

A Perspective of Pemphigus from Bedside and Laboratory-Bench

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Abstract Pemphigus represents a distinct organ-specific acquired autoimmune disease characterized by intra-epidermal blistering, which is induced by autoantibodies against desmosomal cadherins, desmoglein 1 (Dsg1), and Dsg3. Pemphigus is currently divided into three distinct varieties, i.e., pemphigus vulgaris (PV), pemphigus foliaceus (PF) and other variants of pemphigus (mostly associated with inflammation), depending on clinical features, the level of separation in the epidermis, and immunologic characteristics of auto-antigens. Blistering pathomechanisms differ for each of the types of pemphigus. Pemphigus, which results from autoantibodies against desmogleins and possibly to other proteins, binds to the cell surface antigens. This binding may cause steric hindrance to homophilic adhesion of desmogleins, and may, in turn, lead to internalization of desmogleins and inhibition of desmogleins' integration into desmosomes, resulting in the formation of Dsg3-depleted desmosomes in PV or Dsg1-depleted desmosomes in PF. Furthermore, PV-IgG activates an “outside-in” signaling pathway to induce disassembly of desmosomal components from the inside of the cells by phosphorylation of proteins, including Dsg3. On the other hand, Pemphigus-IgG-augmented signaling pathways may be linked to the secretion of cytokines such as in case of pemphigus herpetiformis and chemokines that initiate or activate inflammation. In this article, the classification of pemphigus and the characteristic pathomechanisms for acantholysis will be reviewed, with particular emphasis on the molecular and biochemical cell biology of these diseases.

Keywords Pemphigus · Desmogleins · Desmosomes · Signaling

Introduction

Pemphigus is a very distinct organ-specific acquired autoimmune disease, characterized by the loss of cell-cell adhesion, i.e., acantholysis, in both mucous membranes and epidermis, resulting in the formation of mucocutaneous blisters and erosions. Autoantibodies found in patient sera target intercellular adhesion antigens/molecules, which mediate cell–cell adhesion at/in desmosomes. Desmosomes are characteristic intercellular adhesive junctions, which tightly connect cells to form tough sheets of epithelium and compact tissues such as skin, heart, and liver. The molecular components of desmosomes in the epidermis and mucous membranes consist of the desmosomal cadherins, including desmoglein 1–4 and desmocollins 1–3, and intracellular attachment proteins, including plakoglobin, plakophilins, and desmoplakins, which mediate the binding of keratin intermediate filaments to desmosomal cadherins [1, 2]. Pemphigus patients develop autoantibodies to desmoglein 1 (Dsg1) and Dsg3 antigens [3–5].

In this review, both the clinical and histopathological features that define pemphigus classification, and the characteristic pathomechanisms for acantholysis are reviewed, with the molecular and biochemical cell biology of this disease being highlighted.

Classification of Pemphigus

The disease pemphigus is currently divided into three distinct varieties, including pemphigus vulgaris (PV),

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pemphigus foliaceus (PF), and other pemphigus variants that are defined by clinical features, the level of separation in the epidermis as revealed by light microscopy, and immunologic characteristics of the auto-antigens involved as determined by Western immunoblot and enzyme-linked immunosorbent assay (ELISA) findings [5–7]. Although the classical typing of pemphigus had been based primarily on clinical, histological, and immunofluorescence microscopic features, the identification of the target antigens, Dsg3 and Dsg1 in the early 1990s [3, 8], now allows for more rational disease typing that involves molecular cell biological aspects of the disease. Therefore, classification of pemphigus now depends upon five aspects of the disease: (1) the target antigen molecule(s) involved (i.e., desmogleins, desmocollins, or others); (2) the level within the epidermis at which the separation occurs; (3) the type of antibody involved (i.e., IgG and/or IgA); (4) whether or not inflammation, hyperplasia and/or keratinocyte apoptosis (necrosis) is/are present; and finally, (5) the clinical features of disease expression (Table 1).

The most common and major type of pemphigus is PV, which is characterized clinically by generation of flaccid blisters and erosions both in oral mucous membranes and in the skin. Histologically, PV is characterized by cell–cell detachment, manifest primarily as suprabasal separations; i.e., suprabasilar acantholysis. Western immunoblot analysis reveals two types of PV. The mucous membrane-dominant type is characterized by the presence of anti-Dsg3 antibody only, whereas the mucocutaneous type has both anti-Dsg1 and Dsg3 antibodies [6]. The blister formation in the former is limited primarily to oral mucous membranes, whereas that in the latter is generally localized to both oral mucous membranes as well as any portions of whole body skin [6, 9].

The second major type of pemphigus is PF, which is characterized clinically by very fragile superficial blisters appearing as large leafy scales and by mild erosions on the whole body skin but not mucous membranes. Histological-

ly, separation is typically evident along the granular cell layer, with autoantibodies against Dsg1 evident immunologically. Milder cases of PF can present clinical features similar to seborrheic dermatitis due to disease distribution on the scalp, face and chest, and to the presence of large leafy scales. Severe cases, in turn, exert a clinical and histological resemblance to exfoliative dermatitis and even to systemic staphylococcal scalding syndrome caused by the exfoliative toxin (produced by *Staphylococcus aureus*), which destroys the adhesive function of Dsg1, as seen in PF, by splitting the extracellular domain of Dsg1 [10].

