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VIEWPOINT

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Monopathogenic vs multipathogenic explanations of pemphigus pathophysiology†

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Abstract

This viewpoint highlights major, partly controversial concepts about the pathogenesis of pemphigus. The monopathogenic theory explains intra-epidermal blistering through the "desmoglein (Dsg) compensation" hypothesis, according to which an antibody-dependent disabling of Dsg 1- and/or Dsg 3-mediated cell–cell attachments of keratinocytes (KCs) is sufficient to disrupt epidermal integrity and cause blistering. The multipathogenic theory explains intra-epidermal blistering through the "multiple hit" hypothesis stating that a simultaneous and synchronized inactivation of the physiological mechanisms regulating and/or mediating intercellular adhesion of KCs is necessary to disrupt epidermal integrity. The major premise for a multipathogenic theory is that a single type of autoantibody induces only reversible changes, so that affected KCs can recover due to a self-repair. The damage, however, becomes irreversible when the salvage pathway and/or other cell functions are altered by a partnering autoantibody and/or other pathogenic factors. Future studies are needed to (i) corroborate these findings, (ii) characterize in detail patient populations with non-Dsg-specific autoantibodies, and (iii) determine the extent of the contribution of non-Dsg antibodies in disease pathophysiology.

KEYWORDS

acantholysis, antimitochondrial antibody, autoantibody, autoantigen, autoimmunity, desmogleins 1 and 3, FcRn, pemphigus vulgaris

†This article is a result of a collaborative effort to provide a comprehensive yet non-dogmatic view of pemphigus pathophysiology, even though individual authors may or may not support one view or the other.

1 | INTRODUCTION

Pemphigus vulgaris (PV) is a potentially lethal mucocutaneous blistering disease characterized by IgG autoantibodies (PVIgG) binding to keratinocytes (KCs). Patients with PV develop cell–cell detachment (acantholysis), blisters and non-healing erosions due to suprabasal split within the epidermis. Identification of the nature of proteins targeted by PV autoimmunity and elucidation of molecular mechanism of acantholysis have been a subject of intensive research during the last three decades. While several hypotheses were put forward to explain the mechanism of pemphigus acantholysis, the field has been dominated by studies focused on desmoglein (Dsg) 1 and 3, the results of which gave rise to a strong believe that anti-Dsg antibodies have the primary role. Indeed, as these antibodies are found in the vast majority of pemphigus patients, they may play a pivotal role in disease pathophysiology. The fact that anti-Dsg antibodies are pathogenic, however, does not mean that antibodies to other self-antigens might not also be pathogenic. If non-Dsg antibodies alone were responsible for some cases of PV, one would expect to see a certain number of cases of acute PV with antikeratinocyte antibodies detectable by direct and/or indirect immunofluorescence but negative by Dsg 1/3 ELISA. This was indeed the case in a number of studies. Different authors reported 5,[1] 6,[2] 9,[3] 10,[4] 17,[5] 19[6] and even 33%[7] of patients with PV lacking both anti-Dsg 1 and anti-Dsg 3 antibodies by ELISA. Additionally, the monopathogenic theory of pemphigus has been challenged by reports showing the presence of various species non-Dsg antibodies in PV sera and activation of multiple signalling pathways in KCs exposed to PVIgG, strongly suggesting that non-Dsg pathways are also involved.

In this viewpoint article, we discuss the major controversies about the pathogenesis of pemphigus summarized in Table 1, and review "pros and cons" of the monopathogenic and multipathogenic explanations of disease pathophysiology.

2 | THE MONOPATHOGENIC THEORY OF PEMPHIGUS PATHOPHYSIOLOGY

The monopathogenic theory of pemphigus pathophysiology explains intra-epidermal blistering through the "Dsg compensation" hypothesis. According to this hypothesis, Dsg 1 and 3 antibody profiles and the normal epidermal distributions of Dsg 1 and 3 determine the sites of blister formation within epidermis in PV and pemphigus foliaceus (PF), and either Dsg 1 or Dsg 3 alone is sufficient to maintain keratinocyte adhesion in the upper and lower epidermal compartment, respectively.[8,9] The main postulate of the monopathogenic theory is that antibody-dependent disabling of Dsg 1- and/or Dsg 3-mediated cell–cell attachment of KCs is sufficient to disrupt epidermal integrity and cause blistering.

