSS, indicating that a large proportion were simply asymptomatic carriers (71). Another epidemiological survey revealed that 164 (33%) of 500 pregnant women attending an antenatal clinic carried S. aureus in the nose, axilla, and/or perineum. Of the 184 S. aureus strains isolated, 5 produced ET as determined by the neonatal-mouse assay (54). Other studies reported toxin production in 6% of 2,632 S. aureus strains isolated from hospitalized patients of all ages (203), 4.4% of 194 strains isolated from human diarrhea and wounds (4), 3.9% of 666 strains isolated from apparently healthy animals (4), 19% of 100 S. aureus strains isolated from the mouths of children with dental disease (179), and 40% of S. aureus isolated from superficial skin infections (17).

**EXFOLIATIVE TOXINS**

Although the presence of a soluble toxin in the pathogenesis of SSSS was the subject of speculation as far back as the 1950s (159), the first clue of its existence came only after Melish and Glasgow showed that sterile fluid obtained from intact bullae and from phage group II S. aureus culture medium was able to produce a positive Nikolsky sign in neonatal mice (170). Even intraperitoneal inoculation resulted in exfoliation, indicating that the toxin had its specific target in the skin. Within a year, the active component in the supernatant was isolated, purified, characterized, termed exfoliatin (125), and confirmed by others (13, 171). Soon thereafter, it was realized that at least two different ET serotypes existed (131). The second serotype was isolated and characterized as a heat-labile toxin that has a similar molecular weight to the original toxin. This second ET serotype was able to elicit a positive Nikolsky sign in the neonatal-mouse bioassay; the discoverers termed this toxin ETB (132).

**Serootypes**

At present, at least two serologically distinct toxins, ETA and ETB, can cause disease in humans and are produced by a small but significant proportion of S. aureus strains (205). Although they differ in some physicochemical properties, both toxins produce the dermatological effects described above in neonatal mice. The toxins are species specific, affecting humans, monkeys, mice, and hamsters but not rats, rabbits, dogs, hedgehogs, voles, guinea pigs, chickens, or frogs (24, 68, 125). Both toxins have been identified and fully sequenced; they have 40% sequence homology (21, 145, 196). ETA and ETB consist of 242 and 246 amino acids, respectively, with molecular masses of 26,950 and 27,274 kDa (145). The gene for ETA is chromosomal, while that for ETB is plasmid located (91); both genes have been fully sequenced and cloned into plasmid vectors for protein expression in Escherichia coli (116, 224). ETA is heat stable and retains its exfoliative activity even after being heated at 60°C for 30 min (112, 125). Initial work with EDTA, a metal ion chelator, suggested that ETA but not ETB required a metal ion for activity (113). However, this is now uncertain. Comparison of the primary amino acid sequences revealed that amino acid residues 93 to 107 of ETA were significantly homologous to a region of rat intestinal and placental calcium-binding protein and supported the evidence that ETA but not ETB may require calcium for activity (53). On the other hand, calcium-binding motifs have not been identified in the two recently determined crystallographic structures of ETA (44, 270) (see below).

The prevalence of different ET serotypes shows a significant geographical distribution. In the United Kingdom and Ireland, 32% of 116 S. aureus isolates from patients with suspected SSSS produced ETA, 27% produced both ETA and ETB, and 12% produced ETB only, with neither toxin being detected in 34 strains (56). A large epidemiological study of dermatological patients in Germany reported that 98% of the ET-producing strains produced ETA (71). A French study found that most cases of childhood SSSS were associated with ETA alone (89%) and that only a few were due to both ETA and ETB (8%) or ETB alone (4%) (203, 279). Similarly, a Nigerian study reported that ETA accounted for 91.1% of the 34 toxin-producing strains isolated from a range of human and animal sources (4). In the United States, 40% of S. aureus isolates from SSSS patients produced ETA, 36% produced both, and 16% produced ETB only (174). On the other hand, ETB seems to be the predominant toxin in Japan, with two studies reporting ETB, ETA and ETB, and ETA production rates of 52, 23, and 25% of 61 strains (186) and 35, 37, and 21% of 43 strains isolated from patients with SSSS (133), respectively.

Two other serologically different staphylococcal ETs that affect animals have also been reported (232, 233, 263). Staphylococcus hyicus is known to cause exudative epidermitis in piglets younger than 1 month; the infection is characterized by exudation, exfoliation, and vesicle formation with skin erosion (121, 144). The responsible toxin, termed S. hyicus exfoliative toxin (ShET), has been isolated and purified from culture supernatants of S. hyicus recently (232). This toxin, a monomer...
with a molecular mass of 27 kDa, does not cross-react with ETA or ETB in immunodiffusion tests and causes exfoliation in piglets less than 1 month old and in 1-day-old chickens but not in older pigs, 15-day-old chickens, mice, or guinea pigs (263). While still in its infancy, work on ShET has already shed light on possible receptors for the ETs (see below) and may provide a more acceptable model for studying the mechanism of action of the toxins. A serologically different ET isolated and purified from a horse strain of \textit{S. aureus} was termed ETC by the authors on the basis of its similarities to the human ETs (233). ETC is a heat-labile, 27,000-kDa toxin that can produce intraepidermal splitting in both neonatal mice and chickens. However, the role of these toxins in human SSSS is still to be determined.

**Site of Action**

Most of the work on ETs has been done with ETA and the neonatal-mouse model. The toxins are made during the post-exponential phase of bacterial growth (24) and excreted from colonizing staphylococci before being absorbed into the systemic circulation (173). The toxins reach the zona granulosa of the epidermis by diffusing through dermal capillaries. Work with \textsuperscript{125}I-labelled ET injected into neonatal mice has shown that apart from the skin, there is no significant binding of the toxins to any other body organ (94).

Histological studies have shown that addition of ETs to confluent keratinocyte cultures isolated from pieces of human skin obtained from plastic surgery or mouse organotypic skin cultures results in the disappearance of small vesicles that are usually present between the cells (153, 166); this is followed by formation of intercellular fluid-filled gaps in the granulosa-spinosum interface, which eventually leads to the characteristic midepidermal splitting seen in SSSS (Fig. 3). Furthermore, the biological actions of ETA and ETB are histologically indistinguishable (97). Cytolysis or necrolysis does not occur; an inflammatory response or cell degeneration has not been observed in the neighboring region (58, 68, 109, 285). Although \textit{S. aureus} is sometimes seen in localized forms of SSSS, it is only rarely seen in generalized SSSS (27).

