Staphylococcus aureus exfoliative toxins: How they cause disease.

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Abbreviations:

BI- bullous impetigo
ET- exfoliative toxins
EDIN- epidermal cell differentiation inhibitor
ETA- exfoliative toxin A (epidermolysis A, exfoliatin A)
ETB- exfoliative toxin B (epidermolysis B, exfoliatin B)
ETD- exfoliative toxin D (epidermolysis D, exfoliatin D)
PF- pemphigus foliaceus
SSSS- Staphylococcal scalded skin syndrome, (pemphigus neonatorum, dermatitis exfoliativa neonatorum, Ritter’s disease)
TEN- toxic epidermal necrolysis

Introduction

General Microbiology: Staphylococci are hardy Gram-positive cocci found as bacterial pathogens or commensal organisms in both humans and animals. These organisms are resistant to harsh conditions and can be recovered from non-physiologic environments up to months after inoculation. They grow easily under numerous conditions and are classified based on coagulase activity. Coagulase positive strains are classified as Staphylococcus aureus. Approximately 35% of the general population are commensal nasal carriers and most newborns will be colonized within the first week of life (Dancer and Noble, 1991). Of the staphylococci, S. aureus is the most significant pathogen causing both human and animal diseases. S. aureus causes a wide variety of infections acquired in both the community and hospital settings. It is the most common cause of pyogenic infections of the skin. A variety of predisposing factors may lead to more serious infections such as conjunctivitis, pneumonia, septicemia, osteomyelitis, septic arthritis, empyema, meningitis, pericarditis or endocarditis. Diseases caused by S. aureus can be the result of direct tissue invasion or due to the action of a variety of exotoxins released by the bacteria (landolo, 1989; Marrack and Kappler, 1990). S. aureus strains capable of causing disease express a wide variety of virulence factors including exported toxins (exotoxins), cell surface molecules associated with adhesion and multiple antibiotic resistances including methicillin and vancomycin resistance (Centers for Disease Control and Prevention, 1997; 2000a; 2000b), all contributing to the pathogenicity of these organisms. A minimum of 34 different extracellular proteins are produced by S. aureus, and many of these have defined roles in the pathogenesis of their associated diseases (landolo, 1989). Infectious conditions caused by these organisms can be divided into three major categories: (i) superficial skin infections, skin abscesses and wound infections including bullous impetigo (BI) and furuncles, (ii) systemic or infections of deep seeded tissues including osteomyelitis, endocarditis, pneumonia and sepsis, and (iii) conditions caused by intoxication with one of the excreted toxins. Among the conditions caused by intoxication with an exotoxin are toxic shock syndrome caused by toxic shock syndrome toxin (TSST-1) (Bloster-Hautama et al, 1986), staphylococcal food poisoning caused by the staphylococcal enterotoxins (SE) (Bergdoll, 1972; Bergdoll, 1983; Bergdoll et al, 1981) and staphylococcal scalded skin syndrome (SSSS) mediated by the exfoliative toxins (ETs), primarily ETA and ETB (Arbuthnott et al, 1972). This discussion will focus on the exfoliative toxins of S. aureus and the mechanism by which they cause disease in humans.

General characteristics of the exfoliative toxins.

There are two major biologically and serologically distinct S. aureus ET isoforms that are primarily responsible for the skin manifestations of SSSS and BI in humans (Wiley and Rogolsky, 1977) ETA and ETB. Five percent of clinical S. aureus isolates produce either ETA, ETB or both toxins (Piemont et al, 1984) and there are reported geographic differences in the prevalence of the particular toxins that are expressed. In North America, Europe and Africa ETA is the predominant ET (Adesiyun et al, 1991; Ladhani, 2001), in contrast ETB has been reported to be more common in Japan (Kondo et al, 1975). These toxins exhibit exquisite species specificity, being active in humans, mice, monkeys and hamsters.
but not in rats, rabbits, dogs, hedgehogs, voles, guinea pigs, chickens or frogs (Bailey et al., 1995; Elias et al., 1975; Ladhani et al., 1999).

