A new Perspective of Autoimmune Bullous Diseases: Molecular Cell Biology of Blistering Mechanisms and Logical Treatments

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Pemphigus and pemphigoid are most distinct types of organ-specific acquired autoimmune diseases, and characterized by intraepidermal and subepidermal blistering, which are induced by autoantibodies against desmosomal cadherins, desmoglein 1 (Dsg1) and desmoglein 3 (Dsg3), and a hemidesmosome adhesion protein, type XVII collagen, i.e., bullous pemphigoid antigen II (BP180), respectively.

Blistering pathomechanisms after autoantibodies bind to their antigens are not yet clear. In pemphigus vulgaris (PV), the antibody binding is thought to cause steric hindrance to homophilic adhesion of Dsg3 leading to acantholysis, and/or to activate outside-in signaling pathways to induce phosphorylation of Dsg3, which may, in turn, lead to internalization of Dsg3 and inhibition of Dsg3 integration into desmosomes, resulting in the formation of Dsg3-depleted desmosomes. We have shown that this Dsg3-depletion from desmosomes, which is seen in animal model and patients, decreases the cell-cell adhesive strength, suggesting that this may cause acantholysis.

In bullous pemphigoid, antibody binding is thought to activate complement cascade leading to the generation of inflammation, which may produce proteases and cause dermal epidermal separation. However, this does not yet explain why separation runs specifically along the lamina lucida, but not sub-lamina densa or even not intercellular adhesion of basal cells, because proteolysis due to inflammatory enzymes are not believed to have any specificity to the lamina lucida, if antibody binding to BP180 should exert effects only of complement activation. We have shown that BP-IgG binding to BP180 causes internalization of BP180 in culture and patient basal cells and depletes cultured keratinocytes of BP180 resulting in the reduction of adhesive strength to the bottom of culture plates. These results suggest that BP-IgG reduces the BP180 content from hemidesmosomes resulting in decreasing the adhesive strength to the lamina densa and inflammation generated by BP180 immune-complex tears the weakened lamina lucida due to BP180 deficient. This may cause the BP-specific split at lamina lucida in combination with non-specific inflammatory enzymatic activity exerted at dermal-epidermal zone.

Blistering mechanisms of both pemphigus and pemphigoid appear to have a common point that autoantibody-target antigens are depleted form cells, leading to a decrease in the adhesive function. The depletion of specific adhesion peptides may determine the specificity of those diseases and myriad primary and/or secondary effects to autoantibody binding to antigens may exaggerate the diseases. Treatments should be determined by taking these two aspects into mind, to reduce or deplete pathogenic antibodies and to control the pathogenic reactions caused by an-
WHAT ARE PEMPHIGUS AND BULLOUS PEMPHIGOID?

**Pemphigus**

There are two major common types of pemphigus, that are pemphigus vulgaris (PV) and pemphigus foliaceus (PF). PV is characterized by cell-cell detachment, manifest primarily as suprabasal separations; i.e., suprabasilar acantholysis, demonstrating clinical features characterized by generation of flaccid blisters and erosions both in oral mucous membranes and in the skin. PV is subdivided into the mucous membrane-dominant type and the mucocutaneous type. The former is characterized by the presence of anti-desmoglein 3 (Dsg3) antibody only and the latter is by both anti-desmoglein 1 (Dsg1) and Dsg3 antibodies. The blister formation in the former is limited primarily to oral mucous membranes, while that in the latter is generally localized to both oral mucous membranes as well as any portions of whole body skin.

PF is characterized by histologically acantholysis along the granular cell layer, immunologically by autoantibodies against Dsg1 and clinically by presenting very fragile superficial blisters, appearing as large leafy scales, and mild erosions on the whole body skin but not mucous membranes.