Recently, immunoperoxidase staining on histological specimens by Gentilhomme et al. (97) revealed that cells that bordered both the upper and lower edges of the cleavage site caused by the ET were positive for keratohyalin granules. Electron microscopy of ferritin-labelled toxin and light microscopy of fluorescein thiocarbamyl toxin were previously used to show that the toxin bound intensely to keratohyalin granules in the stratum granulosum of the epidermis and also that ETB had a particularly high affinity toward profilaggrin (360 kDa) and filaggrin (30 kDa) in epidermal extracts (247, 248). However, there is no evidence suggesting that the toxins have an intracellular mechanism of action. Profilaggrin is synthesized during the pathway of keratinocyte development and incorporated into keratohyalin granules, where it is hydrolyzed to intermediate filaggrins and then to amino acids in the stratum corneum (24). The authors speculated that ET binding to these intracellular granular proteins may lead to disruption of intercellular cohesion because the keratohyalin granules are linked to the keratin intermediate filaments, which extend to desmosomes at the cell surface. These observations fitted well with previous electron micrographic observations suggesting that desmosomes, which link adjacent cells, were disrupted (172). However, several histological studies have demonstrated that even though intercellular edema can be demonstrated, the desmosomes are initially intact and desmosomal disruption is a secondary event, occurring only as the edema progresses (70, 109, 153). Along with the above observation, some authors (62, 285) also noted that adding the toxin to mouse epidermis resulted in the development of intracytoplasmic clearing along the cell membrane at the granulosa-spinosum interface, followed by disappearance of the cell membranes. It was speculated that the toxins may act by weakening cell membranes and making them more fragile and that the desmosome disruption
was a secondary event (62). Therefore, the role of the desmosome in the epidermolysis seen in SSSS is still unclear, and it is not likely to be the primary site of action of the toxins.

A more promising primary site of toxin action may be the putative receptor first identified in mice (223). Water-insoluble sodium dodecyl sulfate fractions of neonatal and adult mouse epidermal extracts containing various gangliosides were incubated with ETA for 3 h and then subcutaneously injected into neonatal mice. ETA incubated with neonatal (but not adult) mouse epidermis fractions was unable to induce exfoliation, indicating that an ET-inhibitory substance may be present in neonatal epidermis (223). This ability was not lost after trypsin or pronase digestion, heating at 100°C for 30 min, or neuraminidase treatment, thereby suggesting that the receptor was not a protein but possibly an amino sugar. The presence of the receptor in neonatal but not adult epidermis may explain the increased susceptibility of neonates to SSSS, and the authors speculated that the identified receptor may be masked or changed as the neonate evolves into an adult (223).

More recently, ETA and ETB were shown to bind GM4-like glycolipid extracted from the skin of suckling mice (264). Furthermore, ShET from S. hyicus bound to skin extracted from 1-day-old chickens but not to skin extracted from adult chickens. Exfoliation in the respective neonatal animal models was abolished when the ETs and ShET were preincubated with GM4-like, but not GM1-, GM2-, or GM3-like, glycolipids from the skin of 1-day-old chickens and sucking mice, respectively. Their results strongly suggest that the GM4-like glycolipids that are present in susceptible neonatal but not adult or non-susceptible animals are possible receptors for the toxins and would account for the age and species specificity of the toxins. Furthermore, the majority of adults who develop SSSS have an underlying disease which may alter the normal physiology of adult skin, making it susceptible to exfoliative toxins (48). Inflammation, for example, will induce the expression of major histocompatibility complex (MHC) class II molecules on the surface of keratinocytes (245). However, despite these recent successes, three decades of intensive research have been unable to elucidate the exact mechanisms by which the toxins induce exfoliation.

**Putative Mechanism of Action**

Initial attempts to determine the mechanisms involved in exfoliation were made by studying the enzyme pattern of S. aureus isolated from clinical cases of localized and extensive SSSS. It was initially suggested that the potent dermonecrotic effect of delta-hemolysin may be responsible, but this theory was soon rejected after the discovery of the exfoliative toxins (96). Histological studies on the epidermal effects of ETs led to speculations that the initial appearance followed by the disappearance of intercellular vesicles may be due to the release of proteolytic lysosomal enzymes by nearby cells (153). Lysosome-like lamellar bodies inside adjacent cells are known to release lipids and enzymes into the surrounding extracellular space (92, 102); proteases will produce a similar nonspecific disruption of the extracellular matrix to some extent (24, 252).

Subsequent histochemical studies did not identify any difference between enzyme reactions in ET-injected, saline-injected, and uninjected specimens of mouse epidermis (62). Native ETA was also shown to have no intrinsic arylsulfatase, β-galactosidase, or cathepsin activity (these enzymes are normally present in lysosomes) (278). The toxins did not have any enzymatic activity toward known nonspecific substrates, including casein (134, 218), and using sensitivity plates (15, 21), and their activity was unaffected by any of a wide range of metabolic inhibitors tested (24, 249). Even attempts at crystallizing either ETA (289) or ETB (180) to determine their three-dimensional structure, which might shed light on their mechanism of action, met with little success because of difficulty in obtaining stable crystals.

Takuchi et al. (262) were the first group to provide evidence that ETs may act as proteases. Epidermis from 1- to 3-day-old mice (jla-ddY) was incubated with 200 μg of partially purified ET, ET boiled for 40 min at 100°C, epidermis alone, or ET alone as negative controls. The mixtures were incubated at 37°C for 12 h, and the supernatant was then tested for caseinolytic activity after removing the epidermis by centrifuging at 1,000 × g for 15 min. Incubation of ET with epidermis induced a fourfold increase in caseinolytic activity compared to the three other negative controls; this activity was inhibited by α-2-macroglobulin, indicating the activation of proteases or inhibition of protease inhibitors in the epidermis. Contamination with other proteases is unlikely, because ETA or epidermis alone did not demonstrate any caseinolytic activity. However, the authors did not identify the protease in the supernatant or provide any evidence supporting that ETA was the protease induced.

A major step forward followed after both ETs were shown to have significant primary amino acid sequence homology (25%) to the staphylococcal V8 protease, particularly in the region containing the protease active site (53). V8 protease is a member of the trypsin-like serine protease family; it has an unusual specificity for cleaving the carboxyl-terminal side of acidic amino acid residues and does not possess any disulfide bridges (64). The active site in question consists of a catalytic triad of serine, histidine, and aspartic acid and forms the active site of all known eukaryotic trypsin-like serine proteases. The high degree of sequence conservation around these regions was speculated to preserve the active site of possible protease activity of the toxins. It was also shown that phenylmethylsulfonyl fluoride (PMSF), a specific serine protease inhibitor, was able to delay exfoliation by the ETA in neonatal mice; however, inhibition was not achieved because higher doses of PMSF were lethal to the animals (53).

At the same time, Bailey and Smith (22) observed that radiolabelled diisopropyl phosphorofluoridate (i2Pr2-P-F), which has the ability to covalently modify the active site of known serine proteases such as V8 protease and chymotrypsin, was also able to label the Ser-195 of the putative active site of ETA, supporting the theory proposed by Dancer et al. that the ETs may act as serine proteases. However, it should be noted that i2Pr2-P-F could label only 6% of the total ETA (22), suggesting that the active site of the toxin was not easily accessible to i2Pr2-P-F.

Soon thereafter, several papers appeared which further supported this hypothesis. Two groups independently reported that site-directed replacement of the Ser-195 active site of the catalytic triad of ETA resulted in a complete loss of biological activity when excess amounts were injected into newborn mice (210, 214).