2.1 | Summary of data supporting the monopathogenic theory (see Supplement for details)

The clinical and experimental data supporting the unique pathogenic role of anti-Dsg antibodies sufficient to explain the clinical phenotype and lesion formation in the great majority of pemphigus patients include reports about the presence of antibodies against Dsg 3 and/or Dsg 1 in 95% of pemphigus sera and correlation of antibody titres with disease activity and clinical phenotype, such as Dsg 1 in PF, Dsg 3 in mucosal PV and Dsg 1+Dsg 3 in mucocutaneous PV;[10,11] induction of PF- and PV-like lesions by Dsg 1 and Dsg 3 antibodies, respectively, affinity purified from patients' sera (Refs 12,13 and E. Schmidt,

TABLE 1 Major controversies about pemphigus pathogenesis (modified from Ref. 58,69)*

Major postulates of the monopathogenic theory:		Findings counteracting the respective postulates of the monopathogenic theory:
(i) Epidermal integrity primarily depends on the desmosomal expression of Dsg 1 and Dsg 3	vs	(i) Epidermis in patients with PV who develop both Dsg 1 and 3 antibodies would have disintegrated to a single cell suspension if the integrity of epidermis was based exclusively on Dsg 1 and 3
(ii) Sera of patients with PV contain pathogenic autoantibodies targeting preferentially the Dsg 1/3 targets	vs	(ii) The autoantibodies eluted from certain recombinant Dsg 1 and Dsg 3 protein constructed recognized non-specifically several non-Dsg keratinocyte proteins
(iii) Pemphigus acantholysis results from inactivation of Dsg 1 and/or 3 molecules by corresponding autoantibodies due to steric hindrance and/or internalization	vs	(iii) In addition to blocking function of adhesion molecules on keratinocyte cell membrane, PVIgG elicit pro-apoptotic signalling events causing cell shrinkage, detachment from neighbouring KCs and rounding up—the unique process of PVIgG-triggered signalling events collectively described by the term apoptolysis
(iv) PV phenotype can be reproduced in mice solely by genetically or antibody-mediated inactivation Dsg 3	vs	(iv) Mice with genetically or antibody-mediated inactivation of Dsg 3 develop some but not all clinical–histological manifestations of PV (that is, pseudo pemphigus phenotype)
(v) An interplay between Dsg 1 and 3 antibodies generally determines the mucocutaneous phenotype in PV and PF	vs	(iv) The Dsg 1/3 antibody profile does not always match the predicted clinical phenotype based on the relative Dsg 1 and Dsg 3 expression pattern in skin and mucosa
(vi) Serum concentrations of anti-Dsg 1/3 IgG grossly correlate with the clinical activity of PV and PF	vs	(vi) Serum concentrations of Dsg 1/3 antibodies do not always correlate with the clinical activity of PV and PF

*Abbreviations: KC, keratinocytes; PF, pemphigus foliaceus; PV, Pemphigus vulgaris.

unpublished data); induction of PV-like lesions associated with desmosomal degradation and acantholysis by Dsg 3 antibodies in vitro and in Rag2-deficient mice with splenocytes from Dsg 3-deficient mice;[14–21] induction of acantholysis in vitro/ex vivo by IgG from HLA-DRB1*04:02-transgenic mice immunized with human Dsg 3 or Dsg 3 peptides;[22] and abrogation of acantholytic activity of sera from patients with PF and PV due to depletion of anti-Dsg 1 and anti-Dsg 3 reactivities, respectively.[23–25] Indirect support is provided by reports that apoptosis is not required for the induction of desmosomal degradation and acantholysis by PVIgG in vitro and that apoptotic cells are scarce and mostly absent in lesional skin of pemphigus patients.[26–28] Apoptotic events may arise in later stages of the disease after acantholysis had occurred.[26]

2.2 | Summary of data challenging the monopathogenic theory (see Supplement for details)