Both ETA and ETB have been cloned and their resultant protein products characterized (Lee et al., 1987; O’Toole and Foster, 1987). The primary human active ETs, ETA and ETB, share significant amino acid identity with each other and have similar biophysical properties (Bailey et al., 1980; Lee et al., 1987; O’Toole and Foster, 1987). A comparison of these toxins is shown in Table 1. ETA is a very stable protein that is resistant to extreme heat while ETB is heat sensitive. Both ETA and ETB share amino acid identity with staphylococcal V8 protease (glutamyl endopeptidase I), a well characterized general serine protease of Staphylococcus aureus (26% with ETA and 31% with ETB). Most significantly, this identity includes the key amino acid residues of the V8 protease active site, serine-histidine-aspartate. This catalytic triad is highly conserved and forms the active site of trypsin-like serine proteases (Dancer et al., 1990). The X-ray crystal structures of both of these toxins have been determined (Cavarelli et al., 1997; Papageorgiou et al., 1999; Vath et al., 1999; Vath et al., 1997). These three-dimensional structures show marked similarity with each other and V8 protease demonstrating that the ETs are members of the trypsin-like serine protease family, but do have some significant differences at the amino- and carboxyl-termini.

Recently a new potential human active exfoliative toxin, ETD, was identified from an isolate of S. aureus taken from a wound site. It was initially identified as an open reading frame which had significant homology to the known ETs (Yamaguchi et al., 2002) but has also been associated with a clinical isolate from a patient with BI (Yamaguchi et al., 2002; Yamaguchi et al., 2002). ETD expressed as a recombinant protein product demonstrated specific protease activity in the neonatal mouse model and exhibits cleavage of recombinant mouse or human Dsg1 which is indistinguishable from that of ETA by Western blot analysis (Hanakawa et al., 2002). It was further shown to be serologically distinct from ETA and ETB. A fourth potential human active ET produced from S. aureus, ETC, was isolated from a horse infection and was shown to have activity in the neonatal mouse model (Sato et al., 1994); however, it has not been associated with human disease. The clinical significance of either ETD or ETC has not been established. Other similar exfoliative toxins made by S. hyicus, (ShETA, ShETB and ShETC) have also been identified and partially characterized (Anderson, 1998). These toxins have been associated with comparable skin diseases in different animal populations but are likewise not associated with disease in humans.

The ETs, like most staphylococcal proteins associated with virulence, are encoded on mobile genetic elements (Novick, 2003) allowing for horizontal transfer of these virulence factors. The gene encoding ETA is located on the bacterial chromosome within a 43 kb temperate phage, φETA (Yamaguchi et al., 2000), that was recently shown to be capable of transducing a toxin negative S. aureus strain to positive. ETB is encoded on approximately 38.2-38.5 kb plasmids (Lee et al., 1987; Yamaguchi et al., 2001). ETD was identified within a pathogenicity island which also encodes EDIN-B (Yamaguchi et al., 2002). Pathogenicity islands are varied sized segments of potentially mobile DNA that encode virulence associated genes (Kaper and Hacker, 1999). These islands have been described for numerous types of bacteria and at least three different types of pathogenicity islands have been reported in staphylococcal species (Kuroda et al., 2001). Also like most staphylococcal virulence factors, the ETs are regulated by the staphylococcal accessory gene regulator locus, agr locus (Ji et al., 1995; Novick, 2003). This locus encodes a two-component regulatory system that coordinates the expression of specific virulence factors not required for bacterial viability, including the exotoxins. The ETs have a strong association with a particular agr group, agr group IV, (Jarraud et al., 2000) but the clinical significance of this association remains to be established.