The pathomechanisms for generation of these different clinical features in PV and PF are well explained by the compensation theory of Dsg1 and Dsg3 [8, 9]. The epidermal distribution of Dsg1 and Dsg3 is inversely related. Normally, the expression levels of Dsg1 protein are low within the lower less-differentiated epidermal cell layers and significantly elevated in the upper epidermis, in particular within the granular cell layer. Conversely, Dsg3 protein expression is much higher in the deep epidermis and diminished in the granular cell layer [9, 11]. It should be noted that the expression of Dsg3 predominates throughout all mucous membrane layers, whereas that of Dsg1 is expressed minimally, if at all. Therefore, the reason why PF patients have no oral lesion is that the PF patients' sera contain only anti-Dsg1, but not anti-Dsg3, antibodies so that the integrity of mucous membrane can be maintained by Dsg3, although the adhesive function of Dsg1 has been severely compromised. In contrast, the pathogenesis for mucous type of PV can be explained by that the patients' sera of this type contain only anti-Dsg3 antibody, which can destroy the Dsg3-dependent adhesive function of keratinocytes in all mucous membrane layers, but cannot destroy the Dsg3/Dsg1-dependent adhesive function of keratinocytes in the epidermis. In this latter case, the cell–cell adhesion of epidermis can be maintained with the compensatory function(s) of Dsg1. Thus, in the case

Table 1 Classification of pemphigus

Clinical classification	Specific antigen	Histologic characteristics
<i>P. vulgaris</i>		
Mucosal dominant	Dsg3	Suprabasal split
Mucocutaneous	Dsg3, Dsg1	Suprabasal–granular split
<i>P. foliaceus</i>	Dsg1	Granular cell layer split
Brasilian PF	Dsg1, Dsg3	Granular cell layer split
Other types of pemphigus <i>P. vegetans</i>	Dsg3	Suprabasal split, acanthosis, neutrophilic/eosinophilic infiltration
<i>P. herpetiform</i>	Dsg1, Dsg3	Eosinophilic spongiosis
Paraneoplastic <i>P.</i>	Dsg1, Dsg3 plectin, BPAG1, desmoplakins 1, 2, envoplakin, periplakin, 170 kDa peptide	Necrosis of keratinocytes inflammatory infiltrate
IgA pemphigus neutrophilic IgA dermatosis	Dsg1, Gsg3, Dsc1	Subcorneal pustules and split neutrophilic spongiosis

of PV patients who have both anti-Dsg1 and anti-Dsg3, mucocutaneous lesions can be induced due to the impairment of adhesive function of both Dsg1 and Dsg3 (Fig. 1).

The third major group of pemphigus includes the so-called paraneoplastic pemphigus, pemphigus vegetans, pemphigus herpetiformis, and IgA pemphigus. The common feature of this varied group is that their individual pathomechanisms cannot be attributed simply to the destruction of Dsg1 and/or Dsg3 adhesive function alone, as their characteristic association with intense inflammatory alterations attests.

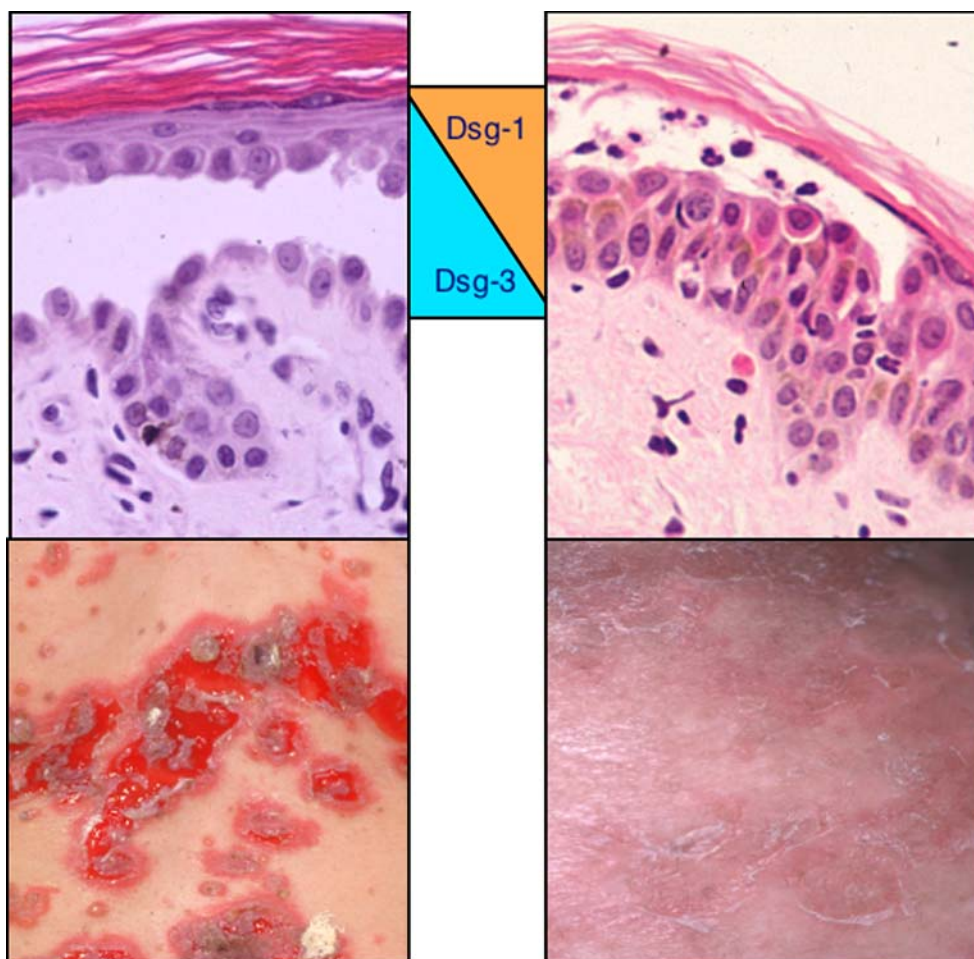
Paraneoplastic pemphigus is a distinct type of pemphigus characterized by antibodies against a variety of antigens (i.e., Dsg3, Dsg1, envoplakin, periplakin, bullous pemphigoid antigen 1, plektin, desmoplakins 1, and desmoplakins 2) and association with internal malignancy, such as non-Hodgkin's lymphoma, chronic lymphocytic leukemia, Casleman's disease, and thymoma [12, 13]. Clinically, this form of pemphigus is characterized by ocular and oral mucosal erosions, which manifest on the eyelids and lips and associate with polymorphous skin eruptions consisting of erythema and papules, which result in blisters and erosions