Several clinical studies demonstrated lack or weak correlations of anti-Dsg reactivity with clinical phenotype. The Dsg 1/3 antibodies can be absent in patients with PV in active stage of disease but present in patients with PV during remission as well as in healthy subjects and patients with irrelevant medical conditions.[1,4,12,29–47] In a number of studies, Dsg 3 antibody levels did not correspond to the presence of cell surface antibodies detectable by indirect immunofluorescence or predict relapse of the disease.[48–51] Furthermore, the Dsg 1/3 antibody pattern did not match the predicted morphologic phenotype of pemphigus.[1 4,6,13,40,46,52] Inactivation of the Dsg 1- or 3-mediated adhesion induces overt blistering—an indispensible feature of pemphigus phenotype—in neither patients with *DSG1* mutations [53,54] nor any hitherto described mouse models of Dsg 3 inactivation, through either genetic manipulations[55–57] or anti-Dsg 3 antibody production (reviewed in,[58] as would be expected if inactivation of Dsg 1 and Dsg 3 was a sole cause of PF and mucosal PV, respectively. Notably, acantholysis per se does not constitute true pemphigus phenotype, because it can be seen in patients with a variety of dermatological conditions who do not develop blisters and erosions characteristic of pemphigus. On the other hand, despite the presence of functional Dsg 3, the *Dsc3*$^{fl/fl}$/K14-Cre mice lacking desmocollin (Dsc) 3 develop extensive skin blistering,[59] indicating that Dsg 3 alone is not sufficient to maintain epidermal cohesion. Furthermore, it has been demonstrated that extracellular domain of Dsg 3 mediates only a weak homophilic adhesion in vitro[60] and that inhibition of Dsg 3 binding is not sufficient to cause loss of cell cohesion.[61] Therefore, not surprisingly, human KCs deprived of endogenous production of Dsg 1 or 3 due to gene silencing *via* RNAi continue to form desmosomes.[62] Noteworthily, results showing that chimeric proteins containing the extracellular epitope of Dsg 1 or 3 combined with the Fc portion of human IgG_1 absorbed out all disease-causing pemphigus IgGs [23,24,63] are interpreted with caution, because patients with PV produce autoantibodies against Fc-IgG_2,[64] and PVIgG eluted from the Dsg/Fc-IgG chimeras react with multiple keratinocyte proteins.[65,66]

3 | THE MULTIPATHOGENIC THEORY OF PEMPHIGUS PATHOPHYSIOLOGY

The multipathogenic theory of pemphigus pathophysiology explains intra-epidermal blistering through the "multiple hit" hypothesis.[67] According to this hypothesis, a simultaneous and synchronized inactivation of the physiological mechanisms regulating and/or mediating intercellular adhesion of KCs is necessary to disrupt their most important phylogenetic function such as maintenance of epidermal integrity. Individual variations within the constellations of pathogenic PVIgG targeting different keratinocyte proteins likely determine the magnitude of the "multiple hit" attack required to disrupt epidermal integrity in a particular patient with PV, and explain the clinical and immunopathological variability of PV. The term "pathogenic" is applicable to the non-Dsg antibodies that can induce one or more of the PV-relevant changes of KCs, such as cell shrinkage, cell–cell detachment and/or pro-apoptotic signalling. In addition, it has been documented that PVIgG synergize with the effectors of apoptotic and oncotic pathways, serine proteases and inflammatory cytokines to overcome the natural resistance and activate the cell death programme in KCs (Fig. 1). The major premise for the multipathogenic theory is the hypothesis that a single type of autoantibody induces only reversible changes, so that affected KCs can recover due to a self-repair. The damage, however, becomes irreversible when the salvage pathway and/or other cell functions are altered by a partnering autoantibody and/or another