Characteristic clinical features in PV and PF are well explained by the compensation theory of Dsg1 and Dsg3. Dsg1 and Dsg3 are inversely distributed in the epidermis, i.e.,

**Key words: Pemphigus, Bullous pemphigoid, Desmoglein, Bullous pemphigoid antigen**

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**Fig. 1**

Types of pemphigus. Pemphigus is divided into pemphigus vulgaris, pemphigus foliaceus and other types of pemphigus, clinically by the nature of blisters and histo-pathologically by the layer of splits in the epidermis. Pemphigus herpetiformis is associated with eosinophilic spongiosis, but rarely with acantholysis, although blisters are formed usually by severe spongiosis.
the former is in the upper and the latter is in the deeper epidermis. In the mucous membrane, however, the expression of Dsg3 predominates throughout all layers of the epithelium, while that of Dsg1 is at a minimum level, if at all. Therefore, in PF, possessing only anti-Dsg1 antibody, no oral lesion is generated because of the lack in the target antigen of Dsg1. In contrast, in mucous type of PV, possessing only anti-Dsg3 antibody, mucous lesions can be caused by 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, resulting in fail to generate blisters 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 of mucocutaneous type of PV characterized by the presence of both anti-Dsg1 and anti-Dsg3, the patients generate mucocutaneous lesions in both mucous membrane and epidermis (Fig. 1).

Bullous pemphigoid

The disease bullous pemphigoid demonstrates characteristic clinical, histological and immunological features, i.e., tense blisters with and/or without erythema all over the body surface, the splits along the lamina lucida beneath the basal cells at the basement zone and the presence of circulating autoantibodies against hemidesmosomal molecules, respectively (Fig. 2). The BP autoantibodies include two major antigens with apparent molecular weight of 230-kDa and 180 kDa, the latter of which is pathogenic. The autoantibody against 180-kDa bullous pemphigoid antigen (BP180), which is a transmembrane collagenous protein, type XVII collagen is the most suspected pathogenic agents for BP, since injection of antibody (rabbit IgG) against noncollagenous domain (NC16a) of mouse BP180 into the mouse has been shown to cause blistering identical to BP in the animal, and most of BP patients have antibody to BP180 when examined by immunoblotting using synthetic NC16 domain as a subject.

PATHOMECHANISMS OF BLISTERING AFTER AUTOANTIBODY- BINDING TO ANTIGENS IN PEMPHIGUS AND PEMPHIGOID

Pathomechanisms of pemphigus

By recent insights into pathomechanisms underlying the generation of acantholysis in PV after autoantibodies bind to Dsg3 and/or Dsg1 on the keratinocyte surface, two principal hypotheses have been raised. The first proposes that anti-Dsg3 antibody-dependent steric hindrance interferes with intercellular adhesive function(s) of Dsg3, leading directly to desmosomal dissociation. The second hypothesis proposes that myriad PV-IgG-induced intracellular signaling events could lead to desmosomal dissociation.

Since the Dsg N-terminal domain is known to play an essential role in the adhesive function of Dsg, and monoclonal antibody to this domain is pathogenic, similar to AK23 in the PV, anti-Dsg3 antibody targeting this domain is likely to cause steric hindrance between Dsg3 and Dsg3/Dsc3. This binding thus
represents a primary trigger for acantholysis, even though this binding itself does not lead directly to desmosomal detachment. However, we have observed no inhibition of Ca\(^{2+}\)-induced desmosome formation by PV-IgG binding to surface PV-antigens\(^{32}\) at early time points. This result suggests that PV-IgG does not directly inhibit desmosome formation, even though antibodies in PV-IgG may also cause steric hindrance between homophilic Dsg3- or heterophilic Dsc3-interactions. However, longer PV-IgG incubation ultimately leads to cell-cell detachment, suggesting that impairment of desmosome remodeling may be involved in the PV-acantholysis.