Redpath et al. used oligonucleotide site-directed mutagenesis to replace the active-site Ser-195 of ETA with glycine and showed that injection of 1 and 10 μg of wild-type ETA into 3-day-old Sha-Sha mice gave a positive Nikolsky sign within 2.5 h whereas the mutated ETA had no effect at doses up to 100 μg, indicating that Ser-195 is essential for the biological activity of ETA (214). Prevost et al. (210) also performed a similar experiment, replacing the Ser-195 with cysteine, and showed that a positive Nikolsky sign was obtained in neonatal mice with as little as 0.1 μg of wild-type ETA but not with up to 300 μg of mutated ETA.
Research on the ETs was hampered by the lack of a suitable model. Neonatal and hairless mice still remain the “gold standard” for demonstrating the toxins as causes of exfoliation. However, this model, as well as isolated epidermis from various sources, does not allow for accurate, quantitative, and reproducible studies of toxin activity (69). A search for an in vitro model for the toxins soon began in an attempt to develop a rapid method to routinely identify the toxins and possibly aid in elucidating their mechanism of action. Bailey and Redpath (23) tried to determine whether the ETs might have an esterolytic activity on the basis that proteases are always esterases and serine proteases tend to have a higher catalytic efficiency toward esters (76). They demonstrated that both ETA and ETB have very low but significant N-t-butoxycarbonyl L-glutamic acid α-phenyl (boc-L-Glu-O-phenyl) esterase activity, which was not present in a mutant form of ETA where the Ser-195 of the putative catalytic site was replaced by glycine (23). Furthermore, the pH dependence of both ETA and ETB in this reaction was typical of the histidine-associated pKₐ found with all serine proteases. On the other hand, Jaulhac et al. (118) modified the original neonatal-mouse model (262) and demonstrated an induction of proteolytic activity by using a supernatant of the epidermis. An epidermal extract was prepared by grinding neonatal-mouse epidermis in liquid nitrogen, suspending it in buffer, and subjecting it to high-speed centrifugation. The supernatant was then incubated with ETA or ETB and different protein substrates, and the appearance of soluble peptides was estimated by the Lowry titration assay. Significant proteolytic activity in the supernatant with ETA and ETB (but not with biologically inactive toxin mutants) was induced after 20 h (118).

Many authors have argued that the proteolytic activity observed by both Takiuchi et al. (262) and Jaulhac et al. (118) was due to initial protease contamination of the toxin preparation (24, 44). However, this is very unlikely, because a threefold induction in supernatant proteolytic activity was obtained compared to the native toxin preparation or epidermis alone, and this activity was no different from the negative controls. Jaulhac et al. (118) also showed that mutants of ETA had no proteolytic activity. This provides strong evidence that ETA is the serine protease in the supernatant, although it is still possible that altering the active site of the toxins also resulted in destruction of their ability to release or activate serine protease(s) from epidermal cells. Furthermore, the authors demonstrated proteolytic activity against whole bovine casein, β-casein, and α-casein but not κ-casein or bovine serum albumin (118). Preliminary purification of one of the digested peptides by high-performance liquid chromatography also revealed that ETA might cleave a peptide bond between glutamic acid and valine, although the authors did not search for other cleaved peptides (118). Their results suggested that the toxin might cleave the peptide bond between a glutamic acid and a hydrophobic amino acid; this has been supported by recent structural findings (see below).

Probably the strongest evidence supporting the toxins as serine proteases comes from some recent spectacular studies on computer models and crystallographic data. By using the structures of known glutamate-specific trypsin-like serine proteases to model the three-dimensional structure of ETA and other serine proteases, a 15 to 20% sequence homology to other known glutamate-specific serine proteases (including V8 protease), particularly in the region of the putative catalytic triad, was demonstrated (25). The computer model predicted that ETA consisted of two domains, S1 and S2, each made up of six antiparallel β-strands that form a β-barrel common to all members of the trypsin family, although the toxin had a larger N-terminal portion than the other members. The model also demonstrated that Thr-190 and His-213 (chymotrypsin numbering), which form the core elements of the S1 pocket, were unconditionally conserved in all serine proteases that are glutamate specific as well as in the ETs. However, because both toxins differed from the others in that they had a Lys-216 protruding into the S1 pocket, it was thought that its positive charge would help stabilize binding to a negatively charged substrate, such as glutamic acid.

Crystallographic data from two independent studies confirmed all these speculations and provided even more insight into the structure and function of the toxins. Vath et al. (270) reported that both toxins were unique in that they possessed a highly charged N-terminal α-helix (αN helix) containing four positive and four negative charges (Fig. 4). This αN helix interacts with loop 2, which forms part of the S1 pocket, in such a way as to partially block the pocket entrance. Furthermore, the Pro-192–Gly-193 peptide bond is flipped 180° compared to that in the other glutamate-specific proteases. This allows the glycine to form a hydrogen bond with Asp-164 of loop D of the S2 pocket and the proline to form a hydrogen bond with the Ser-195 of the active site, thereby preventing any activity by the toxin. The authors proposed that binding of the highly charged αN helix to a specific epidermal receptor results in a conformational change which opens the S1 pocket and flips the proline-glycine peptide bond by 180° to allow the proline to form a hydrogen bond with Asp-164 instead of the glycine. This conformational change then allows the active site to cleave the carboxyl-terminal side of glutamic acid.

Cavarelli et al. (44) confirmed most aspects of the above model with their own crystallographic data (44). Their free-energy simulations showed that the proline-glycine flip requires very little energy, less than 3 kCal per mol, and that there were no steric hindrances to prevent the proline-glycine flip from occurring. The possibility of binding to a specific molecule that induces a conformational change leading to toxin activation had been proposed before (112); this event is not novel, since thrombin (98, 99) and the hepatitis C virus NS3 protease also demonstrate such a phenomenon (130).
However, the highly charged N-terminal region of the toxins have significant sequence homology to that of trypsinogen. It is therefore possible that cleavage (by an as yet unknown mechanism) of, for example, the first few N-terminal amino acids of ETA and ETB is sufficient (as in trypsinogen) to induce the conformational change that activates the toxins (53). However, it should be noted that larger deletions of 10 or 20 amino acids in this region reduce toxin activity (44).

In vitro enzymatic studies by the same authors (44) with synthetic substrates confirmed previous work by Bailey and Redpath (23) showing that the toxins could hydrolyze the carboxyl terminal of glutamic acid of the ester boc-\text{Glu-O-phenyl}. These authors also showed that the toxins were unable to hydrolyze boc-I-Asp-O-phenyl, indicating the glutamic acid specificity of the S1 pocket. This activity, as well as the ability to produce exfoliation in neonatal mice, was lost when any of the three amino acids constituting the putative catalytic triad of ETA was replaced by another amino acid, confirming their importance in the observed activity.

However, there is some evidence, speculated as far back as 1969, that ETs might have intrinsic lipase activity, since analysis of clinical isolates of \textit{S. aureus} from patients with localized or extensive SSSS revealed that a significant proportion of the strains had lipolytic activity toward a range of lipids (12, 261). Soon thereafter, Wiley and Rogolsky (278) demonstrated that incubation of purified ET with either egg yolk emulsion or bovine brain sphingomyelin resulted in a dose-dependent release of phosphate, suggesting that ETs have intrinsic phospholipase activity (278). However, it should be noted that other investigators were unable to detect any lipase activity toward triacylglycerol or phospholipids (210).