### Clinical conditions caused by the exfoliative toxin producing S. aureus.

Staphylococcal scalded skin syndrome and Bullous impetigo. Staphylococcal scalded skin syndrome (SSSS) is an exfoliative dermatitis mediated by infection with S. aureus capable of producing one or both of the exfoliative toxins ETA or ETB. This condition is well described and discussed in several recent reviews (Ladhani, 2001; Ladhani and Evans, 1998; Prevost et al., 2003). It is typically a condition of infants and young children, although it may also affect adults (Cribier et al., 1994; Gemmell, 1995). SSSS is characterized by the separation of extended areas of the upper epidermis specifically at the level of the stratum granulosum by disruption of the desmosomes after specific cleavage of desmoglein 1 (Amagai et al., 2000; Amagai et al., 2002). The condition can initiate with fever and erythema, followed by a positive Nikolsky’s sign and progress to the formation of large bullae and exfoliation of extensive areas of the upper epidermis of the skin. In SSSS this exfoliation occurs at a site that is separate from that of the infection. The infecting bacteria produce the exotoxin and release it into the blood stream where it makes its way to the skin and causes exfoliation/epidermolysis. Infections that result in SSSS may be as serious as pneumonia or sepsis in adults, or as trivial as otitis media or uncomplicated conjunctivitis in children.

### Table 1. Comparison of human active exfoliative toxins

<table>
<thead>
<tr>
<th>Toxin</th>
<th>Size</th>
<th>Molecular weight</th>
<th>Accession #</th>
</tr>
</thead>
<tbody>
<tr>
<td>ETA</td>
<td>242 aa</td>
<td>26.9 kDa</td>
<td>PO9331</td>
</tr>
<tr>
<td>ETB</td>
<td>246 aa</td>
<td>27.3 kDa</td>
<td>AAA26628</td>
</tr>
<tr>
<td>ETD</td>
<td>244 aa*</td>
<td>27.2 kDa</td>
<td>AB036767</td>
</tr>
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*Predicted number of amino acids after removal of signal sequence.
In infants and children the syndrome is usually associated with a trivial infective focus, 3% mortality with appropriate antibiotic therapy, and the patients are rarely bacteremic (Gemmel, 1995). Mortality in children is usually associated with secondary infections and other complications from the loss of the upper epidermis of the skin or failure to appropriately treat the underlying infection. However, hospital nursery outbreaks continue to be reported and are becoming increasingly associated with drug resistant strains of S. aureus (MRSA) (Cribier et al, 1994; Ito et al, 2002; Mackenzie et al, 1995).

In contrast, the syndrome in adults is usually associated with a chronic underlying medical condition. It is most often associated with old age, an immunocompromised state, renal deficiencies or diabetes mellitus (Cribier et al, 1994) but has been described in healthy adults (Oyake et al, 2001). There is often bacteremia and a greater than 50% mortality rate even with appropriate antibiotic therapy (Cribier et al, 1994). The increased mortality rate is likely heavily contributed to by the underlying medical condition that might have predisposed the adult to infection with S. aureus or to the affects of the exfoliative toxin itself. When SSSS has been reported in healthy adults there is usually insignificant morbidity or mortality (Oyake et al, 2001).

In SSSS the bullous fluid is characteristically a sterile transudate. There is no inflammatory cell infiltration associated with the bullous lesions or exfoliated areas unless or until there is a secondary infection at that location. This is in contrast to bullous impetigo, which is a localized skin infection with exfoliative toxin producing S. aureus. In BI there are bullous lesions with purulent exudates, local signs of inflammation, inflammatory cell infiltrates and necrosis, and S. aureus can usually be isolated from the lesions. Recently Gravet and coworkers showed that ET positive strains of S. aureus isolated from patients with impetigo were associated with the leukotoxin, LukE-LukD, in 78% of cases studied (Gravet et al, 2001). These pore forming toxins may contribute to the local inflammation and necrosis seen in BI, but their role in the condition has not been clearly established. To date, no association has been recognized with S. aureus strains isolated from clinical cases of SSSS and the leukotoxin, LukE-LukD. Koning and coworkers have also shown an association of ETB producing strains with P-V leukocidin, a toxin that is linked to necrotic pneumonias (Gillet et al, 2002), and the multidrug resistant plasmid pSK41 (Koning et al, 2003). They report that this combination of virulence factors contributes to the severity of nonbullous impetigo. However no association of these factors with BI or SSSS has been demonstrated. The role of the exfoliative toxins in the pathogenesis of bullous impetigo clearly appears to be the enhancement of local spread of the organism in a relatively protected plane, just under the stratum corneum in the upper epidermis, thus allowing for a successful infection by the organism. The presence of additional toxins such as the leukocidins or other potential virulence factors may augment this process and contribute to a successful infection by thwarting the host’s immune response. In contrast, the role of these toxins to promote bacterial survival and enhance their pathogenicity in SSSS is not so clear and warrants additional research. For a more extensive review of SSSS see references (Ladhani and Evans, 1998; Ladhani et al, 1999).

