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Desmosomes and disease: pemphigus and bullous impetigo

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Desmosomal cadherins are the pathophysiologic targets of autoimmune or toxin-mediated disruption in the human diseases pemphigus and bullous impetigo (including its generalized form, called staphylococcal scalded skin syndrome). Experiments exploiting the production of both pathogenic and nonpathogenic antidesmoglein antibodies in pemphigus patients' sera have afforded data that make an invaluable contribution towards identifying the functional domains of the desmogleins involved in intercellular adhesion. Conformational epitopes of antidesmoglein autoantibodies in pemphigus patients' sera and the specific cleavage site of desmoglein 1 by exfoliative toxin have been identified, implicating the N-terminal extracellular domains of the desmogleins as critical regions for controlling intercellular adhesion. Furthermore, the development of active autoimmune mouse models for pemphigus allows *in vivo* characterization of the disease and its pathogenesis. These studies offer new insight into the potential mechanisms of acantholysis in pemphigus and staphylococcal-associated blistering disease, with implications for the role of desmogleins in desmosomal structure and function.

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Abbreviations

Dsg	desmoglein
ET	exfoliative toxin
FS	fogo selvagem
IgG	immunoglobulin G
mAb	monoclonal antibody
MP	methylprednisolone
PF	pemphigus foliaceus
PV	pemphigus vulgaris
SSSS	staphylococcal scalded skin syndrome

Introduction

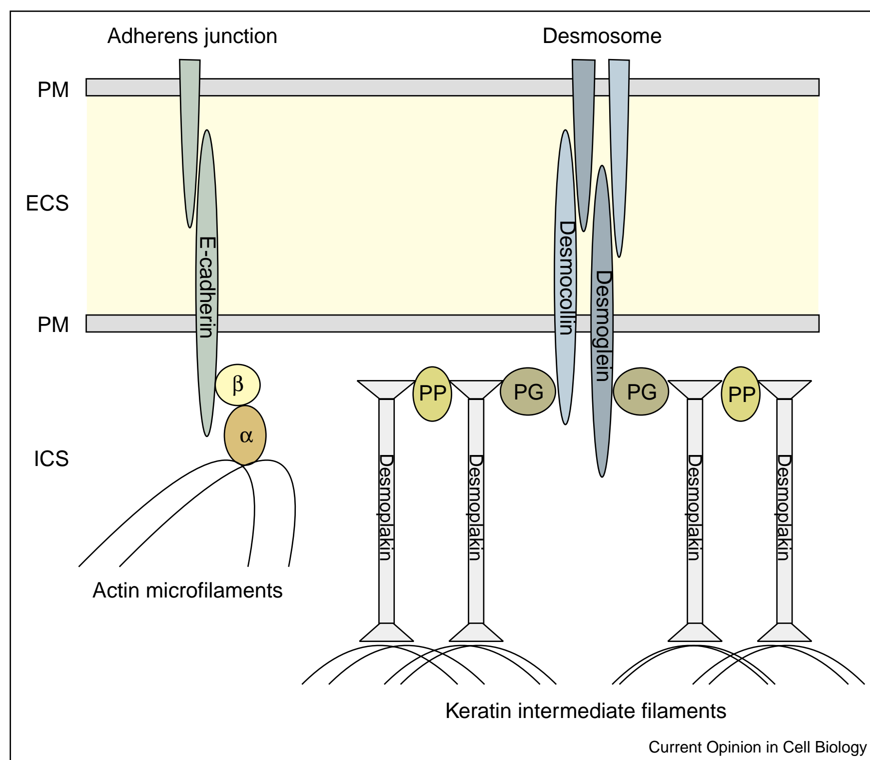
Desmosomes are intercellular adhesive junctions that are structurally linked to the intracellular intermediate filament network. They are expressed by epithelial cells and a few other cell types and are abundant in tissues under mechanical stress, such as the skin, gastrointestinal mucosa, heart and bladder. Desmosomes, together with adherens junctions, provide the major intercellular adhesive forces within simple polarized epithelia. The intercellular space of desmosomes contains two major types of transmembrane glycoproteins, desmogleins and desmocollins, which are homologous to the classical cadherins found in adherens junctions (Figure 1) [1,2]. The cytoplasmic domains of the desmosomal cadherins bind plakoglobin, a member of the armadillo family of proteins [3], which also includes β-catenin (an intracellular component of adherens junctions) and plakophilin. Classical cadherins bind either β-catenin or plakoglobin, but preferentially the former in desmosome-containing cells. Here the structural homology of adherens junctions and desmosomes diverges, as classical cadherins associate with the actin cytoskeleton by binding to the vinculin homologue α-catenin, whereas desmosomal plakoglobin binds to the plakin family member desmoplakin, which links the desmosome to keratin intermediate filaments [4].