over the entire skin surface. Histologically, paraneoplastic pemphigus differs from PV and PF by the prominence of apoptotic or necrotic keratinocytes, as well as intense inflammatory changes with lymphocytic infiltrations in subepidermal regions and often in the epidermis, although acantholysis also is common.

The pemphigus herpetiformis variant is clinically characterized by clusters of vesicles and occasionally pustules in the erythematous areas that are similar to those observed in dermatitis herpetiformis, but not generally seen in PF and PV [14]. This variant also differs histologically from PF and PV due to the presence of eosinophilic spongiosis and microabscesses in the mid- or subcorneal epidermis, as well as to the lack of obvious acantholysis despite the immunologically common features of anti-Dsg1 and/or Dsg3 IgG antibodies [15]. IgA pemphigus is caused by IgA antibodies against Dsg1, Dsg3 and desmocollins 1 and presents characteristic clinical features similar to those of P. herpetiformis and subcorneal pustular dermatosis associated with a marked neutrophilic infiltrate in the epidermis [16, 17].

Pemphigus vegetans, a unique variant of PV that also includes anti-Dsg3 (IgG and IgA), Dsc1 and Dsc3 (IgG, IgA

Fig. 1 Difference between pemphigus vulgaris (*left panels*) and P. foliaceus (*right panels*). In normal epidermis, the expression of Dsg1 increases in the upper epidermis, whereas that of Dsg3 is higher in the lower epidermis and decreases with keratinocyte differentiation and migration toward the upper epidermis. The separation points within the epidermis reflects this distribution of Dsg1 and Dsg3 in PV (basal/spinous) and PF (upper spinous/granular). The *lower panels* demonstrate the gross phenotype of active PV (*left*) and PF (*right*) blistering



and IgE) antibodies [18, 19], has clinically a chronic prognosis with typical PV blisters and hypertrophic vegetations in the flexures such as axillae, the groin and the umbilicus, associated occasionally with pustules at the edge of the lesions with vegetations. *P. vegetans* is clinically divided into two subtypes, the Neumann subtype and the Hallopeau subtype [20]. The former is rather severe, often associated with widely distributed blisters as seen in the ordinary PV and vegetations at the flexures, with the latter being characterized by chronic verrucous vegetations at the flexures and associated pustules or vesicles rather than blisters. Histologically, this form differs from ordinary PV, as marked acanthosis is evident with features of pseudoepitheliomatous hyperplasia and an intense inflammatory cell infiltrate, predominantly containing eosinophils, in the epidermis. Although suprabasal separations are common, they often occur to a lesser extent to that in PV.