In addition to the associations with the leukotoxins LukE-LukD and P-V leukocidin, and the multidrug resistance plasmid pKS41 (Gravet et al, 2001; Koning et al, 2003), there are further reports linking the ETs with other virulence factors. ETD is also clonally linked to EDIN-B (C3stau) (Yamaguchi et al, 2002) and ETB has been linked to EDIN-C (Yamaguchi et al, 2001). There are three EDIN isoforms produced by S. aureus. EDIN-A was the first discovered as an inhibitor of morphological differentiation of epidermal keratinocytes in vitro and thus called epidermal cell differentiation inhibitor (EDIN) (Sugai et al, 1992). It is now accepted that the EDINs belong to the C3 family of bacterial Rhoades-specific monoadophosphoribosyltransferases that specifically modify eukaryotic small GTP-binding proteins belonging to the Rho family (Sugai et al, 1992). Rho GTPases are central regulators of the eukaryotic actin cytoskeleton and their inactivation blocks important cellular functions including differentiation, chemotaxis, phagocytosis and oxidative burst (Aktories et al, 2000). The clinical significance of the association of the EDINs with the ETs and the role of EDINs in the pathogenesis of S. aureus are not clear. Likewise the role of ETD is unclear as it has only been rarely isolated (1 in 88 isolates) from S. aureus obtained from impetigo patients (Yamaguchi et al, 2002; Yamaguchi et al, 2002), but warrants further investigation.

The characteristic separation of the epidermis at the stratum granulosum seen in SSSS is also demonstrated in the neonatal mouse model (Melish and Glasgow, 1970) Figure 1. Animal models of SSSS show identical response to either of the purified ETs. This cleavage is associated with a disruption of the desmosomes, with the surrounding cells remaining intact. The mechanism by which these toxins cause exfoliation is now known to involve cleavage of desmoglein 1 (Dsg1), a desmosomal protein member of the cadherin family of cell adhesion molecules. This is accomplished by a unique serine protease activity of the exfoliative toxins acting at a specific extracellular site on Dsg1, as recently described (Amagai et al, 2000). Dsg1 is the predominant cell-cell adhesion molecule in the upper epidermis where the separation occurs. Dsg1 amino acid sequences are highly conserved among mammalian species and share a high percentage of identity (Koch and Franke, 1994; Nilles et al, 1991). In other studies ETA has been shown to cleave α- and β-melanocyte stimulating hormones after a lengthy incubation period (Rago et al, 2000); however, it is unclear how this might result in or contribute to SSSS or BI. Also, other investigators studying ETB have purified a distinct 20 kDa protease from a supernatant of preparations of neonatal mouse epidermis treated with ETB which was able to reproduce epidermolysis similar to that of SSSS when injected into neonatal mice (Ninomiya et al, 2000). These results would suggest the presence of an additional target for the ETB versus ETA, as Dsg1 has been shown to be