Because the desmosomal glycoproteins were found in the intercellular space of adhesion junctions, it was hypothesized that their function was to provide cell–cell adhesion, analogous to the role of classical cadherins within adherens junctions. This review details how the study of two human diseases, pemphigus and bullous impetigo (and its generalized form staphylococcal scalded skin syndrome), has confirmed this hypothesis and elucidated both the function of these glycoproteins, in particular the desmogleins, and the pathophysiology of these diseases.

Pemphigus: autoimmune diseases of cell adhesion

Pemphigus is a potentially fatal blistering disease of the skin and mucous membranes characterized by the loss of intercellular adhesion (acantholysis) of keratinocytes due to binding of autoantibodies to the cell surface (reviewed in [5]). In pemphigus foliaceus (PF), blisters occur in the granular cell layer of the superficial epidermis and affect only the skin, whereas blisters of pemphigus vulgaris (PV) develop in the suprabasal portion of the deep epidermis and typically affect mucous membranes first, followed by skin. Direct immunofluorescence on perilesional skin in

Figure 1



Simplified diagram of the relationship of the desmosomal proteins as compared to adherens junctions. α, alpha catenin; β, beta catenin; ECS, extracellular space; ICS, intracellular space; PG, plakoglobin; PM, plasma membrane; PP, plakophilin.

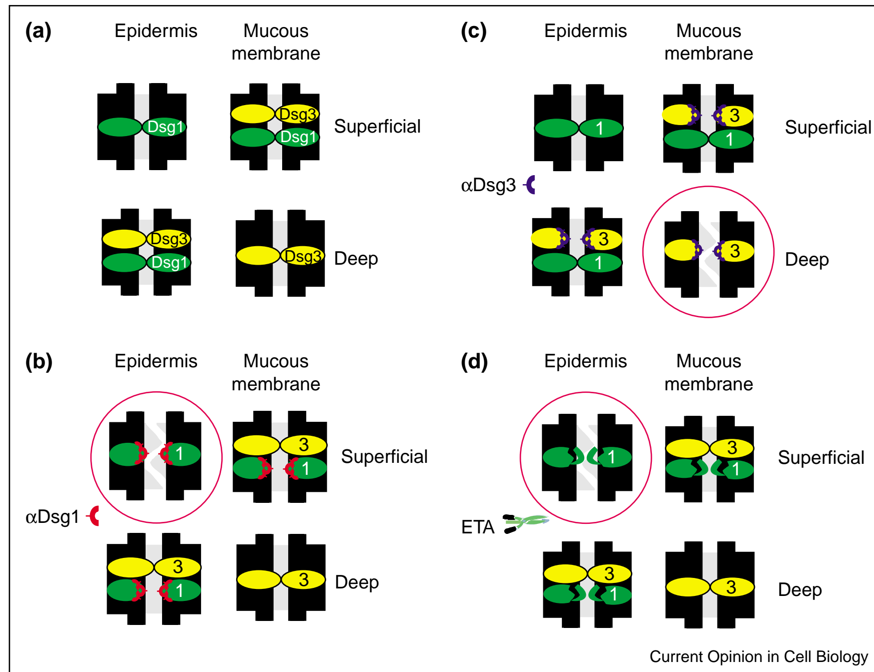
both types of pemphigus detects immunoglobulin G (IgG) on the surface of keratinocytes throughout the epidermis. Indirect immunofluorescence can be used to quantify the titer of antibodies to the cell surface of normal keratinocytes in pemphigus patients' sera.