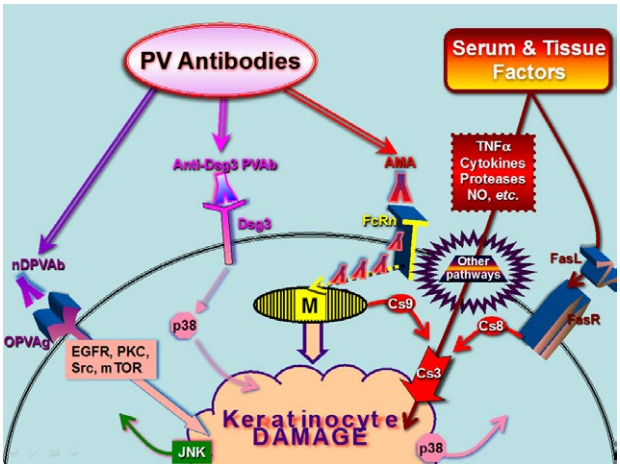

FIGURE 1 Hypothetical scheme of multipathogenic theory of pemphigus (modified from Ref. 81). The process of keratinocyte damage in PV is rather complex and relies upon a synergistic action of antibodies to adhesion molecules, mitochondrial proteins and other kinds of self-antigens, like acetylcholine receptors, as well as humoral factors, such as FasL, TNF-α, cytokines, serine proteases and nitric oxide, that by acting altogether become able to overcome the natural resistance and activate both the extrinsic and intrinsic cell death pathways in KCs. FcRn is an indispensible element of pathogenic action of antimitochondrial antibodies and, perhaps, other PVIgG species that target intracellular self-antigens. AMA, antimitochondrial antibodies; Cs, caspase; FasL, Fas ligand; FasR, Fas receptor; NO, nitric oxide; nDPVAb, other non-Dsg PV antibodies; OPVAg, other PV antigens; PVAb, PV antibody; TNF-α, tumor necrosis factor-α

pathogenic factor, for example Fas ligand (FasL). Thus, a simultaneous and synchronized inactivation of the several physiological mechanisms regulating and mediating intercellular adhesion of KCs is apparently required to irreversibly disable their most important phylogenetic function such as maintenance of epidermal integrity.

3.1 | Summary of data supporting the multipathogenic theory (see Supplement for details)

The first evidence that non-Dsg antibodies are pathogenic was provided in the study of Amagai et al.[12] who demonstrated that gross blisters were induced in mouse skin by the PVIgG depleted of Dsg 3 antibody. Later on, neonatal *Dsg 3*$^{-/-}$ mice developed the PV phenotype featuring widespread blistering only after passive transfer of PVIgG lacking anti-Dsg 1 antibody, indicating that blisters were induced by targeting the non-Dsg 1 and 3 antigens.[68] Thus far, the published evidence directly or indirectly supporting the role for non-Dsg antibodies and other endogenous pathogenic factors in pemphigus pathophysiology include the differences between the pathobiological effects of Dsg 3 antibody and the total PVIgG serum fraction at both the ultrastructural and biochemical levels, over forty different keratinocyte protein bands identified by immunoblotting and/or immunoprecipitation as "pemphigus antigens" and direct pathogenic effects of non-Dsg antibodies on KCs (reviewed in Ref. 69) as well as involvement of apoptotic signalling events in pemphigus acantholysis providing a rationale of the concept of apoptolysis.[70] In addition to Dsg 1 and 3 molecules, recent proteomic analyses of large quantities of pemphigus and normal control sera[47,71] revealed reactivities with Dsc 1 and 3, several muscarinic and nicotinic acetylcholine receptor subtypes, HLA molecules, a number of mitochondrial proteins and some other intracellular molecules including thyroid peroxidase and the Ca^{2+}/Mn^{2+}-ATPase, or hSPCA1, encoded by the *ATP2C1* gene. The highest specificity to PV showed the combinations of autoantibodies to hSPCA1 with C5a receptor plus Dsc 1 or Dsc 3 or HLA-DRA.[47] Indeed, anti-Dsc 3 PVIgGs have been shown to be pathogenic.[72,73] Noteworthily, one copy of *ATP2C1* is mutated in patients with Hailey–Hailey disease (a.k.a. familial benign chronic pemphigus) representing a non-immune phenocopy of cutaneous lesions in PV.[74,75] Both Hailey–Hailey disease KCs[76] and normal KCs treated with PVIgG[77] exhibit altered intracellular calcium metabolism that can lead to abnormal cell–cell adhesion.[78] An expanded discussions of possible pathogenic roles of new pemphigus antibodies detected by in proteomics studies can be found elsewhere.[47,64,71,79]