Pathomechanisms of Pemphigus

The Roles of Autoantibodies to Dsg1 and 3 in Pemphigus Blistering

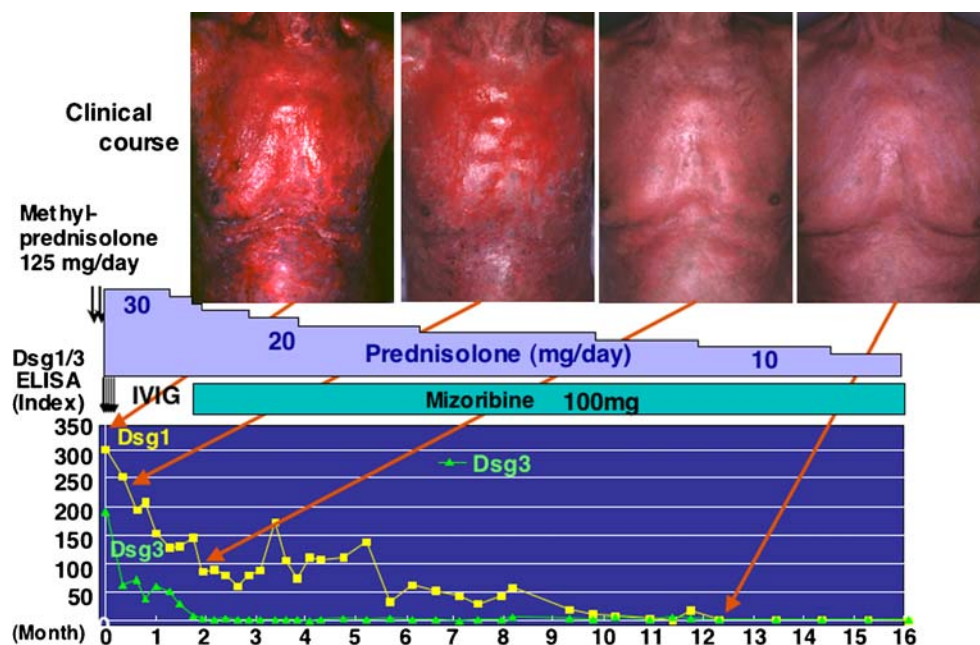
The importance of the levels of Dsg1 and Dsg3 autoantibodies for the generation of blisters in pemphigus reflect directly on both the clinical and treatment courses, as demonstrated in a representative PV case shown in Fig. 2. For example, at the initiation of therapy, when the entire body surface was covered with erosions, the ELISA index levels for anti-Dsg1 and anti-Dsg3 were very high. Intravenous injection of 125 mg/day methylprednisolone for 2 days followed by oral prednisolone at 30 mg/day

(approximately 60 kg body weight patient) markedly reduced the ELISA titers at 1 month. This reduction of the anti-Dsg1 and anti-Dsg3 ELISA titers associated well with the reduction of erosion severity. However, it is of interest to note that no new blister formation was detected 2 weeks after the initiation of glucocorticosteroid therapy, although the levels of anti-Dsg1 and anti-Dsg3 ELISA index were still elevated over the normal range. Two months after initiation of therapy, a point at which all skin erosions had resolved, the anti-Dsg3 ELISA titer was within the normal range, whereas anti Dsg1-ELISA titer remained elevated, taking an additional 10 months to reach the normal range. It appears that the reduction of anti-Dsg1 and anti-Dsg3 antibody levels was achieved by the combination of prednisolone and an oral immunosuppressant, mizoribin (100 mg/day) in this patient. It should be noted that the clinical remission in this case preceded the disappearance of pathogenic autoantibodies, although the ELISA levels for the pathogenic autoantibodies appear grossly proportionate with the clinical severity.

This was more evident in second case, this time for PF. The anti-Dsg1 ELISA titer decreased precipitously to one fifth of the pre-treatment value 2 months after the initiation of oral prednisolone at 40 mg/day. Titers then gradually diminished to within the normal range within another 3 months. However, within 1 month after the initiation of therapy when the anti-Dsg1 ELISA titer was still very high (i.e., approximately 100), nearly all of the patient's skin erosions had resolved, and no new blister formation was evident.

The resolution course observed in these two cases is similar to that seen in many patients. Thus, although anti-Dsg1 and anti-Dsg3 antibodies are essential to cause

Fig. 2 Correlation of anti-Dsg1 and anti-Dsg3 ELISA levels with clinical severity in a PV case during treatment. General correlation of ELISA titers with clinical severity is readily evident, whereas elimination of blistering precedes the normalization of ELISA levels in this PV case



blistering in pemphigus, these observations suggest that: (1) nonpathogenic anti-Dsg1 and anti-Dsg3 antibodies may be contained in patients' IgG; (2) the susceptibility of epidermal keratinocytes to pathogenic antibodies for the generation of blisters may be reduced due to corticosteroid treatment; and (3) other factors might aggravate the blistering process by coordinating with pathogenic anti-Dsg1 and anti-Dsg3 antibodies.

The first possibility is quite feasible based upon a number of findings. First, six of eight anti-Dsg3 monoclonal antibodies (mAbs) generated in a mouse model for PV (i.e., mice received immunized Dsg3-knockout splenocytes) bind to Dsg3 on keratinocytes, but are not pathogenic. Only two of the mAbs generated in this model were pathogenic and induced a PV phenotype as determined by a passive transfer model using neonatal mice [21]. This possibility is further supported by the fact that around 40–71% of healthy individuals, who are immediate relatives of pemphigus patients, have anti-Dsg3 antibodies [22]. This suggests that PV-IgG from patients probably contains both pathogenic and nonpathogenic anti-Dsg3 antibodies as a polyclonal antibody, and their ratios may change from patient-to-patient, and even from time-to-time in individual patients.

The second possibility is also conceivable as demonstrated in an experiment in which PV-IgG and methylprednisolone exhibit reciprocal effects on keratinocytes. Methylprednisolone treatment increased the protein levels of E-cadherin, Dsg1, and Dsg3 by 300, 180 and 40%, respectively, and abolished the phosphorylation of E-cadherin, beta-catenin, plakoglobin, and Dsg3, the latter being induced by PV-IgG [23]. As such, these cellular responses of keratinocytes to methylprednisolone may alter their susceptibility to PV-IgG-induced/linked acantholysis.