The characteristic pathology of the disease and the immunofluorescence findings suggested that desmosomes may be a potential target of autoantibodies in pemphigus patients. Immunochemical studies identified the PF 160 kD antigen as desmoglein 1 (Dsg1) [6], the first member of the desmoglein family. PV antigen was characterized as a 130 kD glycoprotein, which was subsequently cloned using PV patients' sera and shown to be another desmoglein, desmoglein 3 (Dsg3) [7]. Plakoglobin, previously identified as an intracellular protein of adherens junctions and desmosomes, was co-precipitated with both PF and PV sera [8], identifying that this protein is bound to desmoglein in the desmosomal plaque.

Enzyme-linked immunosorbent assay (ELISA) studies have shown that all PF sera contain autoantibodies against Dsg1, and sera from patients with mucosal-dominant PV react mainly against Dsg3 [9–11]. PV patients who progress from mucosal to mucocutaneous lesions develop anti-Dsg1 in addition to anti-Dsg3 antibodies [12]. Even in the rare patient who transitions from PF to PV or vice versa, the antibody profile changes to be characteristic of the disease type [13,14]. The pathogenicity of pemphigus sera is due to antidesmoglein antibodies, since neonatal mouse passive transfer studies have shown that the extracellular domains of Dsg1 and Dsg3 can adsorb out all pathogenic antibodies from PF and PV sera, respectively, and affinity-purified anti-Dsg1 or anti-Dsg3 antibodies cause characteristic disease [15–18].

The different histological sites of blister formation in PV and PF, despite the usually identical immunofluorescence findings in the two diseases, is in part explained by the concept of desmoglein compensation [19,20]. Dsg1 and Dsg3 demonstrate inverse expression patterns in the epidermis, with Dsg1 predominant in the superficial epidermis and less so in the deep epidermis, whereas Dsg3 is only expressed in basal and lower epidermal keratinocytes [19–22]. In mucous membranes, Dsg1 is expressed in the superficial epidermis with little if any expression in the basal layers, whereas Dsg3 is expressed throughout (Figure 2a). PF anti-Dsg1 IgG causes blisters in the superficial layers of the epidermis but not in the deep epidermis or mucosa, where the expression of Dsg3

Figure 2



Desmoglein compensation: explanation for the clinical and microscopic localization of blisters in pemphigus and bullous impetigo. For simplicity, only desmogleins are depicted. **(a)** Expression patterns of Dsg1 and Dsg3 in skin and mucous membranes. **(b)** Localization of blisters in pemphigus foliaceus. Anti-Dsg1 antibodies cause blisters in the superficial skin and not mucous membranes. **(c)** In mucosal dominant PV, anti-Dsg3 antibodies cause blisters in the deep mucous membranes. **(d)** Localization of blisters in bullous impetigo. Exfoliative toxins specifically cleave Dsg1. The presence of Dsg3 in the deep epidermis and mucous membranes results in superficial blisters in only the skin, identical to what is observed in pemphigus foliaceus. In (b), (c) and (d), Dsg1 is denoted by 1 and Dsg3 by 3. Red circles indicate the site of blister formation.

compensates for the antibody-induced functional loss of Dsg1 (Figure 2b). Similarly, in mucosal PV, anti-Dsg3 IgG causes acantholysis in the deepest layer of the mucous membranes where Dsg1 expression is minimal (Figure 2c). In tissues where both Dsg1 and Dsg3 are expressed, both anti-Dsg1 and anti-Dsg3 antibodies are required for pathogenicity, analogous to the autoantibody profile that is observed in mucocutaneous PV. These observations led to the proposition of a 'desmoglein compensation' theory, which suggests that Dsg1 can maintain adhesion without Dsg3 and *vice versa*, at least under baseline non-traumatic conditions. Consistent with this hypothesis is the observation that pemphigus is not unusual in neonates born to mothers with PV, but is very rare in neonates born to mothers with PF. This clinical observation is thought to be due to the expression of compensatory Dsg3 throughout the epidermis in neonatal human skin, similar to the mucosal expression pattern of desmoglein isoforms in adults. Direct experimental confirmation of the desmoglein compensation theory was shown by ectopic expression of Dsg3 on an involucrin promoter in the upper layers of the epidermis, which provided protection from PF antibodies in transgenic but not wild-type mice [23]. Taken together, these studies confirmed that Dsg1 and Dsg3 are specific autoimmune targets in pemphigus and can compensate for one another with regard to epidermal keratinocyte adhesion.