Although the adhesion molecules targeted in PV may potentially induce downstream signalling events,[80] the PVIgG-induced activation of epidermal growth factor receptor and Src was not affected in *Dsg3*$^{-/-}$ KCs.[81] Src activation is an early acantholytic event[82] triggered by non-Dsg PVIgG.[62] In addition to activating the oncosis effector,[83] both extrinsic and intrinsic apoptotic signalling pathways are activated in KCs treated with PVIgGs,[83,84] with the principal effector molecules being FasL[84,85] and cytochrome c,[81,86] respectively. Both acantholysis and apoptotic signalling can be triggered by the same signal effectors activated by PVIgG and mediated by the same set of cell death enzymes, because, on the one hand, inhibitors of Src, EGFRK, p38 MAPK and mTOR can block both acantholysis and apoptotic events,[62,70,87–92] and, on the other hand, caspase inhibitors can prevent acantholysis both in vitro and in vivo.[83,90,93,94] Apoptotic enzymes can cleave Dsg 1, 2 and 3,[95–97] thus representing the principal effector of apoptolysis—a unique paradigm of keratinocyte damage in PV that is non-synonymous with apoptosis.[70] Antimitochondrial antibodies (AMA) target the mitochondrial nicotinic acetylcholine receptors that protect KCs from apoptolysis.[98] Noteworthily, Dsg 3 does not serve as a surrogate antigen allowing AMA to enter KCs.[81] The susceptibility of KCs to apoptolysis in PV may be increased due to the presence of a polymorphic variant of the *ST18* gene encoding a pro-apoptotic molecule.[99]

4 | UNRESOLVED ISSUES

1. Little is known about the cutaneous inflammation which follows binding of PVIgG to KCs and may contribute to intra-epidermal loss of adhesion and blister formation. Local factors, such as innate immune mechanisms, may enhance a proinflammatory environment with an IL-1β-/TNF-α-dominated signature that facilitates blister formation presumably via production of IL-6.[100,101] This contention is supported by previous findings that injection of pathogenic anti-Dsg antibodies into newborn mice did not result in intra-epidermal loss of adhesion in mice deficient for IL-1 receptor 1, the receptor for the proinflammatory cytokines IL-1α and IL-1β;[102]
2. While intramolecular and intermolecular epitope spreading among extracellular domains on Dsg 1 and Dsg 3 is rare in PV and has no correlation with disease course,[103] a possibility that epitope spreading may play a role in generation of autoantibodies that target a variety of desmosomal autoantigens in PV needs to be explored;
3. The largely accepted critical role of autoreactive T-cell interaction with autoreactive memory B cells and the induction of pemphigus-specific plasma cells is also not yet fully elucidated;[104]
4. The presence of IgA, IgM and IgE antibodies to KCs in some pemphigus patients[105–107] suggests their direct pathogenic role with or without synergy with IgG autoantibodies and warrants future research in this unexplored direction.

5 | EVIDENCE OF SYNERGY OF ANTI-DSG AND NON-DSG ANTIBODIES IN PEMPHIGUS

The synergy within the pool of pathogenic PVIgG may stem from functional cooperation of autoantibodies to proteins mediating either the same and/or separate biologic functions of KCs. For example, through the first scenario, simultaneous blockade of the Dsg and Dsc molecules mediating their heterophilic *trans*-interactions within the desmosome[108] may distort epidermal integrity more efficiently, compared to interference with *cis*-interactions of single-type desmosomal proteins. Through the second scenario, simultaneous attack on molecules that regulate shape and motility of KCs, such as acetylcholine receptors,[109] and molecules that mediate cell–cell adhesion, such as desmosomal cadherins, will inactivate the adhesive function

of desmosomal cadherins due to their phosphorylation and internalization, leading to shrinkage of KCs,[87] and prevent formation of new desmosomes due to steric hindrance at nascent desmosomes, respectively. In other words, non-Dsg antibodies may potentially "amplify" the activity of anti-Dsg antibodies.