More recently, a triacylglycerol acylhydrolase of \textit{Mucor miehei} (37) has been speculated to have structural homology to serine proteases, with His-257, Asp-203, and Ser-144 forming the catalytic triad of the lipase. As with serine proteases, lipase activity could be reduced with the serine protease inhibitor PMSF. A chemically analogous but structurally different aspartate-histidine-serine catalytic triad has also been found in human pancreatic lipases which hydrolyze triglycerides into di- and monoglycerides and free fatty acids (280). Regarding the ETs, ETA also has the sequence Gly-Asp-Ser195-Gly-Ser-Gly, which resembles the Gly-Xpe-Ser-Xpe-Gly consensus sequence of all known mammalian and microbial lipases (201). More recently, on the basis of the Lys-216 residue unique to both the ETs, computer models for the toxins were used to demonstrate that the Ser-195 active site of the toxins might act on lipids by cleaving phosphodiester bonds in a similar manner to alkaline phosphatase (25). The model showed that a phosphonyl group of a phosphodiester, instead of a peptide, would fit into the active site of the toxin and that Lys-216, along with His-213, would serve to stabilize the negative charge on the phosphonyl group. Similarly, lipases with a serine protease catalytic triad may also have an active site that is covered by a protein region displaced during the activation process (37, 41, 280).

Although the ETs might have no inherent lipase activity, it is possible that enzyme activity is induced only after activation in the epidermis. For example, the human pancreatic lipase in its native form has little enzyme activity, and data from its three-dimensional structure suggest that the serine residue representing the catalytic site is covered by a short one-turn $\alpha$-helix surface loop which undergoes a conformational change to expose the active site at a lipid-aqueous interface (280).

Recently, preliminary work was presented in which an octapeptide derived from ETB prevented the formation of a precipitation line between ETB and anti-ETB serum. Furthermore, a subcutaneous injection of 2 mg of the peptide resulted in a positive Nikolsky sign in neonatal mice 16 h later (225). This work has not been published in a peer-reviewed journal; it is unlikely that such a small peptide could simulate the whole toxin molecule, and the positive Nikolsky sign may be due to a toxic rather than an exfoliative effect.

In summary, current evidence suggests that ETs induce exfoliation by possessing an atypical glutamic acid-specific trypsin-like serine protease activity which may be activated only locally by an as yet unknown mechanism. The GM4 gangliosides, toward which the ETs have a strong affinity, are unlikely to be the activating factors, since such binding abolishes exfoliation in the neonatal-animal models. It is, however, possible that binding to cell surface GM4 gangliosides results in internalization of the toxin by keratinocytes to reach keratohyalin granules; during this journey, the toxin is activated either by partial cleavage by an intracellular protease or by attachment to coreceptor to induce the exfoliation seen. On the other hand, the possibility of ETs acting as lipases, as well as their potential ability to activate or release other serine proteases from surrounding cells, still exists. In the endless search to identify the complex mechanism of action of the toxins, simpler explanations should not be forgotten. A very specific substrate in the epidermis, the requirement of specific microenvironment conditions for toxin activation, or physical disruption of the intercellular matrix by excess fluid-filled vesicles are only a few such possibilities.

### SUPERANTIGENIC ACTIVITY

Whether staphylococcal ETs possess superantigenic activity is currently under considerable debate. Superantigens are a group of bacterial and viral proteins that can activate a large number of T-cell clones simultaneously. They have been reviewed recently (137). Conventional antigens require digestion by antigen-presenting cells (APCs) followed by surface presentation of a small peptide fragment complexed to an MHC protein in order to activate a small proportion (less than 0.1%) of T cells bearing specific T-cell receptors (245). In contrast, superantigens can bypass this process of intracellular processing (80, 81) and can bind directly to the MHC molecule on the surface of the APC outside the antigen-binding groove (59). This complex can then cross-link with the variable V$\beta$ region of the $\beta$ chain of the T-cell receptor (77, 124), resulting in polyclonal CD4$^+$ and CD8$^+$ T-cell activation (up to 20% of all T cells) that is characteristic of superantigens (245).

\textit{S. aureus} produces several exotoxins that also possess superantigenic activity, including TSST-1 (2, 209) and the staphylococcal enterotoxins (SE), including SEA, SEB, SEC1-3, SEG, and SEE (77, 109, 137, 198, 234, 260); new toxins continue to emerge (259). While the ETs are globular proteins with molecular masses of 24 to 30 kDa, similar to the masses of other superantigens, the degree of primary sequence and structural homology such as that seen between other superantigens is poor (165). Initial reports indicated that the ETs had mitogenic activity toward murine T lymphocytes (181), and ETA has been shown to preferentially stimulate V$\beta$2 human (46) and V$\beta$3 murine T cells (106).

The main argument against the ETs being superantigens arises from work by Fleischer and Bailey (82). Cloning ETA into a non-toxin-producing strain of \textit{S. aureus} by using a shuttle vector, these investigators showed that purified recombinant ETA (confirmed by ETA-specific rabbit antisera on Western blots), and even crude concentrated extract of the recombinant toxin, was unable to stimulate human or murine T cells even at concentrations of 10 $\mu$g/ml (83). On the other hand, 10 ng of
commerially available ETA, as well as TSST-1 and SEB, per ml could stimulate activity that was consistent with superantigens (46, 124). The authors speculated that because superantigens exert their effects at concentrations that are not detectable by conventional biochemical methods, previous demonstration of superantigenic activity with ET was probably due to contamination by other staphylococcal enterotoxins (82, 83), as was demonstrated with protein A (235). They also mentioned that another group of researchers (107) could not precipitate MHC class II molecules with commercial ETA, possibly because the toxin did not bind to the molecule. Similarly, ETA and ETB purified from S. aureus strains could stimulate spleen cells of transgenic mutant mice deficient in the expression of MHC class II antigens (45), as well as MHC class II deficient macrophages, but did not bind to class II molecules (28). Further arguments against superantigenic activity presented by Cavarelli et al. (44) are as follows: (i) the characteristic symptoms of cytokines (including rash, fever, and hypotension) induced by the action on lymphocytes of other superantigens such as TSST-1 and the enterotoxins is not seen with the ETs; (ii) histological examination of skin from patients with SSSS does not show any recruitment of immune system cells; and (iii) none of the other known superantigens possess the speculated hydrolytic activity of the ETs.

On the other hand, Vath et al. (270) cloned ETA into a superantigen-free strain of S. aureus and showed that the purified toxin had mitogenic activity toward T cells. The authors argued that since superantigenic activity could be demonstrated only after cloning ETA into the superantigen-free strain of S. aureus, ETA must be the source of the superantigenic activity and contamination by other superantigens was unlikely. Also, recombinant ETA with a mutated active site, Ser195Cys-ETA, was shown to possess a similar mitogenic activity, indicating that the mitogenic activity was separate from the exfoliative activity of the ETs. This dual action is also seen with the separate emetic activity and mitogenicity of the enterotoxins (33) and with the lethality and mitogenicity of TSST-1 (188). The ETs also activate lipopolysaccharide-responsive murine macrophages to produce tumor necrosis factor alpha and high levels of interleukin-6 (IL-6) (compared to TSST-1 and SE) and nitric oxide (which was not produced by either TSST-1 or the SE) resulting in contact-dependent cytotoxicity of transformed embryo fibroblast cells, although the level of killing was lower than that for TSST-1 and SE (84).