The third possibility is supported by the findings of autoantibodies against antigens other than Dsg1 and Dsg3, which have been described in certain PV patients. For example, antibodies against keratinocyte acetylcholine receptor and a non-Dsg1/3 antigen, pemphaxin, which is a novel 75-kDa annexin, have been reported in patients with active PV, although it is unclear how they are involved in blistering mechanisms [24–26]. In this regard, it is of interest to note that PV-IgGs, eluted from rDsg3-Ig-His, also cause blisters in Dsg3-knockout neonatal mice (but not in normal mice), and depletion of anti-cholinergic receptor antibodies impairs the ability of PV serum to induce blisters in mice [24]. Taken altogether, Grando et al. have proposed a “multiple-hit” hypothesis that blistering in PV is the result of synergy between anti-desmoglein antibodies and anti-cholinergic receptor antibodies [26–28].

Other factors besides autoantibodies may also be involved in disease pathogenesis, leading to acantholysis as a coordinated effect(s) with anti-Dsg1 or anti-Dsg3 antibodies. For example, PV model mice, which are

produced by passive transfer of monoclonal anti-Dsg3 antibodies or complete loss of Dsg3 function (e.g., Dsg3-knockout mice) require inactivation of both desmogleins 1 and 3 using anti-Dsg3 simultaneously with either anti-Dsg1 PF antibody or exfoliative toxin A, the latter two being disruptive to Dsg1 function(s) to generate spontaneous blistering as seen in PV patients [9, 21, 29]. As PV patients having only anti-Dsg3, with no evidence for anti-Dsg1, also display spontaneous suprabasal blisters in skin as well as oral mucous membrane, other factors that destroy Dsg1 to generate spontaneous suprabasal blistering must be involved, e.g., urokinase plasminogen activator (uPA)-dependent proteolysis or intracellular signaling events leading to dissociation of intracellular desmosomal assembly [2, 30]. To elucidate these possibilities, further laboratory and clinical studies are required.

Mechanisms of Cell–Cell Detachment, Acantholysis

Anti-Dsg3-Induced Steric Hindrance of Dsg3 Adhesive Function and Intracellular Signaling

Two principle hypotheses for the mechanism(s) responsible for PV acantholysis have emerged over the past several years. The first proposes that anti-Dsg3 antibody-dependent steric hindrance interferes with intercellular adhesive function(s) of Dsg3, leading directly to desmosomal dissociation [9, 21]. The second suggests that the myriad intracellular signaling events that are linked to the generation of acantholysis could be activated by PV-IgG binding to the antigen on the keratinocyte [30].

It is evident that Dsg1 and Dsg3 play (an) essential role(s) in the maintenance of keratinocyte adhesion in the epidermis. Most notably, exfoliative toxin of *S. aureus*, an enzyme that is strictly specific for digestion of Dsg1 [10], causes acantholysis in the granular cell layer in the epidermis. In addition, Dsg3-knockout mice mimic a phenotype of PV in oral mucous membranes, revealing a suprabasal split [31, 32]. Furthermore, injection of PF-IgG (i.e., anti-Dsg1 antibodies) into Dsg3 knockout mice causes severe skin blistering [9], suggesting that Dsg1 plays an important adhesive function in the upper, and Dsg3 in the lower, epidermis, as introduced above in desmoglein compensation hypothesis [33].

It is also evident that exertion of steric hindrance between Dsg3–Dsg3 or Dsg3–Dsc3 [by Dsg3 autoantibodies] is reasonable because antibody against the “kissing point” of Dsg3 (AK23) is highly pathogenic, and human anti-Dsg3 monoclonal antibodies cause acantholysis in vivo and in vitro even when stimulated with single-chain variable-region fragments [29]. In this hypothesis, acantholysis specific for PF and PV might be explained by steric hindrance of Dsg1 and

Dsg3 antibodies to the adhesive function of desmogleins, leading directly to desmosome splitting.

However, it does not appear that PV-IgG binding itself splits desmosomes, although it may cause steric hindrance of Dsg3–Dsg3 or Dsg3–Dsc3 binding, as Dsg3 knockout mice have the capability to form morphologically normal, albeit functionally impaired, desmosomes and display histologically normal intercellular keratinocyte adhesion [9, 31]. Furthermore, we have previously demonstrated, using a cell culture system of Ca^{++} -induced desmosome formation, that PV-IgG binding to cell surface antigens does not inhibit desmosome formation [34, 35]. Desmosomes are absent when cells are cultured in low Ca^{++} (<0.09 mM), but formed within 2 h when the Ca^{++} concentration in the medium was switched from low to high (i.e., >1.0 mM). This Ca^{++} -induced desmosome formation was not inhibited by PV-IgG binding to the surface PV antigen before Ca^{++} switch, suggesting that PV-IgG does not directly split the desmosome. However, this does not exclude a role for steric hindrance of PV-IgG in the homophilic adhesion of Dsg3 molecules.