Direct evidence of synergy between anti-Dsg and non-Dsg antibodies in PV is illustrated by complementary activities anti-Dsg 1/3 and AMA in organ culture of neonatal mouse skin (OCNMS)—the highly sensitive in vitro model of PV.[110] Preabsorption of PVIgG with either non-mitochondrial or mitochondrial proteins in both cases prevented acantholysis, indicating that both AMA and non-AMA antibodies were indispensible for acantholysis.[110] However, treatment of mouse skin only with AMA did not cause acantholysis. Herein, it should be clarified that despite the demonstrated ability of anti-Dsg 1/3 monoclonal antibodies to induce keratinocyte monolayer fragmentation in vitro and microscopic blisters in ex vivo model and in neonatal mice (reviewed in Ref. 111), there exists a possibility of non-physiological interference with desmosome formation and function due to application of suprapharmacological doses of such anti-Dsg antibodies. In fact, recent experiments with OCNMS demonstrated that while high concentrations of the human anti-Dsg monoclonal PV antibody scFv could induce acantholysis on its own, its dilution down to ~0.16 μg/μL could not,[110,112] which allowed to investigate its synergy with AMA. As expected, acantholysis was induced when AMA were added to the non-acantholytic dose of scFv.[110] Likewise, acantholysis developed when AMA were combined with commercial anti-Dsg antibodies. The AMA/anti-Dsg 1 combination induced subcorneal splitting and AMA/anti-Dsg 3—a suprabasal one, consistent with respective predominant localization of Dsg 1 and Dsg 3.[9]

In a separate study, a commercially available antithyroid peroxidase antibody and the mouse monoclonal anti-Dsg 3 antibody AK23 produced increased fragmentation in keratinocyte dissociation assays in vitro when used in combination, as compared to each individual antibody.[113,114]

Thus, the pathogenic role of non-Dsg antibodies is complementary, not alternative, to that of Dsg antibodies, and both anti-Dsg and non-Dsg types of autoantibodies appeared to be required to disrupt integrity of the epidermal barrier.

The multipathogenic theory also explains why KCs remain the only cell type affected by PV autoimmunity despite the fact that the majority, if not all, PV autoantigens have been found in other cell types. Recent studies of the role of FcRn in the pathogenic action of AMA in PV have implicated KCs as a single target in pemphigus based on the fact that these cells have in place all essential elements of the cellular machinery implementing apoptolysis upon binding of PVIgG. Briefly, in keeping with the notion that FcRn ("neonatal" Fc receptor for IgG) can bind IgG on the cell membrane,[115–119] it was demonstrated that PVIgG physically associates with FcRn on the cell membrane of KCs, following which the PVIgG-FcRn complexes become internalized, trafficked through the cytosol to the mitochondria where the complexes are liberated from endosome and dissociate allowing AMA to damage mitochondria, which triggers apoptotic signalling associated with cell shrinkage.[110] Furthermore, while it had been known that FcRn deficiency caused by either its blockade with a neutralizing anti-FcRn antibody or gene knock-out in both cases interferes with the ability to induce phenotypes of humorally mediated autoimmune diseases in mice, including PV and PF,[120–126] it has been recently demonstrated that AMA alter mitochondrial respiration only in those cell types that express FcRn.[110] Lack of FcRn prevented both PVIgG internalization and its ability to reach mitochondria. Furthermore, functional inactivation of FcRn due to pretreatment of KCs with mouse anti-FcRn antibody prevented AMA-dependent alterations of mitochondrial metabolism and integrity, as well as keratinocyte shrinkage.[110]

KCs with damaged mitochondria shrink because they run out of energy and because caspases activated due to cytochrome c release can cleave structural and adhesion molecules resulting in the cytoskeleton collapse.[70] The AMA-induced damage, however, is reversible.[110] During recovery, when KCs extend their cytoplasmic aprons towards neighbouring cells, re-assembly of desmosomes is blocked by steric hindrance of anti-Dsg antibodies that leads to irreversible acantholysis, or apoptolysis. The fact that FcRn is predominantly expressed within the basal epidermal layer[127] may render basal KCs a preferred functional target for PVIgG to intracellular antigens, thus explaining why they shrink more than suprabasal KCs,[128] despite the fact that both basal and suprabasal KCs are targeted by PVIgG.[129] Thus, FcRn appears to be an indispensible element of the autoimmunity against intracellular antigens in KCs but not other cell types.