Furthermore, SSSS does share many clinical features with other superantigen-mediated syndromes, including the tender erythematous rash, tachycardia, hypotension, fever, and shock (29, 114, 157, 174, 245). Since S. aureus in SSSS produces ETs at a site distant from the exfoliation (174), it is very likely that these systemic symptoms, particularly the characteristic rash, are a direct result of the toxins.

**Exfoliative Toxins and the Skin**

Recently, ETs were shown to stimulate the skin associated lymphoid tissue (SALT) by demonstrating that the toxins activated murine splenic T cells in the presence of Langerhans' cells and MHC class II-bearing keratinocytes to produce a range of cytokines, including IL-1 and tumor necrosis factor alpha, capable of stimulating other accessory and immune system cells (268). Furthermore, binding of ETs to Langerhans' cells resulted in dose-dependent depletion of these cells (as measured by the number of ATPase-positive cells per square millimeter) in cultured mouse skin, presumably due to migration of the cells to draining lymph nodes, where they may act as APCs for T lymphocytes (202). SALT is part of the immune system that deals with antigenic challenges common to the skin and includes antigen-presenting Langerhans’ cells, keratinocytes, and T-cell subsets with skin-homing receptors (258). Despite the presence of MHC class II molecules, which are induced on the cell surface during inflammatory processes (273), keratinocytes are unable to act as conventional APC and seem to function only in the presence of superantigens (268). Activation of SALT by ETs may therefore result in the erythematous rash associated with SSSS. For example, there is some evidence that the ability of the superantigen TSST-1 to activate cutaneous lymphocyte-associated antigens may be responsible for the rash and desquamation seen in TSS (149), just as the superantigens of group A streptococci might play a role in the development of the characteristic cutaneous swelling, erythema, and desquamation seen in streptococcal infections (254). However, the lack of immune cells on histological specimens from patients with SSSS cannot be easily explained and requires more research. It may be that the very specific (possibly intracellular) site of action of the toxins does not allow them to exert superantigenic activity in terms of duration or location or that activation of the toxins to proteases, which probably requires a structural change (25, 44, 270), results in loss of superantigenic activity at the site of action.

The ability of the ETs to activate the SALT subpopulation of the immune system has important consequences for several dermatological conditions, including cutaneous T-cell lymphoma and atopic dermatitis. ETA is capable of inducing the proliferation of Vβ2.1-bearing cutaneous T-cell lymphoma cells in vitro, and this proliferative response is enhanced by adding IL-1. This fits in with observations showing that activation of SALT by ETA results in IL-1 release (268), and IL-1, along with IL-6, plays an important role in enhancing the activity of other superantigens (136).

Patients with atopic dermatitis have a higher staphylococcal colonization rate than does the general population (8, 152). Antibiotic treatment of patients with atopic dermatitis has resulted in significant clinical improvement of the condition (150), suggesting that staphylococci play a role in the pathogenesis of the disease. Recently, it has been shown that a large proportion of S. aureus strains isolated from patients with atopic dermatitis release superantigens, including enterotoxins, TSST-1, and ETs (147, 167). It has been speculated that superantigens bind to surface MHC class II molecules found on keratinocytes during inflammation and thereby activate polyclonal T cells locally (257).

**Exfoliative Toxins and Other Human Diseases**

The Vβ-expansion of T cells characteristic of superantigens has also been demonstrated in other conditions, where the role of staphylococcal toxins is still unclear. Kawasaki disease, also known as mucocutaneous lymph node syndrome, is a multisystem disorder in children that is characterized by fever, rash, conjunctivitis, mucosal inflammation, erythematous peeling of the hands and feet, and, rarely, coronary artery aneurysms (147, 175). A selective expansion of Vβ2- and Vβ8-expressing T cells has been detected in the blood of patients with Kawasaki disease (1), and, while TSST-1 has been detected in a proportion of these patients (146, 147, 148), the potential role of the ETs has not been studied. Other conditions where expansion of Vβ-expressing T cells, often by staphylococcal superantigens, has been demonstrated include hematogenously acquired staphylococcal nephritis (138, 271), staphylococcal septic arthritis (38, 290), various autoimmune diseases (137, 184), rheumatoid arthritis (197), multiple sclerosis (39, 256), contact sensitivity (228), and guttate and chronic plaque pso-
riasis (151, 288); another possible role for the expansion of Vβ-expressing T cells is in the gradual decline in the number of CD4+ T cells in human immunodeficiency virus infection (143). Unfortunately, many of the studies on the superantigenic effects of ETs in various diseases have used commercially prepared toxins, which, as many authors have pointed out, may be contaminated by other superantigens. Future studies should take this factor into consideration and use appropriate precautions to eliminate the problem.

Several other conditions have demonstrated a significant association with staphylococcal toxins; sudden infant death syndrome (SIDS) is probably one of the most important (31). S. aureus strains producing multiple toxins have been isolated from infants with SIDS (265). In addition, S. aureus is the most common organism isolated from 2- to 4-month-old infants, being found in almost 50% of the infants (222). This age group is also associated with the highest incidence of SIDS (31). Lee et al. (145) have shown that bacterial isolates, particularly S. aureus and Escherichia coli, from infants with SIDS were lethal to gnotobiotic rats. Therefore, some authors have proposed that common bacterial toxins absorbed through the respiratory tract might be involved (182). Furthermore, staphylococcal infection in combination with influenza virus infection was shown to greatly increase the production of SE, resulting in an exaggerated inflammatory response in ferrets (117). This activity might explain the higher prevalence of SIDS in winter (43). Although TSST-1 was demonstrated in renal tubular cells of some infants with SIDS but not control infants (189), the role of the ETs in SIDS has, unfortunately, never been studied. It is possible that an abnormal inflammatory response due to the superantigenic properties of the ETs, which may be produced locally in the nasopharynx and then systemically absorbed (189), coupled with enhancement by concurrent viral infection will result in sudden death in infants.

**SUSCEPTIBILITY AND SEVERITY**

A whole range of host and organism factors govern the development and severity of SSSS, and many of these are not fully understood. Furthermore, it is still not clear why neonates are more susceptible to SSSS than adults. Initially, anatomical differences in the skin were believed to be the reason, based mainly on the fact that the toxins induced exfoliation in neonatal but not adult mice and that, in humans, the condition was seen primarily in neonates. However, this premise has now been refuted for several reasons: (i) healthy adults of the hairless mice strain will develop SSSS when injected with ET (126); (ii) adult mice can also develop localized SSSS at the site of toxin injection (93) and can even develop generalized SSSS if they are immunosuppressed with drugs such as prednisolone and azathioprine (277); (iii) SSSS cases have been reported in healthy human adults and in adults with underlying disease (48, 194); and (iv) intradermal injection of purified ET into the forearm of a healthy human adult will result in blister formation characteristic of SSSS (127, 277). Nevertheless, anatomical differences are the most likely candidates in determining species susceptibility (69).

