Acantholysis in pemphigus
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IgG–induced clustering of desmogleins 1 and 3 in skin of patients with pemphigus fits with the desmoglein nonassembly depletion hypothesis

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Abstract

Background In pemphigus circulating IgG is present directed against the desmosomal cadherins desmoglein (Dsg)1 and Dsg3. In the epidermis of patients, this IgG deposits in a pattern that is often partly granular and does not reflect the normal Dsg distribution.

Objective To understand why the IgG deposits in a granular pattern in the skin of patients with pemphigus.

Patients/Methods We analysed the distribution of IgG and desmosomal adhesion molecules in skin biopsies of 18 patients with pemphigus vulgaris (PV) and 10 with pemphigus foliaceus (PF) by double staining immunofluorescence. The effect of IgG on desmosomal proteins was studied in an in vitro skin model.

Results In PF skin Dsg1, but not Dsg3, was aberrantly distributed in the same partly granular pattern as the IgG. Vice versa, in skin of PV patients with anti–Dsg3 antibodies, Dsg3, but not Dsg1, colocalized with the granular IgG. Plakoglobin (PG) also coclustered with IgG and Dsg, but this was far more prominent with Dsg1 than with Dsg3. In areas of heavy Dsg1 clustering, but not in areas of heavy Dsg3 clustering, intercellular widening between keratinocytes was present. Patient IgG, but not Fab fragments, induced the same Dsg clustering in vitro.

Conclusions The IgG–induced clustering of the Dsg autoantigens underlies the granular IgG deposition in patient skin. In PF and in mucocutaneous PV, Dsg1 clustering, but not Dsg3 clustering, correlates with nonacantholytic intercellular widening between desmosomes. In the patient the Dsg becomes sequestered from desmosomal components which fits in with the desmoglein nonassembly depletion hypothesis, indicating that targeted non-junctional Dsg is no longer available to be incorporated into desmosomes and this leads to disturbed assembly, and Dsg–depleted desmosomes.

Introduction

Pemphigus is an autoimmune disease characterized by the intraepidermal deposition of IgG and blistering of skin and/or mucous membranes. The two major types of pemphigus comprise pemphigus vulgaris (PV), always affecting mucosa and in approximately half of the cases skin, and pemphigus foliaceus (PF) that affects only skin. In PV the IgG against desmoglein (Dsg)3 causes suprabasal acantholysis, while in PF IgG against Dsg1 provokes subcorneal acantholysis.1–4 Desmogleins, belonging to the cadherin family, are desmosomal proteins that link keratinocytes. Two opposing desmogleins bind each other with the extracellular N–terminal domains. Their cytoplasmic domains bind plakoglobin (PG), which can bind desmoplakin (DP) that can link to
the keratin intermediate filaments. Dsg3 is more abundantly present in the lower epidermis, but absent in the upper layers, while Dsg1 expression increases from the basal to the granular layer. This differential distribution is the basis of the Dsg compensation hypothesis that explains the difference in separation levels between PV and PF by Dsg isoform redundancy.5,6
The mechanism by which IgG induces acantholysis remains a matter of debate. Four concepts prevail currently: (i) binding of IgG to junctional Dsg disrupts homophilic transinteraction, leading to lengthwise splitting of desmosomes and loss of cell–cell adhesion (steric hindrance hypothesis);7 (ii) binding of IgG to non-junctional Dsg disturbs intracellular signalling pathways ending with cytoskeleton collapse or desmosome disassembly (cell signalling hypothesis);8–12 (iii) binding of IgG induces loss of junctional Dsg3 from desmosomes from endocytosis and dismantling of the desmosomes (disassembly depletion hypothesis);13 and (iv) binding of IgG induces endocytosis of the non-junctional Dsg3 pool which stagnates the assembly of Dsg into desmosomes (nonassembly depletion hypothesis).13,14 Supportive evidence is present in the literature for all concepts and underlines the vast complexity of investigating the pathogenic process of acantholysis.
Most of today’s concepts originate from experimental models; relatively little attention has been given to patient skin. Those who perform diagnostic immunofluorescence microscopy know that there is a strange discrepancy between in vivo IgG deposition by direct immunofluorescence (DIF) on patient skin and the intercellular substance (ICS) pattern by indirect immunofluorescence (IIF) that is described as typical for pemphigus. The ICS pattern is a smooth staining around the epidermal cells, also called honeycomb or chicken wire pattern, and is consistent with the normal distribution of the desmogleins. In patient skin however the IgG deposits are often partly granular, especially in the lower layers.15 The reason for this is unknown, but could be related to the clumping of desmosomal components, which has been described for pemphigus, but not for the other acantholytic skin diseases.16,17 In drug–induced pemphigus the absence of such a patchy pattern is considered an indicator of good prognosis.18
In the present study we investigated multiple patient skin biopsies in order to understand why the IgG is deposited in vivo in such an aberrant granular fashion, and whether the granular deposition is connected with changes in cell morphology and acantholysis.
Materials and methods

Patient samples

Forty biopsies of 28 patients with pemphigus, three mucosal-dominant PV, 15 mucocutaneous PV and 10 PF, were included. Biopsies were immediately frozen in liquid nitrogen and stored at -80°C. All diagnoses were based on clinical criteria and laboratory data, including histology and immunofluorescence. The anti-Dsg antibody profiles were determined by Dsg1 and Dsg3 enzyme-linked immunosorbent assays (ELISA) (MBL, Nagoya, Japan). The site of biopsy included lesional and/or non-lesional skin. Skin was considered non-lesional if it seemed healthy on physical examination and Nikolsky’s sign was negative. Biopsies from redundant breast reduction skin served as healthy controls. Electron microscopy was performed for two glutaraldehyde-fixed biopsies, from Nikolsky-negative (N-) skin of a patient with mucosal-dominant PV and from Nikolsky-positive (N+) skin of a patient with PF. For the in vitro studies four PF and four mucocutaneous PV sera were used.