For example, although both breast and urinary bladder epithelial cells express Dsg 1 (http://www.proteinatlas.org/ENSG00000134760-DSG1/tissue), they are not damaged in the Dsg 1 antibody-positive patients with PV. The synergy of Dsg 1 antibody and AMA in these cells apparently does not occur because neither cell type expresses FcRn.[110] Additionally, an indirect evidence of important role of FcRn in PV is rendered by the facts that an excess of normal IgG that can saturate FcRn protects KCs from PVIgG-induced apoptolysis in vitro,[83] and that high-dose IVIg is therapeutic in patients with PV (reviewed in Ref. 130). Thus, it is becoming increasingly evident that an array of interconnected signalling cascades emanating from different cell surface and intracellular proteins simultaneously targeted by genetic abnormalities, such as the *ST18* SNP, pathogenic antibodies and some other endogenous pathogenic factors contribute to the evolving pemphigus phenotype.

6 | KEY POINTS

1. Epidermal acantholysis resulting from null mutation of *DSG1* in patients with SAM syndrome or in mice with null mutation of *Dsg3* or *Rag2*$^{-/-}$ mice producing anti-Dsg 3 antibodies does not cause gross blistering, indicating that neither anti-Dsg 1 nor anti-Dsg 3 antibodies can be solely responsible for blistering in patients with PF and PV, respectively.
2. Reports about the presence and pathogenic relevance of a non-Dsg antibodies are accumulating and have questioned the monopathogenic theory. The presence of multiple autoantigen–autoantibody

systems in PV and interpatient variations may explain, in part, known problems with reproducibility of certain results. Therefore, future studies are needed to (i) corroborate these findings, (ii) characterize in detail patient populations with non-Dsg-specific autoantibodies, and (iii) determine the extent of the contribution of non-Dsg PVIgG in disease pathophysiology.

3. Patients with PV develop antibodies against multiple organ-specific and non-organ-specific proteins, some of which are also targeted in other types of autoimmune diseases. Both anti-Dsg and non-Dsg antibodies are pathogenic in a sense that they are elements of the multifactorial pathophysiological mechanism of PV. Various PVIgG species may concur to cause blistering by acting synergistically. The preferential signalling pathway downstream of targeted self-antigens is apparently determined by a unique composition of the pool of antikeratinocyte antibodies produced by each patient with PV. Distinct constellations of autoantibodies developed by different patients with PV determine clinical severity of the disease, its natural course and response to treatment.
4. The mechanism of pemphigus apoptolysis encompasses several tiers of events triggered through distinct signalling pathways, including genetic predisposition to production of antikeratinocyte antibodies and increased sensitivity of KCs to the tissue and serum factors that trigger extrinsic and/or intrinsic apoptotic pathways. Thus, during apoptolysis, KCs become both a target and a source of various inflammatory and pro-apoptotic factors. Understanding of PV pathophysiology, however, is still incomplete, because the ancillary pathways triggered by pathogenic antibodies and other pathogenic factors remain largely unknown.
5. Although there are no known clinical and pathological differences between PV patients with vs without anti-Dsg antibodies, the immunopathology may be different. The growing evidence about acantholytic activities of non-Dsg antibodies in PV urges mechanistic studies of keratinocyte damage in the anti-Dsg antibody-negative patients with PV. Therefore, IgGs from the sera of patients with PV, who developed mucocutaneous blisters in the absence of anti-Dsg1/3 antibodies, provide a unique experimental tool to elucidate novel aspects of PV pathophysiology.
6. Although multiple hits sustained by a constellation of autoantibodies seem to be required to breach epidermal integrity, elimination of or pharmacological protection from a single type of pathogenic antibody may suffice to abort development of the disease. Therefore, elucidation of the repertoire of pathogenic antibodies in individual patients will further improve our understanding of immunopathogenesis of their disease.

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CONFLICT OF INTERESTS

The authors have declared no conflicting interests.

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

Data S1 Supplementary references
Data S2 Supplementary Mater ials and Methods