Figure 1. Panel A: normal newborn Balb/c mouse skin; Panel B, Newborn Balb/c mouse injected with purified ETA.
Relative protection of healthy adults against the effects of the toxins. SSSS and BI are primarily diseases of infants and children and SSSS only rarely develops in adults (Cribier et al, 1994; Gemmell, 1995). In the mouse model of SSSS it was originally shown that newborn mice would exfoliate in response to injection with ET producing *S. aureus*, but that mice greater than 7 days old would not exfoliate. A time course of susceptibility of newborn mice to these toxins was recently generated and showed that until day 7 of life, mice were sensitive to the affects of a single injection of a low dose of the toxin. However, after 8 days of life there is a dramatic decrease in response to these exotoxins such that these mice failed to respond to even 10 times the dose that caused exfoliation in the population 24 hours prior (Plano et al, 2001). The mechanism of the relative protection in healthy adults who are exposed to the ETs is not completely understood but likely is a multifactorial process. It is known that in mice the adaptive immune response matures within the same time frame as they developed resistance to ETA and ETB (Adkins, 1999). It has been shown that over time healthy humans will produce antibodies that recognize the exfoliative toxins. In one study, more than 50% of persons over the age of 10 years had antibodies that recognize ETA (Melish et al, 1981), however it was undetermined whether these were specific antibodies raised against ETA or against a cross reacting material. The fact that only about 5% of all clinical *S. aureus* isolates are capable of producing ETA argues against these being specific antibodies. In a recent study conducted in our labs we investigated the role of the adaptive immune response in the protection of adult mice and concluded that it does not play a primary role in defense of 10 day old mice against the effects of the ETs (Plano et al, 2001). The role of the adaptive immune response was investigated using two immune compromised mouse populations: (i) mice deficient in T cells (BALB/c mice thymectomized as newborns and allowed to grow to adulthood) and (ii) mice deficient in both T and B cell (CB-17 SCID mice) and therefore unable to produce antibodies. Neither of these immunodeficient populations of mice was affected by exposure to the exotoxin. Furthermore, attempts to protect newborn mice from the effects of the ETs with intravenous injections of normal adult mouse spleen cells either prior to or at the time of exposure to the ETs failed. From these data it was concluded that although clearly the adaptive immune response can play a role in protecting against the exotoxins once specific antibodies are made, it does not play a primary role in the protection of adult mice from the effects of these toxins.

Rapid clearance of the ETs from the serum was however shown to play a key role in protection of adult mice (Plano et al, 2001). Serum clearance from 1 day and 10 day old mice were compared after a single injection with ETA. While the maximal level of toxin found in the serum was similar for both ages, levels peaked earlier and were cleared earlier in the 10 day old mice, which showed no signs of response to the toxin, as compared to the one day old mice which began to exfoliate after 2 hours of exposure to the toxin. Therefore, ETA was not cleared as efficiently in the one-day old mice as in the 10 day old mice. To address whether a sustained high level of ETA would result in exfoliation, 10 day old mice were given repeated injections of toxin so that the maximal level of toxin was maintained for a period of time (4 hours). Exfoliation occurred in these mice confirming the presence and accessibility of the target for ETA in this population and the ability of the toxins to affect adults under certain conditions. It was concluded that whereas the adaptive immune response is not needed for protection of adult mice from SSSS, efficient clearance of the toxin from the bloodstream is a critical factor (Plano et al, 2001). The acuteness with which the mice were no longer susceptible to the affects of the toxin suggests that in addition to the development of the system required to clear the toxin (probably renal), other events, perhaps differential expression of unidentified components in the skin, may be contributing to the protection of adults.

**Mechanisms of action:**

**Exfoliative toxins as potential superantigens.**

There was an ongoing debate as to whether the exfoliative toxins function as superantigens that contribute to the pathogenesis of SSSS, and there are several publications addressing this question (Bailey et al, 1995; Monday et al, 1999; Plano et al, 2000; Vath et al, 1997). Bacterial superantigens are a family of proteins able to bind simultaneously to the major histocompatibility complex class II and to the T-cell receptor (TCR), resulting in stimulation of a large number of T-cells expressing specific Vβ subsets of the T-cell repertoire (Marrack and Kappler, 1990). This stimulation then leads to the release of numerous cytokines and subsequent systemic symptoms. Classic superantigen mediated diseases, such as toxic shock syndromes, are associated with erythematous rash, hypotension and multiorgan system failure. Although SSSS can include erythema, fever and purulent infective foci, these are not always present. The controversy as to whether the ETs were superantigens stemmed from the fact that although for over 30 years it has been known that the ETs are responsible for exfoliation/epidermolysis (Melish, 1971) in SSSS, the precise mechanism by which this occurs was not known until recently (Amagai et al, 2000). Although stimulation of specific Vβ populations of T-cells has been demonstrated (Rago et al, 2000), the SSSS generally lacks the severe systemic symptoms associated with a superantigen mediated syndrome. It is unlikely that this activity plays a major role in the pathogenesis of SSSS but may contribute to other disease processes. It is now generally accepted that in SSSS and BI the exfoliative toxins function as unique serine proteases that specifically cleave desmoglein 1.