As such, the proposed steric hindrance theory cannot absolutely eliminate involvement of the latter hypothesis, i.e., additional “outside-in” signaling activated by PV-IgG. In this regard, we have shown that by using keratinocytes and DJM-1 cells (a human squamous cell carcinoma cell line) metabolically labeled with ^{32}Pi , followed by stimulation with IgG fractions from five PV patients or normal individuals for 20 min, PV-IgG stimulation alone induced the phosphorylation of Dsg3 and dissociation of plakoglobin from Dsg3 [36]. These changes were linked to Dsg3 degradation within 20 min [37]. More recently, we have demonstrated that stimulation with a pathogenic monoclonal anti-Dsg3 (AK23) augmented the phosphorylation levels of Dsg3 and p38MAPK [38], the latter of which being stimulated with PV-IgG [39]. Furthermore, we have shown that stimulation with four different monoclonal anti-Dsg3 antibodies in DJM-1 causes a diverse tyrosine phosphorylation of the novel Dsg3-binding p120ctn, which is a new member of the desmosomal catenins [40], both at different sites of, and/or different p120ctn isoforms [41]. These findings suggest that monoclonal anti-Dsg3 antibody binding to Dsg3 can itself generate outside-in signaling in keratinocytes. These results suggest that PV-IgG activates intracellular signaling leading to the aberrant phosphorylation of a variety of molecules in keratinocytes, including Dsg3.

Evidence to support the concept that PV-IgG activates intracellular signaling is accumulating. In DJM-1 cells labeled with ^{32}Pi , PV-IgG stimulation lead to markedly increased phosphorylation of E-cadherin, beta-catenin, plakoglobin, and Dsg3 [24, 26]. A central role for plakoglobin (PG) in the pathogenicity of PV-IgG has been suggested by recent in vivo work in which PG knockout

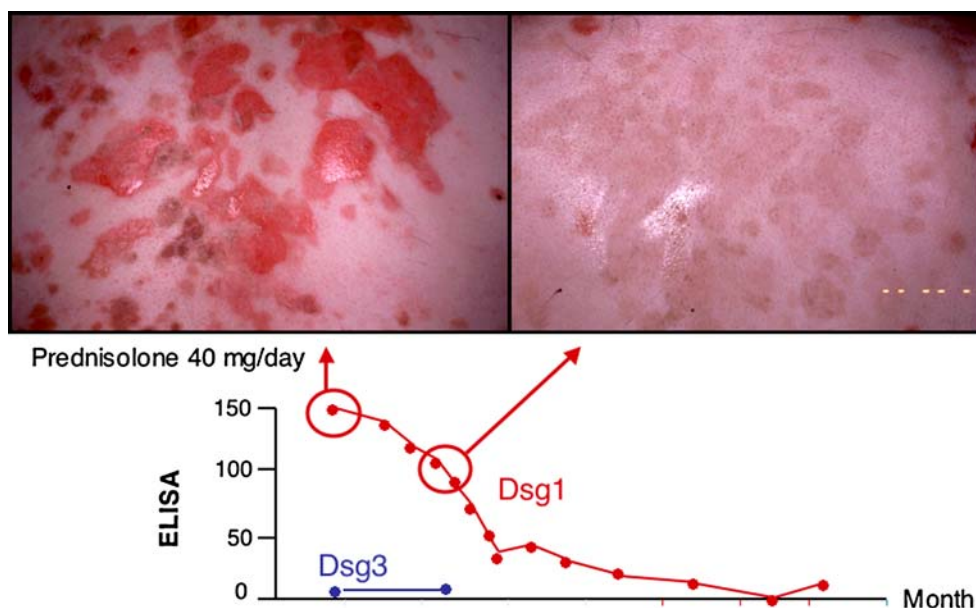
(PG $^{-/-}$) and normal (PG $^{+/+}$) keratinocytes were employed [42]. In addition, other cellular responses suggesting involvement of PV-IgG-induced intracellular signaling events have been reported, including apoptosis signaling [43–45], modulations of plakoglobin [42], p38MAPK, heat shock protein 27 (HSP27) [39], and c-Myc [46] functions.

We also have reported several lines of evidence for the activation of intracellular signal transduction events in response to PF- and PV-IgG binding to cultured keratinocytes [30]. These results indicate that PV-IgG generates specific signal transduction events that induce phospholipase C (PLC) activation, IP_3 -dependent Ca^{2+} mobilization, and protein kinase C (PKC) activation. However, this signaling pathway does not appear to mediate the phosphorylation of Dsg3 [36], but rather the activation of urokinase-type of plasminogen activator receptor and plasminogen activator system (uPAR/uPA), which may have no primary role in the generation of pemphigus-associated acantholysis. Thus, despite the evidence that PV-IgG causes activation of the uPA/uPAR system in cultured keratinocytes [47, 48] and that inhibitors of this system prohibit cell–cell detachment both in vitro and in vivo [49], the involvement of this system in PV may not be essential, but a secondary or an epiphenomenon, because PV-IgG is still pathogenic in both uPA and tissue-PA knockout mice [50].

