See related article on page 2921

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Commentary

Cleavage Isn't Everything

Potential Novel Mechanisms of Exfoliative Toxin-Mediated Blistering

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Bullous impetigo and staphylococcal scalded skin syndrome are blistering skin diseases caused by infection with strains of *Staphylococcus aureus* that elaborate exfoliative toxins, resulting in the cleavage of the keratinocyte cell adhesion protein desmoglein 1.[1] Desmoglein 1 is a desmosomal cadherin expressed in the superficial epidermis and is the target of antibodies in the autoimmune blistering skin disease pemphigus foliaceus. Skin histology is often indistinguishable between the autoimmune and staphylococcal-mediated conditions, as both exhibit loss of intercellular adhesion in the superficial layer of keratinocytes. In pemphigus foliaceus, the autoimmune nature of the disease can be distinguished by immunofluorescence studies that detect the binding of patient autoantibodies to the cell surface of skin keratinocytes. In contrast, immunofluorescence findings are negative in staphylococcal-mediated disease. Bullous impetigo represents a localized skin infection with *S. aureus*, resulting in superficial blisters that quickly break open, leaving classic "honey crust" erosions. Rarely, localized infections with *S. aureus* can progress to bacteremia or sepsis with more widespread blistering due to systemic elaboration of toxin in staphylococcal scalded skin syndrome; in these cases, bacterial cultures of blister fluid are usually negative for growth of *S. aureus*. In this issue of *The American Journal of Pathology*, Simpson et al shed light on the cellular mechanism leading to loss of cutaneous integrity in bullous impetigo and staphylococcal scalded-skin syndrome.

Pemphigus Neonatorum: Identification as a Desmosomal Disease

The first cases of staphylococcal-mediated blistering disease appeared in the literature as early as 1773 and were astutely termed *pemphigus neonatorum* by 19th century physicians.[3,4] In 1878, Gottfried Ritter von Rittershain published the largest series of patients to date with staphylococcal scalded skin syndrome.[5] He described a nearly 50% mortality rate among 279 patients, thereby distinguishing "Ritter's disease" from the more benign course associated with localized disease. In the 1970s, Marian Melish and Lowell Glasgow isolated staphylococcal exfoliative toxin and demonstrated it was the causative agent of bullous impetigo and staphylococcal scalded skin syndrome.[6–8] Subsequently, the genes for exfoliative toxins A and B were cloned,[9–11] and the crystal structure of exfoliative toxin A (ETA) suggested its function as an atypical serine protease.[12] In 2000, desmoglein 1 was identified as a target of exfoliative toxin proteolytic cleavage.[13] Further studies showed that exfoliative toxins cleave the desmoglein 1 ectodomain after glutamic acid residue 381, leaving a truncated transmembrane protein lacking the *trans*-adhesive interface.[14] Collectively, these studies described staphylococcal exfoliative toxin as a novel virulence factor that cleaves off the desmoglein 1 adhesive ectodomain, thereby disrupting cell-cell adhesion. The resulting skin blister creates a portal of entry for *S. aureus* to access the superficial epidermis where it can further proliferate. Thus, more than two centuries after the first descriptions of disease, the molecular pathogenesis of bullous impetigo and staphylococcal scalded skin syndrome appeared to be solved. There is beauty in simplicity. But is that all there is to the story?

A Role for Plakoglobin in Staphylococcal-Mediated Blistering Disease

Simpson et al[2] now propose an additional mechanism for exfoliative toxin-mediated blistering through sequestra-

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tion of plakoglobin by ectodomain-truncated desmoglein 1. Plakoglobin, also known as γ-catenin, is a cytoplasmic plaque protein that can associate with cadherins in both desmosomes and adherens junctions. Demonstrating its predominant role in desmosomes, plakoglobin is required to confer strong desmosomal but not adherens junction adhesion on otherwise nonadherent fibroblasts,[15] and plakoglobin-null keratinocytes demonstrate delayed desmosome formation, leaving adherens junctions relatively unaffected.[16] Plakoglobin's essential role in human desmosome biology is evidenced by Naxos disease, characterized by wooly hair, palmoplantar keratoderma, and potentially fatal arrhythmogenic right ventricular cardiomyopathy due to decreased adhesion of cardiac myocytes leading to ventricular rupture. In pemphigus vulgaris, an autoimmune blistering disease characterized by autoantibodies to the desmosomal cadherin desmoglein 3, plakoglobin remains associated with internalized desmoglein 3, which is ultimately targeted for lysosomal degradation.[17] Previously, it was shown that mice expressing a mutant desmoglein 3 lacking the adhesive extracellular domain exhibit defective desmosomes that are reduced in number, aberrant membrane clusters of plakoglobin, and hyperproliferation and abnormal differentiation of the epidermis.[18] Based on these studies, the authors sought to determine whether pathophysiologic mechanisms of exfoliative toxin-cleaved desmoglein 1 may extend beyond simple ectodomain cleavage.