Further evidence for the involvement of desmogleins in pemphigus derives from targeted disruption of Dsg3 in mice and from the naturally occurring Dsg3-mutant *balding* mouse, both of which show clinical and histopathological lesions similar to those seen in PV [24,25]. Homozygous mutant mice are normal at birth but develop oral erosions (manifested as runting at day 8–10) and cutaneous erosions at sites of trauma. In addition, mice develop alopecia due to loss of anchoring of the telogen hair in the follicle [26]. This latter finding is consistent with the desmoglein compensation hypothesis because the telogen hair club, which anchors the hair in the follicle, contains Dsg3 but not Dsg1. Recent studies have shown that inactivation of both Dsg1 and Dsg3 results in the loss of anchorage of anagen hairs in the follicle, underscoring the importance of desmogleins in follicular cell adhesion throughout the hair cycle (Y Hanakawa, H Li, C Lin, JR Stanley, G Cotsarelis, unpublished.) The phenotypic similarity of the Dsg3-deficient mouse to spontaneous autoimmune PV argues for a primary role of autoantibodies in the disruption of Dsg3-mediated adhesion. However, the Dsg3-mutant mouse model of

pemphigus does not address the primary autoimmune aspects of the disease.

To investigate the role of the immune system in pemphigus, active autoimmune mouse models have been developed through adoptive transfer of splenocytes from Dsg3-deficient mice into Rag-2-deficient mice that express Dsg3 [27,28••]. Both naïve splenocytes and those obtained after immunization of donor mice with recombinant extracellular Dsg3 result in stable expression of anti-Dsg3 antibodies in Rag-2-deficient mice after transfer. Recipient mice display oral and cutaneous erosions as well as telogen hair loss, mimicking the phenotype of the Dsg3-deficient mice, although naïve transfer delays the phenotypic onset by approximately two weeks. Histologically, immunohistologically and ultrastructurally these mice are an excellent model of human disease [29,30]. They also provide additional, very convincing evidence that autoimmunity against Dsg3 is central to the pathophysiology of pemphigus. In addition, the adoptive transfer model allows characterization of the role of lymphocytes in initiating the immune response in pemphigus. In reconstitution experiments with T and B cells from Dsg $3^{-/-}$ mice, both cell lineages are needed to initiate the immune response against Dsg3 [31]. This model has also allowed the isolation of pathogenic monoclonal antibodies that can be used to define pathogenic epitopes on Dsg3 [32••].

Bullous impetigo and staphylococcal scalded skin syndrome: toxin-mediated diseases of cell adhesion

Bullous impetigo, a common bacterial skin infection in children, is caused by localized infection with *Staphylococcus aureus* strains that produce exfoliative toxins. Although most cases of *S. aureu*s skin infection remain limited, a generalized form of disease called staphylococcal scalded skin syndrome (SSSS) can develop, especially in newborns, young children and adults who are immunocompromised or have renal failure. SSSS is characterized by blisters that can progress to confluent areas of erosions (due to loss of the blister roofs), with no mucous membrane involvement despite widespread skin involvement.

By clinical resemblance alone, nineteenth century physicians recognized the relationship of SSSS to pemphigus and aptly named it pemphigus neonatorum. Melish and Glasgow in the 1970s showed that bullous impetigo and SSSS are caused by an exfoliative toxin (ET) elaborated by certain strains of *S. aureus* (reviewed in [33]). Injection of ET into neonatal mice causes blistering in the granular cell layer of the superficial epidermis, with split desmosomes apparent on electron microscopy.