Poor renal clearance of the toxins by neonates and by adults with impaired renal function is a major risk factor for developing SSSS and may partly explain why neonates may be more susceptible to developing SSSS. Adult mice excrete one-third of a test dose of intravenous ETA within 3 h compared to 1/15 of a test dose in newborn mice (94). As a consequence, toxin levels reach a higher peak in the newborn and decline more slowly. Furthermore, nephrectomized (but not hepatectomized) adult mice develop generalized SSSS when challenged with ET. However, renal function alone cannot explain why injection of very high doses of ET into adult mice, which would be expected to more than compensate for the rapid renal clearance, does not result in exfoliation (94).

Antibody status may also have a protective function, and this has been shown in mice, in which inoculation of small doses of toxicogenic S. aureus, for example, resulted in exfoliation only in neonatal mice that had not previously been immunized with purified extracts of ET (173). A large epidemiological study looking at the antibody status of patients over the whole range of SSSS spectrum was also performed (173). Anti-toxin antibodies were found in 88% of infant cord blood samples. These findings reflect passive maternal antibodies, which, in turn, may protect newborn infants. This finding is further supported by the observation that infants involved in neonatal-nursery outbreaks tend to have the localized rather than the generalized form of the disease. In addition, work with mice showed that maternal antibodies play an important role in protecting offspring from subcutaneous challenges of ETA (163).

The large epidemiological study also showed that antibody levels fell to 30% in patients between 3 and 24 months of age and then rose steadily to 50% in those older than 10 years and to 91% in those older than 40 years. Furthermore, the same authors noted that 13 (62%) of 21 and 15 (100%) of 15 patients with localized SSSS had anti-toxin antibodies in acute-phase (5 days) and convalescent-phase (14 days) sera, respectively, in contrast to 0 of 10 and 5 of 5 patients with generalized SSSS (173). The authors concluded that while antibodies may not always protect against exfoliation, they may serve to localize the infection around the port of entry of the responsible S. aureus strain. In support of this, the blisters of localized SSSS, such as bullous impetigo, contain S. aureus while the blisters of generalized SSSS, where ETs are produced by S. aureus at a distant site, tend to be sterile (70). Although the study involved ETA only, the overall conclusions are unlikely to be affected by excluding ETB, and at least two other studies have confirmed the above findings (173).

Protective antibodies may also explain why only a proportion of nasal carriers of toxin-producing S. aureus are infected. However, it is possible that the strains harbored by these carriers are not active producers of the toxins. The antibody theory also provides an explanation for observations that breastfeeding is protective in neonates.

While adult SSSS is very uncommon, more than 30 cases have been reported (this is likely to be a gross underestimation, since the disease was linked to the ETs only in 1970) and have been reviewed recently (48). Of the 32 reported cases, 21 (66%) were in males, and the ages of the patients ranged from 19 to 91 years; more than half of the patients were older than 60 years. Most of the adults with SSSS had an underlying disorder such as poor renal function (63); immunosuppression with immunosuppressive drugs; infections such as HIV and AIDS (63); malignancy including Hodgkin’s and non-Hodgkin’s lymphomas, metastatic breast carcinoma, and acute and chronic myeloid leukemias; and other less common causes such as chronic alcohol abuse, heroin misuse, diabetes mellitus, cachexia, and rheumatic fever (96, 173). Another review of adult SSSS found that 78% of patients had factors suggesting possible impaired immunity such as those described above (32). Furthermore, renal impairment was found in 73% of those who had their renal function tested, and many had frank renal failure.

There have been only two reports of apparently healthy immunocompetent young adults developing SSSS, and both cases were due to ETB (185); it is not known whether this is merely a coincidence or whether the ETB subtype is more
pathogenic. Another epidemiological study (185) did note that although both toxins were equally responsible for localized SSSS such as bullous impetigo, ETB was more frequently isolated from children with generalized SSSS than was ETA alone (20 of 24 and 2 of 24, respectively). Recently, a case of SSSS was reported in an immunocompetent 73-year-old man who had very mild renal impairment and who was taking ibuprofen (103). Nonsteroidal anti-inflammatory drugs have been previously reported to predispose to SSSS (129), possibly by a combined effect of inhibiting prostaglandin-mediated renal vasodilation and inhibiting neutrophil function. However, as the authors correctly point out, because such drugs are so widely used, this association may be purely coincidental.

DIFFERENTIAL DIAGNOSIS

While the mortality rate from SSSS in neonates and children is low, it is important to make a correct diagnosis in order to initiate early and appropriate treatment, as well as to prevent medicolegal implications, since the disease may result in allegations of burns by neglect (160) or child abuse (231). Differential diagnoses of generalized SSSS in neonates are few and include drug- or virus-mediated TEN, burns, epidermolysis bullosa, bullous erythema multiforme, listeriosis, syphilis, diffuse cutaneous mastocytosis, and graft-versus-host rejection (65). Chemical burns from petrol, paraffin, boric acid, ethylene, acrylonitrile fumigant spray, tribromofluorone, and monosulphiram, which act at the dermoepidermal junction, must also be excluded (162). TEN, also known as Lyell’s syndrome after the author who first described it (159), is probably the most important differential diagnosis of SSSS, since their treatments are very different (236). TEN is an uncommon but life-threatening cutaneous disorder that occurs as frequently in children as in adults (215). More than 100 different drugs, including antibiotics and anti-inflammatory drugs, and occasionally immunizations and infections such as with members of the family Herpesviridae, have been implicated in TEN (236). TEN occurs 1 to 3 weeks after drug ingestion, although reexposure to the offending drug may result in a more rapid and very severe presentation, with involvement of multiorgan systems including the liver, kidneys, gut, and lungs (212, 236). The dermatological features are characterized by an initial prodrome of pyrexia, malaise, anorexia, pharyngitis, and a tender, morbilliform rash followed by epidermal exfoliation in sheets (236). As in SSSS, fragile blisters are also seen in TEN and the Nikolsky sign is positive (18, 236). However, unlike SSSS, eroding mucosal lesions of the mouth, conjunctiva, trachea, bronchi, esophagus, anus, and vagina (18) are almost always present in TEN, as demonstrated in one study of 87 patients (215), and can be differentiated histologically from SSSS lesions (see below).

Localized SSSS such as bullous impetigo is usually characterized by blisters that dry up without producing any marked crusting (162, 178). On the other hand, in streptococcal impetigo there is copious exudation from raw surfaces that dries into a characteristic thick, dirty yellow crust, which soon reforms if removed. Furthermore, glomerulonephritis is a known complication of the latter but not the former condition (162, 174). However, staphylococcal impetigo and streptococcal impetigo are often clinically indistinguishable, and antibiotic treatment should cover both groups of organisms.

The initial generalized erythematous rash seen in SSSS may be confused with streptococcal scarlet fever, but the very tender skin, exfoliation, lack of a strawberry tongue, and possible perioral and periorbital crusting seen in SSSS should confirm the diagnosis (174). Congenital epidermolysis bullosa can present with exfoliation of the skin upon slightest mechanical pressure, but, unlike SSSS, it presents from birth. Other congenital bullous diseases include acrodermatitis enteropathica and bullous ichthyosis. Occasionally, bacterial (staphylococci, streptococci, E. coli, or, rarely, Haemophilus spp.) (207), fungal (Candida spp.), and viral (herpesviruses, vaccinia virus, varicella-zoster virus) infections resemble SSSS. Isolated cases of diffuse cutaneous mastocytosis (195), Kawasaki disease (79), porphyria (217), and TSS (66) have been reported to mimic SSSS.