**Exfoliative toxins are unique serine proteases.**

For many years the mechanism of action of the exfoliative toxins was unclear, however there was mounting indirect evidence that ETA and ETB were serine proteases (Dancer et al, 1990). Both ETA and ETB share amino acid identity with staphylococcal V8 protease (glutamy l endopeptidase I), (26% with ETA and 31% with ETB) and most significantly, this identity includes residues of the V8 protease serine-histidine-aspartate catalytic triad. This catalytic triad is a signature sequence common to trypsin-like serine proteases. Mutant forms of ETA that contain amino acid substitutions at any one of the residues of the catalytic triad (S195, H72, D120, ETA numbering) have no activity as exfoliants in the animal model of SSSS (Prevost et al, 1992; Prevost et al, 1991).
Both ETA and ETB have esterase activity versus a synthetic substrate, a common property of serine proteases (Bailey and Redpath, 1992). In addition, as stated above, after prolonged incubation times, ETA and ETB are able to cleave α and β-melanocyte-stimulating hormone (Rago et al., 2000). Until the recent report that ETA cleaves desmoglein 1, the strongest evidence that ETA and ETB are proteases came from the X-ray crystal structures of these proteins. (Cavarelli et al., 1997; Papageorgiou et al., 1999; Vath et al., 1999; Vath et al., 1997). Comparisons of the X-ray crystal structures of both of these toxins as well as an inactive ETA with a mutation at the active site serine (Ser195Ala variant) with that of glutamyl endopeptidase I clearly demonstrate that the ETs are members of the trypsin-like serine protease family. The structures also show that they have significant differences at the amino- and carboxy- termini and loop regions when compared to other like serine proteases (Papageorgiou et al., 1999). The catalytic site of both ETs also are very similar to those of glutamate-specific serine proteases (V8) suggesting a similar catalytic mechanism. However, differences in the structures show that a part of the catalytic site is in an inactive conformation in crystalized ETA, but in an active conformation in ETB. This suggests that some action is necessary to put ETA into an active confirmation. It has been postulated that this could be accomplished by the binding of ETA to a specific target possibly involving the unique amino terminus (Papageorgiou et al., 1999). Analysis of the structure of the inactive Ser195Ala variant showed no significant perturbation at the active site, suggesting that its loss of biological activity as an exfoliant is attributed solely to disruption of the catalytic serine residue (Papageorgiou et al., 1999).