The depletion of Dsg3 from desmosomes affects desmosome-remodeling

We also have provided several lines of evidence for the second hypothesis involving outside-in signaling\(^{7, 25, 33}\) and the phosphorylation of Dsg3, effects exerted by PV-IgG stimulation of cultured keratinocytes.\(^{15}\) This Dsg3 phosphorylation was associated with PG dissociation from Dsg3, with degradation occurring within 20 min of binding.\(^{1}\) This result may explain in part, the Dsg3-depleted desmosomes that are subsequently formed (Fig. 3).\(^7\) This degradation of Dsg3 may be explained by endocytosis of Dsg3 induced by PV-IgG binding to the Dsg3 free on the cell surface before integration into desmosomes.\(^{14, 37}\) This appears also true in case of PF, as HaCaT cells treated with PF sera show a similar internalization.\(^{36}\) These results suggest that PV-IgG activates intracellular signaling leading to the aberrant phosphorylation of Dsg3, which appears to be linked to Dsg3-depleted desmosome formation and cutaneous blistering in PV (Fig. 4).\(^7\) In this regard, other investigators have demonstrated reduced Dsg3 half-life, although a decrease in Dsg3 content was not detected, in PV-sera treated HaCaT cells.\(^{37}\) In addition, Dsg1 was reduced in the Triton X-100-soluble pool in PF-IgG treated Dsg1-rich keratinocytes.\(^{38}\) Furthermore, we have also shown that PV-IgG activates another intracellular signaling pathway in which Ca\(^{2+}\) and PKC\(^{39, 40}\) are significantly involved, events that are linked to urokinase-type plasminogen activator secretion.\(^{41}\) Thus, the

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**Fig. 3**

Desmoglein 3 (Dsg3)-depletion from desmosomes by pemphigus vulgaris (PV)-IgG and anti-Dsg3 antibodies.\(^7\) Treatment of keratinocytes with PV-IgG and pathogenic anti-Dsg3 antibodies deplete cells of Dsg3, whereas desmoplakins (DPK) and Dsg2 were not depleted.
signaling-related events thought to be involved in the pathomechanisms of PV include not only phosphorylation of Dsg3\(^{33}\) and a Ca\(^{2+}\)/PKC pathway,\(^{24}\) but also apoptosis signaling,\(^{42-44}\) as well as modulations of PG,\(^{45, 46}\) p38MAPK, heat shock protein 27 (HSP27)\(^{47}\) and RhoA.\(^{48}\) These observations are summarized in Fig. 5.\(^{49}\)

The depletion of Dsg3 from desmosomes thus-generated may change the ratio of Dsg3 to other desmosomal cadherins, which in turn could modulate or impair the proper desmosome remodeling for migration and keratinization of keratinocytes. Actually, it has been shown that over-expression of human Dsg3 under the control of the keratin 1 (K1) promoter in the suprabasal epidermis of transgenic mice, causes hyperproliferation associated with acanthosis, hypergranulosis, hyperkeratosis, localized parakeratosis, and abnormal hair follicles, all of which are features in common with human ichthyosiform diseases.\(^{50}\) Thus, the ratio of Dsg3 to other desmosomal cadherins appears critical for the proper regulation of epidermal differentiation. In addition, transgenic mice with Dsg3 expression under the control of the involucrin promoter, express Dsg3 in the epidermis with a distribution pattern as found in normal oral mucous membranes. The subsequent dysadhesion of corneocytes leads to increased transepidermal water loss and death of these aminols.\(^{51}\) The desmosomal cadherin Dsc1 is expressed in upper epidermis, where strong adhesion is required. In mice with a targeted disruption in the Dsc1 gene, the epidermis is fragile, and acantholysis in the granular layer, demonstrates that Dsc1 is required not only for strong adhesion and barrier maintenance in the epidermis, but also that Dsc1 contributes to epidermal differentiation.\(^{52}\)

Thus, an imbalance between Dsgs and Dscs can lead to reduction of adhesion. In this regard, as mentioned in the section on intercellular desmoglea (above), cell-cell adhesion was formed only at the appropriate ratio of Dsg1

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**Fig. 4**
Desmoglein 3 (Dsg3)-depleted desmosome formation.
to Dsc1, suggesting this ratio of Dsg and Dsc is an important factor in reconstitution adhesion. Actually, we have shown that PV-IgG significantly reduces the ratio of Dsg3 to Dsc3 in cultured keratinocytes and in PV-patient skin. These findings suggest that differential expression of desmosomal cadherins affects the major functions of the epidermis, including the stability of desmosomes. Furthermore, it is of interest to note that a unique case of an acquired palmoplantar keratoderma with anti-Dsc3 antibody has recently been reported, with eosinophilic spongiosis and suprabasal acantholysis of blistering as in PV associated with marked hyperkeratosis. Taken together, including both hypotheses presented above, pemphigus might better be characterized as a disease of desmosome remodeling, which is caused by impairment of physiological and dynamic desmosome remodeling required for normal epidermal keratinization, resulting in generation of blistering as a major symptom.