Three homologous *S. aureus* ETs have been cloned, termed A, B, and D [34,35]. The crystal structures and amino acid sequences of ETs show homology to chymotrypsin-like serine proteases [36,37]. Structural features suggested that a specific substrate might be required to activate the catalytic site, but until recently this target has been elusive. The recognition that the histopathology of bullous impetigo/SSSS and PF are identical, together with the desmoglein compensation theory, led to the hypothesis that Dsg1 would be a logical target of cleavage by ET [38]. In other words, inactivation of Dsg1 by either PF anti-Dsg1 antibodies or ETs should result in the same pathology, namely a blister only in the superficial epidermis, with no mucous membrane involvement (Figure 2b,d). Confirmation of the target of ET was first shown by immunofluorescence of skin biopsies from neonatal mice after ETA injection, demonstrating disrupted cell-surface staining of Dsg1 [38]. Additional studies showed direct cleavage of Dsg1 in mouse skin, on cultured cells and in solution by all ET types [35,39].

Further studies showed that ETA, ETB and ETD all directly cleave Dsg1 at only one site, located after glutamic acid residue 381 in the N terminus between extracellular domains 3 and 4 [40]. This cleavage is efficient, as shown by its having K_{cat}/K_m values in the range of 10^4-10^5 $M^{-1}sec^{-1}$ [41•]. Proteolysis depends upon an intact catalytic site in ET, as mutation of the presumed catalytic serine 195 in ET decreases or eliminates cleavage of Dsg1. ETs are specific for Dsg1 and do not cleave closely related molecules such as Dsg3 and E-cadherin. At least part of this specificity is due to specific binding of ETs to Dsg1 both *in vivo* and in solution [40]. The specificity of ETs depends on the calcium-dependent conformation of Dsg1, as no cleavage is observed with denatured or calcium-depleted substrate [42]. By site-directed mutagenesis of canine Dsg1, which is highly homologous to human Dsg1 but not hydrolyzed by ETs, it was shown that five amino acids 100 residues upstream of the scissile bond are necessary for cleavage. Furthermore, ET is resistant to a variety of serine protease inhibitors, perhaps because the enzymatic cleavage site may be inaccessible or inactive prior to substrate binding [41•]. The requirement of specific substrate binding for activation of the native enzyme catalytic domain may account for the fastidious enzyme–substrate specificity of the ETs, which is consistent with the induced-fit model of enzyme–substrate recognition.

Mechanisms of acantholysis

The mechanism by which exfoliative toxin and the autoantibodies in pemphigus cause acantholysis has been at least partially elucidated. Administration of ET and Fab' monovalent pemphigus antibodies to neonatal mice results in gross blisters within three hours, suggesting that loss of adhesion does not require transcriptional regulation of desmosomal proteins. Presumably, ET results in loss of adhesion by cleaving the N-terminal region of Dsg1 that is predicted to mediate its extracellular binding interactions on the basis of

homology to the crystal structure of the classic cadherins [43,44,45••].

Studies to define the pathologic epitopes on desmogleins have elucidated potential mechanisms of acantholysis. The function of cadherins is dependent on their calcium-stabilized conformation. Many studies have shown that pemphigus antibodies bind conformational epitopes on desmogleins [46] and that these epitopes are stabilized by calcium [47]. Finer mapping with Dsg1–Dsg3 chimeric molecules has shown that almost all pemphigus sera bind conformational epitopes formed by the N-terminal 161 amino acids, and that binding to this area correlates with pathogenicity [48,49••,50,51]. A pathogenic monoclonal antibody isolated from the adoptive transfer mouse model of pemphigus discussed above has been shown to recognize a specific calcium-dependent conformational epitope within the desmoglein adhesive interface, whereas nonpathogenic antidesmoglein antibodies map to a more C-terminal extracellular domain [32••]. These epitope mapping data support a direct role for pathogenic autoantibodies in the disruption of desmoglein extracellular interactions.