The authors expressed a mutant desmoglein 1 protein lacking the amino-terminal 381 amino acid residues (Δ381-Dsg1) in primary human keratinocytes to mimic the exfoliative toxin-cleaved protein. Ectodomain-truncated desmoglein 1 localized to the keratinocyte cell surface, disrupted the keratinocyte cell surface staining of the desmosomal proteins desmocollin 3 and desmoplakin, reduced desmocollin 3 protein levels in whole cell lysates, and compromised the adhesive strength of keratinocytes in a cultured cell dissociation assay. Because plakoglobin has been shown to regulate levels of desmosomal cadherins in plakoglobin-deficient keratinocytes,[16] the authors hypothesized that the negative effects of ectodomain-truncated desmoglein 1 on cell adhesion were due to sequestration of plakoglobin. Consistent with this hypothesis, a triple point mutation of the cytoplasmic plakoglobin binding site of Δ381-Dsg1 abolished its interaction with plakoglobin and rescued defects in desmosomal protein levels and cell adhesion. Interestingly, expression of the desmoglein 1 cytoplasmic domain alone, or a chimeric protein combining the extracellular and transmembrane domains of the interleukin 2 receptor with the cytoplasmic domain of desmoglein 1, did not compromise cell adhesion, suggesting that desmosomal localization is necessary for the dominant negative effect.

As an alternative strategy to rescue desmosomal adhesion, exogenous expression of plakoglobin restored cell surface localization of desmosomal proteins in keratinocytes expressing ectodomain-truncated or toxin-cleaved desmoglein 1, and in part rescued the cell adhesion defects. Recent studies have indicated that plakoglobin mRNA and protein levels are up-regulated in epithelial cells after histone deacetylase inhibition.[19,20] To determine whether histone deacetylase inhibitors similarly up-regulate plakoglobin in human keratinocytes, the authors treated primary keratinocytes with trichostatin A. Trichostatin A slightly up-regulated plakoglobin expression, but more significantly up-regulated protein levels of desmoglein 1 and desmocollin 3 in human keratinocytes. Trichostatin A also rescued the adhesive defects in primary keratinocytes expressing Δ381-Dsg1 or treated with exfoliative toxin.

From Bench to Bedside

Simpson et al provide convincing evidence that adhesive defects caused by ectodomain-truncated desmoglein 1 are dependent on plakoglobin sequestration. Increasing the level of cellular plakoglobin rescues desmosome organization and cell adhesion defects caused by ectodomain-truncated or toxin-cleaved desmoglein 1, potentially through up-regulation of desmocollin 3 expression. These findings add to the complexity of exfoliative toxin-induced pathology and provide scientific support for novel therapeutic strategies. As with all good lines of inquiry, the study highlights several avenues for further investigation.

One issue relevant to the pathophysiology of staphylococcal scalded skin syndrome is whether the ectodomain-truncated desmoglein 1 accurately reflects the fate of exfoliative toxin-cleaved desmoglein 1 *in vivo*. The authors show that in organotypic raft cultures, exfoliative toxin-cleaved desmoglein 1 is internalized together with plakoglobin and desmocollin 1, although desmoglein 1 in the basal, nonblistered layers of the epidermis remains detectable at the cell surface. Previous studies have also suggested that acutely toxin-cleaved desmoglein 1 is internalized.[13] A recent study examined the localization of desmosomal proteins in the skin of patients with staphylococcal scalded skin syndrome, including the desmoglein 1 ectodomain and endodomain.[21] In nonlesional skin, cell surface desmoglein 1 endodomain staining was preserved, even in areas where ectodomain staining was lost. However, nearer to the blister, disruption of both cell surface ectodomain and endodomain staining occurred. No changes in desmocollin 3, desmoglein 3, or plakoglobin localization were observed. Further studies are necessary to clarify the fate of the desmoglein 1 endodomain *in vivo*.

Whether histone deacetylase inhibition can be used effectively as adjunctive therapy for staphylococcal mediated blistering disease, or autoimmune blistering disease, is an intriguing concept. Traditional therapy for staphylococcal scalded skin syndrome uses double antibiotic coverage including the bacterial protein synthesis inhibitor clindamycin, which interferes with toxin production. Several histone deacetylase inhibitors currently in clinical use, such as vorinostat and romidepsin, are associated with significant side effects including immune suppression, which would be undesirable in cases of fulminant infection. However, other classes of histone deacetylase inhibitors, such as valproic acid and nicotin-

amide, are not associated with significant immune suppression. Vorinostat has been effective in treating the autoimmune blistering disease bullous pemphigoid.[22] There is also anecdotal support for the use of nicotinamide, either topical or oral, as an adjunctive therapy for pemphigus.[23–25] Future studies may further delineate the specific protein targets of the different histone deacetylase inhibitors, leading to more effective and potentially safer therapies for these life-threatening skin diseases.

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