**Pathomechanisms of bullous pemphigoid**

Regarding with pathomechanisms of bullous pemphigoid, Liu et al. have suggested that complement activation by anti-BP180 antibodies is required to cause blistering in a mouse model by comparing the effects of anti-BP180 antibodies on blistering in C5-sufficient and C5-deficient mouse strains. Therefore, autoantibodies against BP180 itself can not cause blisters in skin (Fig. 6). However, this does not yet explain why splits to generate blisters run specifically along intra-lamina lucida, but not sub-lamina densa or even intercellular adhesion of basal cells, because there are no reasons that proteolysis and tissue damages due to complement-induced inflammation accurately limit to the lamina lucida as such a narrow domain as 500 nm in width. The complement activation in cases of epidermolysis bullosa aquisita usual-

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**Fig. 5**

Schematic model illustrating plausible pemphigus vulgaris (PV)-IgG-induced cellular events that may be linked to PV-blasting. PV-IgG-induced outside-in signaling will be initiated by PV-IgG binding to desmoglein 3 (Dsg3), cholinergic receptor and/or other membrane surface molecules. Question 1 (?-1) indicates PV-IgG autoantibodies binding to antigens and receptors other than Dsg3. Question 2 (?-2) indicates a variety of unknown signaling cascades, that mediate to serine kinase to phosphorylate Dsg3, PLC/ Ca++/PKC pathway linked to uPA/uPA activation, p38MAPK and apoptosis pathways. It has been shown that pathogenic monoclonal anti-Dsg3 antibodies cause Dsg3 degradation leading to formation of Dsg3-depleted desmosomes (illustrated at the left part of the figure). Although Dsg3-depletion from desmosome may be implicated to reduce the adhesive mechanical strength and uPA/plasmin proteolysis to be implicated to digest desmosomes at outside of the cell, possible mechanisms underlying how to link p38MAPK and apoptosis signaling to cause cell-cell detachment are unknown (question 3: ?-3).
ly causes sublamina-densa blistering and that in case of systemic lupus erythematosus (SLE) is associated with basal cell degeneration and/or blistering at the sublamina densa region, but not along the lamina lucida.

In this regard, we have previously shown that BP180 is distributed on the lateral-apical plasma membrane as a pool without bound to keratin filaments as well as on the basal plasma membrane at hemidesmosomes bound to keratin filaments, and that the binding of BP-IgG to BP180 causes the internalization of BP180 from the lateral-apical plasma membrane in cultured keratinocytes. This may suggest that this internalization of BP180 play an initial BP-characteristic role in pathogenesis of BP.

We also demonstrated that BP-IgG/serum can deplete cells of BP180 by semi-quantitative, resulting in the reduction of adhesive strength of cells to the bottom of the culture plate in culture plate, as shown by a cell-detachment assay from the dish by vibration. This suggests the presence of an insufficient adhesive function of hemidesmosomes due to shortage of BP180 to be integrated into hemidesmosomes. Thus, the inflammation generated by BP180 immune-complex formation tears the weakened lamina lucida due to BP180 deficient. This may cause the BP-specific split at lamina lucida in combination with non-specific inflammatory enzymatic activity exerted at dermal-epidermal zone (Fig. 7).