Exfoliative toxins specifically cleave the cadherin protein Dsg1 but not the closely related Dsg3. The autoimmune condition pemphigus foliacious (PF) is characterized by blistering in the superficial epidermis, essentially identical to SSSS (Amagai, 1999; Mahoney et al., 1999; Stanley, 1993). This blistering is caused by the disruption of desmosomes after loss of function of the cadherin protein Dsg1 because of the interaction with a specific autoantibody to Dsg1. The desmosomal cadherin proteins, desmogleins and desmocollins, are the major cell adhesion molecules and Dsg1 is the predominant desmosomal cadherin protein in the upper epidermis of the skin while Dsg3 is found in the lower epidermis (Wu et al., 2000). With the evidence that the ETs appeared to be potential serine proteases, and the knowledge that PF antibodies injected into neonatal mice cause blistering in the superficial epidermis, like that caused by the ETs, Amagi and coworkers reasoned that a logical target for ETs would be Dsg1. They initially examined skin from neonatal mice injected with ETA by immunofluorescence using antibodies against Dsg1 and Dsg3 as a control. They focused on the deeper layers of the epidermis, basal and immediate suprabasal areas that have Dsg1 and Dsg3 but do not blister. Treatment with ETA caused significant changes in Dsg1 staining consistent with cleavage and subsequent internalization of the cleavage products (Amagai et al., 2000). Dsg3 staining was not affected by ETA, supporting its role in maintaining adhesion at this level of the epidermis after exposure to an ET, (desmoglein compensation) (Mahoney et al., 1999). To demonstrate actual cleavage of Dsg1 they used a human keratinocyte cell line to express cloned mouse Dsg1 or Dsg3. They treated the transfected cells with ETA and showed by Western blot analysis, that only Dsg1 was cleaved. To confirm these findings in vivo they took extracts of skin from neonatal mice that developed blisters after injection with ETA and showed by Western blot analysis using antibodies against Dsg1, Dsg3 and epithelial cadherin that only Dsg1 was cleaved. These data confirmed that treatment with ETA resulted in Dsg1 cleavage but did not demonstrate direct cleavage by the ET. To confirm that ETA was directly responsible for Dsg1 cleavage they isolated the extracellular domains of both mouse and human Desg1, and Dsg3 as a control, using a eukaryotic expression system to produce these protein products. Using these purified proteins they successfully demonstrated that ETA directly cleaved the extracellular domain of both mouse and human Dsg1 but not Dsg3 in a dose dependent manner. In similar experiments these investigators have now demonstrated that ETB (Amagai et al., 2002) and ETD (Hanakawa et al., 2002) also specifically cleave Dsg1 but not Dsg3 of mouse and humans. They have also identified a single cleavage site within the extracellular segment of Dsg1 at glutamate 381 (E381) between extracellular cadherin domains 3 and 4. Previous studies have demonstrated that the first three extracellular cadherin domains of desmoglein are needed for calcium dependent heterophilic adhesion to the desmocollins (Chiaev and Troyanovsky, 1997). Cleavage at this site, E381, would release the portion of the Dsg1 involved in this heterophilic adhesion disrupting the desmosome. In additional experiments using immunofluorescence colocalization and communoprecipitation, these investigators have also demonstrated that an exfoliation inactive mutated ET specifically binds to Dsg1 (Hanakawa et al., 2002). These data together clearly demonstrate that the mechanism of action of these toxins is specific recognition and proteolytic cleavage of the structurally critical adhesion molecule, Dsg1.

Differential diagnosis of the conditions caused by the ETs. Distinguishing between SSSS and toxic epidermal necrolysis (TEN) is critical as misdiagnosis of one for the other could lead to harmful therapies. Like SSSS, TEN is a generalized blistering condition that can involve erythema and skin tenderness. The blisters in TEN are deeper in the epidermis, primarily in the basal cell layer, and are brought on by reactions, mainly to drugs. Antibiotic treatment indicated for the S. aureus infection associated with SSSS could worsen the prognosis in TEN. A hallmark difference in these conditions is the lack of mucous membrane involvement in SSSS. This difference along with the clinical history are usually sufficient to make the diagnosis, although definitive diagnosis can be made by histology from a full thickness skin biopsy. The mechanism of action of the ETs, specific cleavage of Dsg1, along with the distribution of this precise target explains the clinical differences seen in SSSS versus TEN. Desmoglein 1 is expressed in the epidermis; therefore, it is now clear that there is no mucous membrane involvement in SSSS because there is no target available for the toxins to act upon.

Pemphigus foliacious and vulgaris are blistering conditions caused by reaction of auto-antibodies to Dsg1 and Dsg3 respectively. In either of these conditions antibodies to both desmogleins can be present and the depth of the lesions will be affected based on the predominant
antibodies. Therefore, PF with only antibody to Dsg1, will look indistinguishable from SSSS by histology. Clinical history is usually sufficient to make the diagnosis versus SSSS. One would need to establish a history of erythema, fever and try to identify the infectious focus with S. aureus to establish the diagnosis of SSSS.

It was recently reported that there is differential expression of Dsg3 in human neonatal versus adult skin (Wu et al., 2000). These investigators reported that in the neonate Dsg3 is expressed throughout the epidermis while in the adult it is limited to the basal and suprabasal layers. The knowledge of the differential expression of Dsg1 and Dsg3 in neonatal versus adult skin can explain the clinical finding that newborns of mothers with PF appear to be protected against blistering from maternal anti-Dsg1 antibodies. It has been postulated that the presence of Dsg3 in the upper epidermides compensates for antibody induced loss of Dsg1 function thereby inhibiting blister formation. These facts could be confusing when trying to explain the lack of desmoglein compensation in SSSS in this same population. Likewise, if the primary desmoglein in the adult upper epidermis is Dsg1 why are adults not more sensitive to the affects of these toxins? Recent studies showed that sustained high levels of ETs were sufficient to overcome any desmoglein compensation that protected 10 day old mice from the affects of the ETs (Plano et al., 2001).