The Final and Direct Events Linked to PV Acantholysis: Anti-Dsg3 Antibody-Induced Depletion of Dsg3 from Desmosomes

Experiments that trace the fate of Dsg3 and other desmosomal molecules after PV-IgG binding to cell surface Dsg3 in normal human keratinocytes and DJM-1 cells have revealed that internalization and degradation of Dsg3 leads to the formation of Dsg3-depleted desmosomes [36, 37]. Immunoblotting and immunofluorescence microscopy using antibodies against Dsg1, Dsg3, PG, desmoplakin 1 (Dpk1) and cytokeratins demonstrated that Dsg3 is markedly depleted from the membrane (Triton X-100 soluble) fraction within 20 min after PV-IgG, whereas no reduction of Dsg3 was detected in the cytoskeleton (Triton X-100 insoluble) fraction [37]. However, a 24- to 30-h incubation with these antibodies caused a marked depletion of Dsg3, but not other desmosomal molecules, from both cytoskeleton fractions and desmosomes. Double-staining immunofluorescence microscopy reveals that PV-IgG and pathogenic anti-Dsg3 monoclonal antibodies deplete the desmosomes of Dsg3, while maintaining the desmosomal contacts containing Dsg1, Dpk1, PG, and keratin filaments, but almost no Dsg3, 30 h after antibody treatments. This finding of Dsg3-depleted desmosome formation is also supported by immunoelectron microscopic studies. Dsg3 molecules, which are freely floating on the non-desmosomal cell surface, are laterally

Fig. 3 Correlation of anti-Dsg1 and anti-Dsg3 ELISA levels with clinical severity in a PF case. General correlation of ELISA titers with clinical severity is again evident, and the diminished blistering also precedes the normalization of ELISA levels in this PF case when anti-Dsg1 ELISA titer is still very high (i.e., approximately 100)



aggregated and internalized as endosomes into the cell within 60 min after PV-IgG binding to Dsg3 [51]. Within 30 min after PV-IgG binding, the ratio of the amount of Dsg3 to Dsc on the plasma membrane (before being integrated into

desmosomes) is markedly reduced to one tenth, as revealed by double-staining immunoelectron microscopy [52]. The idea that PV-IgG-induced internalization and degradation of free Dsg3 on the plasma membrane is a direct and immediate

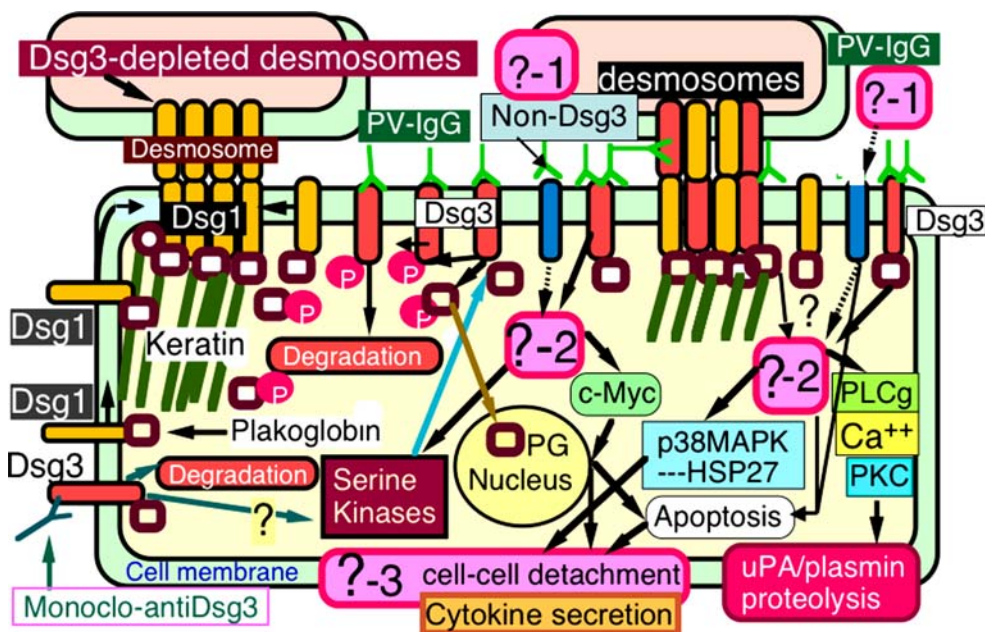


Fig. 4 Schematic model illustrating plausible PV-IgG-induced cellular events that may be linked to PV-blistering. In this proposed model, PV-IgG-induced “outside-in” signaling initiated by PV-IgG binding to Dsg3, cholinergic receptors and/or other membrane surface molecules, and even non-immunoglobulin factors, which activate or perturb intracellular signaling, may be involved in the induction of blistering. A number of questions remain unresolved. First (see ?-1), do PV-IgG autoantibodies bind to antigens and/or receptors other than Dsg3, and/or are non-immunoglobulin factors possibly involved in outside-in signaling? Second, (see ?-2) what are the signaling cascades that mediate serine kinase phosphorylation of Dsg3, the PLC/ Ca⁺⁺/PKC pathway linked to uPA/uPA activation, and p38MAPK and apoptosis pathways? Third and finally, pathogenic monoclonal anti-Dsg3