DIAGNOSIS AND INVESTIGATIONS

Most cases of SSSS are diagnosed on clinical grounds and are easily treated with antibiotics, which rapidly eliminate the staphylococci producing the toxin. Laboratory investigations are required only if the clinical findings are equivocal or when outbreaks occur. Because the condition is the result of exotoxins which may be produced by staphylococci at a distant site, the blister fluid in generalized SSSS tends to be sterile whereas the fluid in localized bullous impetigo will contain S. aureus (70, 158, 208). Staphylococcal producing ET can usually be cultured from the nares, conjunctiva, or nasopharynx (50, 158, 229). Blood cultures are usually negative because the organisms are frequently noninvasive, particularly in children (49, 114, 157, 208, 211). In one study, only 3% of children had a positive blood culture (101), in contrast to 20 (62.5%) of 32 adults (48). Of note, children with renal disease tend to have bacteremia with generalized SSSS rather than the localized form (34). Bacteriological studies of the skin are not usually useful in differentiating SSSS from TEN, since the skin of patients with generalized SSSS is usually sterile and that of patients with TEN is often colonized by S. aureus (67, 236). Indeed, up to half of the deaths in patients with TEN are due to secondary bacterial infection of the denuded skin (18).

Phage typing is not routinely available in hospital laboratories. In the United Kingdom, bacterial swabs of S. aureus can be sent to the Public Health Laboratory at Colindale, London, for phage typing, despite this test having poor reproducibility and requiring special reagents and a large number of tests (95).

Many authorities assert that the presence of characteristic skin lesions, including a positive Nikolsky sign, along with the isolation of phage group II S. aureus from a distal site is sufficient for the diagnosis of SSSS (for a review, see reference 72). However, it is now generally agreed that the production of ET is not associated with any particular phage type, since phage types other than group II will also produce the ETs and only a proportion of phage II strains produce ETs (56, 85, 174).

A biopsy of the blister is one of the most definitive diagnostic tests in SSSS. One study revealed a positive blister biopsy result with intraepidermal cleavage in all 30 adults with SSSS (48). Blister biopsy is also essential in ruling out other bullous dermatoses (42, 75, 269). Histological testing will clearly exclude conditions involving subepidermal separation, including erythema multiforme and TEN, bullous lichen planus, polymorphic light eruptions, graft-versus-host rejection, and non-inflammatory processes such as bullous amyloidosis, Wilson’s disease, drug ingestion, epidermolysis bullosa acquista, and
porphyria (75). To rapidly differentiate TEN from SSSS, a Tzanck preparation from a freshly denuded area will show large polygonal epithelial cells with large nuclei and no inflammatory cells in SSSS and only a few rounded or cuboidal epithelial cells with many inflammatory cells in TEN (9, 73).

Histologically, SSSS needs to be distinguished from other intraepidermal conditions, including TSS and nutritional disorders. TSS is usually diagnosed clinically (90), with or without laboratory tests to detect TSST-1 and other superantigens (178). Histological tests for the diagnosis of TSS are only occasionally required and show spongiosis, scattered necrotic keratinocytes, and scattered neutrophils, with a perivascular and interstitial mixed-cell infiltrate and dilatation of the superficial plexus and eftema in the dermis (111). Nutritional deficiency syndromes, including pellagra, zinc deficiency, biotin deficiency, essential fatty acid deficiency, and the glucagonoma syndromes (neurotic migratory erythema), may present with erythematous, scaly, and vesicular eruptions (3). All have a histologically similar picture, with intercellular edema in the upper half of the stratum spinosum that may be severe enough to cause intraepidermal vesiculation (3). Along with SSSS, subcorneal pustular dermatosis and pemphigus foliaceus are two other causes of subcorneal blistering; they can be distinguished from SSSS by the presence of a large number of neutrophils in the blister and by distinct immunohistochemical findings respectively (75). Lesions from bullous impetigo are characterized by stratum granulosum epidermal cleavage with a local infiltrate of staphylococci and polymorphonuclear leukocytes (174).

If further confirmation is required, the Public Health Laboratory in the United Kingdom may attempt to detect the ETS by using the Ouchterlony method. This method is known to be crude, unreliable, and time-consuming with poor sensitivity and is unsuitable for batch testing of large samples (139). Furthermore, our work (139) and work with diagnostic kits for TSST-1 (219, 238) have shown that protein A in culture supernatants is produced by more than 90% of S. aureus strains (88, 89), resulting in high rates of false-positive results due to nonspecific binding to the detecting antibodies (36, 255). While several techniques have been proposed in the past to study the effects of ETs, few have been developed. Subcutaneous injection of small amounts of the partially purified toxin into neonatal (<5-day-old) Sh-Sha, BALB/c, CD-1, TA, ICR, Swiss-Webster, or Park mice (56, 57, 170) or adult hairless mice (14, 126) results in a positive Nikolsky sign 2 to 120 min after inoculation, although the adult hairless-mouse model is unsuitable for batch testing of large samples (139). Furthermore, our work (139) and work with diagnostic kits for TSST-1 (219, 238) have shown that protein A in culture supernatants is produced by more than 90% of S. aureus strains (88, 89), resulting in high rates of false-positive results due to nonspecific binding to the detecting antibodies (36, 255).

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More recently, PCR with specific ETA and ETB primers has been used to identify the toxin genes in suspected strains of S. aureus (120, 226). For example, Sakurai et al. correctly identified all 30 ETA-, 20 ETB-, and 6 ETA- and ETB-producing strains within 3 h (226). Thus, this technique is rapid and sensitive and has been used to confirm the diagnosis of SSSS in suspected clinical cases (288). Random amplified polymorphic DNA analysis, which is simple and rapid to perform, has also been used to demonstrate that the S. aureus strain isolated from a mother’s breast abscess was identical to the strain causing SSSS in her breast-feeding infant (213).

**TREATMENT**

Although SSSS can be very severe, it is easily managed with antibiotics to eradicate the offending toxin-producing S. aureus strain and appropriate nursing care to prevent and/or treat any secondary symptoms. Neonates must be isolated from other babies and strict barrier precautions must be applied to prevent cross-infection (79). A focal source of infection, which may be absent in more than half the patients (48), must be sought and treated to prevent further spread of the disease. This includes blood, wound, eye exudates, and throat swabs initially, followed by osteomyelitis, septic arthritis, and occult foci if the source is not obvious (174). Potential carriers of toxin-producing strains must also be identified and treated to prevent cross-transmission, which may result in outbreaks. This involves culturing nasal swabs for S. aureus from all personnel who have handled the infected baby (79).