The protection of adults is directly related to serum clearance of the toxins most probably due to a maturing renal system, but the timing of protection demonstrated during the mouse time course (Plano et al., 2001) was extremely acute and might not be completely explained by a gradual maturation of the system required for clearance. This is also supported by the occurrence of SSSS by a gradual maturation of the system required for clearance and not extremely acute and might not be completely explained by a gradual maturation of the system required for clearance. This would suggest that maturation and or expression of as yet unidentified or uncharacterized skin components might also be contributing to the relative protection of adults from the affects of the toxins. Recently newly identified desmogleins were reported in mice (Whittock, 2003). The newly identified mouse Dsg5 has significant nucleotide homology, 96%, with Dsg1 and has identical amino acid sequence at the cleavage site of Dsg1. No information is available at this time about levels of expression in the adults or humans, however given the highly conserved nature of this family of proteins one would expect that similar proteins could be found in human skin and might be contributing to protection in the adult. Whittock and Bower have also reported genetic evidence for an additional desmoglein isoform (Dsg4) in the human genome (Whittock and Bower, 2003). These data considered together supports the need for further studying this area.

**Future areas of research.** One of the most significant remaining questions and an area of potential ongoing research is what causes these toxins to be targeted to the skin. In 1976, Fritsch and colleagues, using radioactive iodine labeled ET, showed that the toxins preferentially concentrate in the skin in newborn mice (Fritsch et al., 1976). In recent studies in our laboratory we have shown the presence of ETs in the skin of both newborn susceptible species and non-susceptible species (newborn Balb/c mice and newborn rats respectively) after injection with purified toxin. This suggests that targeting to the skin may be governed by a process or mechanism separate from those that determine target specificity (Plano unpublished data). The crystal structure of the ETs revealed unique areas in both the amino and carboxy-terminal regions that could potentially play a role in specifically targeting these toxins to the upper epidermides.

**Acknowledgements**

Part of the work presented was supported by grant #K08 AI01466 from the National Institutes of Health. I would like to thank Michelle Perez for help in preparation of the manuscript and Dr. George Munson for critical review.

**References**


Fritsch et al., 2001) was...


STAPHYLOCOCCUS AUREUS EXFOLIATIVE TOXINS

In recent studies published after submission of this manuscript, Hanakawa, et al have further characterized the mechanism of action of the exfoliative toxins and aspects of the interaction of the toxins and their targets desmoglein 1, that contribute to their specificity. They have shown that not only do the toxins recognize a specific amino acid sequence, they also require a calcium dependent conformational change in desmoglein 1 for cleavage. They showed that exfoliative toxins could not cleave Dsg1 after pretreatment at 56°C or at high or low pH, suggesting that a proper conformation was needed for cleavage. They further confirmed that calcium depletion of Dsg1 caused a conformational change that resulted in the inability of a previously active toxin to cleave its target (Hanakawa et al., 2003). In a subsequent study they report initial kinetics with kcat/Km values that suggest very efficient proteolysis by these toxins. Using truncated mutant human Dsg1 and chimeric Dsg’s generated with human Dsg1 and either human Dsg3 or canine Dsg1 they identified regions of Dsg1 upstream from the cleavage site important for both binding and specific cleavage by these toxins (Hanakawa et al., 2004). These data support the complexity of the toxin target interactions that govern the exquisite species specificity and probably contribute to the age specificity of the syndromes caused by these toxins and validate the importance of continued investigation of these unique toxins.


Papageorgiou AC, Plano LRW, Collins CM, Acharya KR: Structural differences in Staphylococcus aureus exfoliative toxins A and B as revealed from their crystal structures. Submitted 1999


Staphylococcus aureus exfoliative toxins: How they case disease.

Lisa R W Plano

Update of recent studies

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