antibodies cause Dsg3 degradation leading to Dsg3-depleted desmosomes (as illustrated in the left portion of the figure) as well as phosphorylation of Dsg3, p38MAPK, and p120-catenin. Although this Dsg3 depletion from desmosomes is implicated in the reduction of adhesive mechanical strength and uPA/plasmin proteolysis also is implicated, albeit secondarily, in the digestion of desmosomes outside of the cell, the possible mechanisms that might link p38MAPK and apoptosis signaling to cell-cell detachment remain unknown (question 3: ?-3). At one end, PV-IgG-induced secretion of cytokines may also be involved. This constellation of events then results in the observed acantholysis, which is profoundly and directly linked to the generation of Dsg3-depleted desmosomes. This figure is a modification of our previous model [64]

cause for the formation of Dsg3-depleted desmosomes is also supported by the finding that PV-IgG induces endocytosis of Dsg3 as a complex with PG, but not with desmoplakins [53]. In addition, PV-IgG treatment also lead to rapid depletion of soluble Dsg3 cell surface pools, followed by loss of detergent-insoluble Dsg3, suggesting the formation of Dsg3-depleted desmosomes [53]. PV-IgG has been also shown to both reduce the half-life of Dsg3 and to perturb its de novo assembly at desmosomal sites, whereas Dsg3-depleted desmosome formation was not detected 36 h after the PV serum treatment [54]. Furthermore, the depletion of Dsg3 from desmosomes is caused by treatment with anti-Dsg3 monoclonal antibodies [55], suggesting that the depletion of Dsg3 is a specific effect of the anti-Dsg3 activity of such antibodies.

The complete depletion of Dsg3 as seen in Dsg3 knockout mice exerts the generation of PV phenotype, especially friction blisters, and even a partial depletion (30%) of Dsg3 from the epidermis exhibits suprabasal acantholysis in passive PV-IgG transfer mice [56]. In addition, 30 to 90% depletion of Dsg3 from cultured DJM-1 cells impairs the strength of cell–cell adhesion as determined by a dissociation assay using the pipetting method [55]. Together, these results suggest that Dsg3 depletion from desmosomes is the most direct event linked to acantholysis.

Pathomechanisms of Inflammatory Group of Pemphigus

The inflammatory group of pemphigus, i.e., paraneoplastic pemphigus, pemphigus vegetans, pemphigus herpetiformis, and IgA pemphigus, commonly exhibit an intense inflammation that may or may not be associated with acantholysis. Therefore, depletion or destruction of the adhesive function(s) of Dsg1 and Dsg3 cannot completely explain the pathomechanisms involved in generation of inflammation in this group of pemphigus. It is tempting to speculate that PV-IgG-augmented signaling pathways may be linked to the secretion of cytokines and/or chemokines that are in turn responsible for the initiation or to activation of inflammation. In fact, it has been reported in *P. herpetiformis* that PV-IgG stimulation of cultured keratinocytes causes secretion of IL8 [57]. Additional research on this aspect of the inflammation associated with this group of pemphigus is clearly warranted.

A Perspective of Blistering Mechanisms in PV

Taken together, a complex interaction of possible cell biologic events that are associated with PV blistering is

illustrated in Fig. 3. The candidate receptor(s) that interact with PV-IgG and mediate a variety of intracellular (i.e., outside-in) signaling events include Dsg3, cholinergic receptors [27], and pemphaxin [26], as well as other transmembrane molecules as potential autoantibody targets. Even non-immunoglobulin factors cannot be excluded from involvement in the activation of intracellular signaling, which then can mediate increased in keratinocyte susceptibility to acantholysis.

Conversely, the final outcome(s) linked to the cell–cell detachment in PV is/are thought to be Dsg3 depletion from desmosomes, which is essential. The uPA/uPA receptor proteolysis activation may induce spontaneous blistering by digesting Dsg1 and other cadherins, even if such events are not essential and even if not caused specifically by anti-Dsg3 antibody. However, other mechanisms underlying cell–cell detachment involving p38MAPK, HSP27, plakoglobin, c-Myc, and apoptotic signaling events have yet to be fully explored.

PV-IgG, which consists of autoantibodies to desmogleins and possibly to others, binds to the cell surface antigens. This binding may cause steric hindrance to homophilic adhesion of desmogleins, which may, in turn, lead to internalization of desmogleins and the inhibition of the integration of desmoglein protein(s) into desmosomes, resulting in the formation of Dsg3-deleted desmosomes. Furthermore, such binding may also activate an outside-in signaling pathway to induce disassembly of desmosomal components from the inside of the cells by phosphorylation of proteins, including Dsg3, and as well, other signaling pathways mediated by calcium and/or PKC linked to the secretion of plasminogen activator and expression of plasminogen activator receptor. This plasminogen activator is active only when bound to its receptor on the cell surface, and thus, it can generate plasmin in the micro-vicinity of the cell surface, possibly resulting in the digestion of extracellular domains of desmosomal cadherins in situ. Even if this proteolyses process is not essential for acantholysis, it may take part in the late stage of the cell–cell detachment process (Fig. 4).

Although we did not review the possible role of immune tolerance against the production of anti-Dsg3 antibody, a number of recent studies have begun to shed light on this aspect of PV disease [58–64].

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