However, antibody-mediated disruption of desmoglein interaction alone may not be sufficient for acantholysis, as polyclonal PV IgG has been shown to cause retraction of keratin intermediate filaments and intercellular detachment in cultured keratinocytes derived from wild-type but not plakoglobin-deficient mice, suggesting that plakoglobin plays a critical role in keratin retraction [52]. Additionally, studies in the squamous carcinoma cell line DJM-1 have demonstrated that Dsg3 is serine-phosphorylated in response to pemphigus IgG, and that phosphorylation is associated with loss of plakoglobin binding [53], which is known to be essential for targeting Dsg3 to the desmosome [54]. Antibody binding results in loss of Dsg3 from membranes and the formation of Dsg3-depleted desmosomes [55]. Subsequent experiments have demonstrated that the pathogenic effects of PV antibodies may be coupled to the internalization of Dsg3 (A Kowalczyk, personal communication). However, recent *in vivo* immunoelectron microscopy studies in mice do not show loss of Dsg3 in split desmosomes or keratin retraction in acantholytic areas, suggesting that Dsg3 is not depleted from the desmosome before acantholysis occurs [56•]. Future studies to address the potential structural and signaling roles of the desmosomal components will help to determine the role of desmosomes—whether they serve as simple 'spot welds' or as dynamic intercellular sensors and mediators of adhesion, analogous to their adherens junction counterparts.

Recently, desmoglein 4 (Dsg4) was identified via linkage analysis in humans with a heritable form of hypotrichosis (analogous to the *lanceolate* mouse) and was proposed to be an autoantigen in PV on the basis of the reactivity of PV sera to the recombinant N-terminal portion of Dsg4 after denaturing protein electrophoresis [57•]. However, current data argue against pathogenic involvement of Dsg4 in blister formation in acquired PV or bullous impetigo, as depletion of Dsg4-reactive IgG from pemphigus sera does not alter pathogenic activity in neonatal mice and Dsg4 is not cleaved by exfoliative toxin (T Nagasaka, K Nishifuji and M Amagai, unpublished). Further studies will be needed to elucidate the role of Dsg4 in follicular and nonfollicular epidermal adhesion.

Treatment of pemphigus based on modulation of cell adhesion

Patients with pemphigus respond rapidly to corticosteroid treatment. Several lines of evidence suggest that the therapeutic effects of corticosteroids are not due to direct immunosuppression alone, since clinical improvement often occurs within 48 hours of dosing despite no change in titers of anti-desmoglein antibodies, and synthetic corticosteroids have been shown to directly inhibit pemphigus-sera-induced acantholysis in organ culture models [58,59]. Methylprednisolone has recently been shown to abrogate phosphorylation of Dsg3 in response to PV IgG, with an associated increase in cell-surface staining of E-cadherin, Dsg1 and Dsg3 on keratinocytes [60••], suggesting a direct role for corticosteroids in modulating epidermal adhesion. Future studies dissecting the immunosuppressive and anti-acantholytic roles of corticosteroids may lead to the development of novel nonsteroidal therapeutic agents. Agonists of acetylcholine receptors have been suggested to be such agents, although this is highly speculative. Smoking has been observed to improve PV, possibly through nicotine [61]. Furthermore, acetylcholinesterase inhibitors have been associated with some decrease in blistering in PV in mice and one patient [62]. These clinical findings lend support to recent experiments showing that nicotinic and muscarinic forms of the acetylcholine receptor can modulate cell adhesion in cultured keratinocytes, perhaps via increased cadherin and catenin expression and signaling [63].

Conclusions

Desmogleins are essential in maintaining the integrity of the epidermis, and their disruption by autoantibodies or direct toxin cleavage results in loss of intercellular adhesion and causes clinical disease. More detailed studies of the basic cellular biology of desmogleins are needed to identify transcriptional regulators of desmoglein synthesis and the factors that regulate desmoglein membrane expression and endocytosis, and to determine the effects of protein phosphorylation on potential downstream signaling cascades. The studies of pemphigus and bullous impetigo/SSSS discussed here define the desmogleins as central mediators of cellular adhesion and better clarify the pathophysiologic basis for human desmosomal disease.

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