Mild forms of SSSS such as bullous impetigo should be managed with cleaning and drying of the lesions with compresses and administration of oral antibiotics that are effective against penicillin-resistant staphylococci as well as streptococci. Meticillin, nafcillin, dicloxacillin, cloxacinil, and flucloxacinil have all been used to treat S. aureus, and other proposed regimens include cloxacinil plus tobramycin, and erythromycin (48). Multidrug-resistant strains are rarely encountered in phage group II S. aureus (48, 279). One recent study of 61 ET-producing S. aureus strains revealed that 7% were resistant to tetracycline, 5% were resistant to gentamicin, and 2% were resistant to chloramphenicol. None of the strains were resistant to meticillin, cephalosporins, or vancomycin (186). Another epidemiological study of 82 meticillin-resistant S. aureus (MRSA) strains isolated from outbreaks in neonatal intensive-care units in Japan revealed that only 1 strain produced ETA and none produced ETB (287). However, the same authors also reported a case involving a 6-month-old infant with SSSS due to a meticillin-resistant ETB-producing phage II S. aureus strain in Japan, who was successfully treated with minocycline (287).

Topical antibiotics are often not effective and should be avoided due to unpredictable absorption through denuded skin; even localized forms of SSSS such as bullous impetigo should be treated with oral antibiotics, which have been shown to be more effective than topical antibiotics (61). Steroids are contraindicated (157, 208) on the basis of both experimental (171) and clinical (220) evidence.

Severe forms require more aggressive treatment with intravenous antistaphylococcal antibiotics and extra care of denuded skin to prevent secondary infection and fluid losses and to maintain body temperature, especially in neonates. Additional broad-spectrum antibiotics should be considered if secondary skin infection is suspected, and they should cover *Pseudomonas* species (110). Intravenous crystalloids should be
used to replace fluids, and an incubator should be used to maintain body temperature. Sterile wrapping and minimal handling may be used to decrease conductive heat, fluid losses, and the risk of secondary infections. Semipermeable dressings, such as those used to cover graft donor sites, have been used to provide a barrier against evaporative fluid losses (177). Topical applications such as silver sulfadiazine, which are sometimes used for burns and TEN, are not recommended for SSSS because of enhanced systemic absorption through denuded skin (86). Because the complications of severe SSSS resemble those of severe burns, many clinicians prefer treating such patients in specialized burns units (243). A few severe cases of SSSS have benefited from extracorporeal detoxification involving sorption detoxification and separation plasmapheresis (250).

Within 2 to 3 days, fever usually subsides, the erythema begins to resolve, and no new bullae form (157). Desquamation of the skin occurs 3 to 5 days after onset, and complete resolution occurs in most cases, usually within 2 to 3 weeks, with no permanent sequelae (100, 114, 157). Serious complications, including lung abscesses, staphylococcal pneumonia, and osteomyelitis, have been reported (87, 104). Mild emollients may be used if the skin becomes very dry during the healing process (114).

The prognosis of SSSS in children who are appropriately treated is good, with a mortality of less than 5% (48, 96, 174). Nevertheless, some children will die despite antibiotic therapy (211). In contrast, mortality rates in adults are very high, usually due to underlying diseases, despite aggressive treatment. One review of adult SSSS reported that 19 (59%) of 32 patients reported in the literature died despite antibiotic therapy (48): all but 2 of those who died had severe underlying disease, and the prognosis was not necessarily better in those with localized forms of SSSS.

PREVENTION

Hospitals remain the most important source of \textit{S. aureus}, and simple barrier nursing techniques are still considered to be the most effective method of preventing transmission and outbreaks of infection. Using MRSA as a parallel example, reinforcement of hygiene measures has consistently led to a decrease in MRSA colonization and infection rates (35, 119, 155, 185). Simple measures such as hand-washing, minimal handling, cleaning objects such as stethoscopes and thermometers before use, and avoiding unnecessary invasive catheterization all contribute to prevention of cross-infection (119). Such aseptic technique should be encouraged among all health care professionals and reinforced at all levels.

Any patient developing SSSS should be immediately isolated. Anyone else at risk of developing SSSS who may have been exposed to ET-producing \textit{S. aureus} strains will benefit from prompt administration of prophylactic anti-staphylococcal antibiotics, which, in mice, have shown to abort exfoliation if given early enough (172). Furthermore, carriers of toxin-producing strains should be rapidly identified and treated to prevent outbreaks. It is important to emphasize that any one outbreak may have more than one carrier (49, 52, 55). Unfortunately, a rapid diagnostic kit for SSSS is not yet available. As a consequence, there is often a delay in diagnosis as well as identification of carriers. The organism has to be isolated and sent to the Public Health Laboratory for phage typing and then possibly for culturing to produce the ETs, which are then tested for by the Ouchterlony method (140).

FUTURE DIRECTIVES AND CONCLUDING REMARKS

Research into SSSS is now reaching an exciting era. Elucidation of the three-dimensional structure of the ETs has given us an insight into the mode of action of the toxins. The precise mechanisms by which they split the epidermis will be determined in the very near future, and their ability to target to a very specific region of the epidermis can be exploited in many ways (140). In particular, the targeting domain of the toxins may be used to deliver drugs to precise intraepidermal locations for the treatment of dermatological conditions such as neoplasms, as has already been demonstrated with \textit{Corynebacterium diptheriae} and \textit{Pseudomonas} toxins (283). Furthermore, if the ETs are confirmed to be superantigens, they may play a wider role in a whole range of dermatological and other diseases.

Similarly, there have been studies showing that antitoxin antibodies will protect neonatal mice from lethal doses of ET, even if the antitoxin is administered late during the course of the disease (127). Because antibiotics are targeted to the offending organism, exfoliation will continue for 24 to 36 h after antibiotic administration. Development of an effective antitoxin based on the structure and function of the toxin may be invaluable in halting the progression of exfoliation in severe cases of generalized SSSS, particularly in adults, in whom mortality rates are high (140). Although the rarity of the disease would not support large-scale immunization, the use of toxoids in immunization against SSSS may also play a role in at-risk groups such as neonates with immune deficiency or adults with cancer or renal failure.

The need for a rapid diagnostic kit for SSSS can no longer be ignored. Current methods of diagnosis are neither sensitive nor specific, and delay in diagnosing the disease will result in increased morbidity and mortality, particularly among adults. Most hospital laboratories have facilities to detect a wide range of microbial toxins, and the ETs should be no exception. Rapid detection of the ETs is important not only in diagnosing SSSS but also in detecting carriers and screening at-risk patients for ET-producing \textit{S. aureus} carriage to prevent the development of SSSS.

Clinicians should also be aware of the need for increased surveillance of staphylococcal infections in general. The pressure to discharge babies early from hospitals, along with the recent trend to stop using antiseptic neonatal umbilical cord care, is likely to increase the prevalence of staphylococcal infections in the community. Therefore, it might be useful to develop a central reporting system, whereby primary-care physicians report to the local hospital any neonatal infection in the community that might possibly be related to SSSS. The community would be rapidly recognized and contained and emerging trends such as antibiotic resistance would be quickly detected and dealt with.

It is hoped that the relative rarity of the disease will not lull researchers and clinicians into a false sense of security and that the recent exciting developments in the field will strengthen efforts to conquer the disease once and for all.

REFERENCES