**PRINCIPLE TREATMENTS FOR PEMPHIGUS AND PEMPHIGOID**

Auto-antibodies against Dsg1 and Dsg3 and BP-180 are pathogenic factors for PV and BP, respectively, as mentioned above. There-
fore, depletion or reduction of these pathogenic IgGs is a principal target for treatments. On the other hand, after these antibodies bind to the antigens on the cell surface, they underwent a series of processes, including depletion of adhesion molecules, which are target antigens, and inflammation, leading to blister formation. Therefore, to modify or prevent these processes linked to blistering is also a target of treatments for auto-immune blistering diseases.

Typical therapies for pemphigus and pemphigoid include oral corticoids (~1 mg/kg/day) and pulse therapy with methylprednisolone (1,000 mg/day I.V.), which reduces primarily titers of pathogenic auto-antibodies taking several weeks and prevent the inflammatory process within a few days. These therapies are usually followed by immunosuppressive agents, such as mizoribin (100 mg/day), azathioprine (75-100 mg/day, Japanese are generally more susceptible to this drug than Caucasians) and mycophenolate mofetil (1,000-1,500 mg/day) in order to reduce production of pathogenic auto-antibodies as adjuvant therapies.

As an alternative therapy, plasmapheresis is one of the most effective therapeutic methods to deplete sera of immunoglobulins (Ig), including pathogenic autoantibodies, in autoimmune diseases. However, it is not always easy to prevent feedback-induced rebound with rapid pathogenic antibody synthesis after plasmapheresis. We have reported a patient having severe pemphigus vulgaris (PV), who did not respond to pulse therapy with intravenous (IV) methylprednisolone (1,000 mg/day for 3 days), but was successfully treated with a com-

![Fig. 7](image)

**Fig. 7**
Non-inflammatory mechanisms of bullous pemphigoid (BP) blistering. Binding of BP antibodies to BP180 causes internalization of free BP180 molecules into the cells as immune-complexes, resulting in depletion of BP180 from hemidesmosomes. These BP180 depleted hemidesmosomes may be more susceptible to proteases than other tissues at dermo-epidermal adhesions.
combination of plasmapheresis immediately followed by high-dose IVIg, eventually leading to complete suppression of the rebound increase in pathogenic PV-IgG, as monitored by weekly enzyme-linked immunosorbent assay (ELISA) for desmoglein (Dsg) 1 and Dsg3. Our experience with weekly Dsg-ELISA demonstrates a distinct difference in the alteration curves of serum levels of pathogenic IgG after plasmapheresis with and without high-dose IVIg (unpublished data). We usually employ double filtration plasmapheresis (DFPP), because this method requires only albumin solution as a substitution fluid, which minimizes the risk of infection and anaphylactic shock, in contrast to plasma exchange, which requires fresh-frozen plasma.

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 two days, followed by oral prednisolone at 30 mg/day (approximately 60 kg body weight patient), markedly reduced the ELISA titers at one 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 two weeks after the initiation of corticosteroid therapy, even though 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 ten 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 (Fig. 8).

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, two months after the initiation of oral prednisolone at 40 mg/day. Titers then gradually diminished to within the normal range within another three months. However, within one 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 (Fig. 9).

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 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.

Thus, it is of special importance for pemphigus and pemphigoid treatments to take it into consideration whether the current medicines that we use are targeting to reduction of pathogenic auto-antibody titers or prevention of inflammation, which may be directly lead to blister formation.

CLOSING THOUGHTS

Pemphigus and bullous pemphigoid are diseases of impairments of desmosome and hemidesmosome remodeling, caused by auto-antibody binding to adhesion molecules of desmosomes and hemidesmosomes, respectively.

Fig. 9
Correlation of anti-desmoglein 1 (anti-Dsg1) and anti-desmoglein 3 (anti-Dsg3) ELISA levels with clinical severity in a pemphigus foliaceus (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 1000).
Binding of auto-antibodies to these molecules are thought to generate aberrant activation of signaling pathways, which may then result in impairments of desmosome and hemidesmosome remodeling. Intensive study into the dynamics of desmosomal and hemidesmosome complex interactions and the myriad consequences of altered protein-protein interaction within these key structures are required to resolve the pathomechanisms and develop new strategies for treatments.

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