Purification of IgG and generation of Fab fragments

IgG was purified by HiTrap protein G sepharose chromatography (GE Healthcare, Uppsala, Sweden) according to the manufacturer’s protocol. Purified IgG was divided into two equal fractions. One fraction was dialysed against phosphate buffered solution (PBS) (10 mmol L⁻¹ sodium phosphate, 150 mmol L⁻¹ NaCl, pH 7.2) and concentrated to 0.5 mL by ultrafiltration (Amicon Ultra 100K NMWL; Millipore, Co Cork, Ireland). From the second fraction Fab fragments were prepared using immobilized papain (Pierce, Rockford, IL, U.S.A.) according to the manufacturer’s protocol. The Fab fragments were separated from the undigested IgG and the Fc-tail by HiTrap protein A sepharose chromatography (GE Healthcare). The flow-through containing the Fab fragments was concentrated (Amicon Ultra 30K NMWL) to 0.5 mL. Both the IgG and Fab were mixed with 3 mL DMEM/HAM 1 : 3 medium containing 2 mmol L⁻¹ glutamine, 100 µg mL⁻¹ penicillin and 100 U mL⁻¹ streptomycin and were stored at -80°C.

In vitro model

Six-millimetre biopsies from redundant breast reduction skin were placed on transwell inserts in a 24-well plate (Corning, New York, NY, U.S.A.). The IgG and Fab fractions were added to the wells, such that the medium contacted the bottom of the insert, and the plate was incubated at 37°C under cellculture conditions. After 24 h the biopsies were taken out, briefly rinsed with Hank’s balanced salt solution (HBSS) (Gibco, Glasgow, U.K.), frozen in liquid nitrogen and stored at -80°C.
Immunofluorescence microscopy
The procedures for immunofluorescence staining and image collection have been described before in detail. The following monoclonal antibodies were used: Dsg1–P23 and 27B2 (Dsg1), 10G11 (Dsg2), Dsg3–G194 (Dsg3), U100 [desmocollin 1 (Dsc1)], U114 (Dsc3), 15F11 (PG), DP2.15 (DP), PKP3–270.6.2 [plakophilin 3 (PP3)], NCH–38 (E–cadherin), 9G2 (β–catenin) and HD121 (plectin). Double staining of deposited IgG and adhesion molecules was performed with fluorescein–conjugated Fcγ–specific goat Fab’2 antihuman IgG (Protos Immunoresearch, Burlingame, CA, U.S.A.) and Alexa 568–conjugated goat antimouse IgG (Molecular Probes, Eugene, OR, U.S.A) as secondary steps. For Fab fragments, we used fluorescein–conjugated rabbit antihuman light chain kappa and lambda Ig (DakoCytomation Denmark, Copenhagen, Denmark). For double staining with two different mouse monoclonal antibodies we used Zenon Mouse IgG Labeling kits Alexa Fluor 488 and Alexa Fluor 568 (Molecular Probes – Invitrogen, Eugene, OR, U.S.A.) following protocols from the company.

Electron microscopy
The biopsies were fixed in 2% glutaraldehyde and postfixed with 1% osmium tetroxide and 1.5% potassium ferrocyanide in 0.1 mol L⁻¹ sodium cacodylate buffer. After dehydration in alcohol they were embedded in epon and ultrathin sections were cut. These were stained with uranyl acetate and lead citrate and examined with a Philips CM100 transmission electron microscope (Philips ⁄FEI Corp., Eindhoven, Holland).

Results
All biopsies had intraepidermal deposition of IgG. This IgG did not have the overall smooth ICS distribution that is seen when pemphigus serum is brought on to a section of normal skin. Although some depositions at first glance had a smooth appearance, a closer examination revealed some additional condensed IgG clumps or clusters, especially in the lower layers. In most biopsies this clustering was more apparent giving the overall pattern an irregular, somewhat speckled or punctate, appearance. Sometimes the IgG was present as interrupted lines but more often it had concentrated in a limited number of fine or coarse clusters per individual cell. We next investigated the distribution of desmosomal and non-desmosomal adhesion molecules.

Clustering of IgG, Dsg3 and plakoglobin in the non-lesional skin of patients with mucosal-dominant pemphigus vulgaris with IgG to Dsg3 only
The normal smooth ICS distribution of Dsg1 and 3 in healthy human control skin is shown in
Figure 1a–c. In the skin of a patient with mucosal-dominant PV, i.e. skin that has never blistered, Dsg3 had lost its normal smooth distribution and was clustered, while Dsg1 was still present in a normal smooth pattern (Figure 1d–f). Desmocollin (Dsc)3 (Figure 1g–j) and Dsc1 (not shown) also remained smoothly distributed. The overall Dsg3 fluorescence intensity was reduced compared with normal human skin. IgG in vivo deposits colocalized with the clustered Dsg3 (Figure 1j–l). The plaque proteins DP, PP3 and plectin remained smoothly distributed (Figure S1a). PG was largely normal but some slight concentrations followed the Dsg3 clusters (Figure 1m–o). The distribution of the adherens junction proteins such as β–catenin remained smooth (Figure S1j–l). Clustering of in vivo deposited IgG can thus be explained by an altered distribution of IgG–bound Dsg3.

Clustering of IgG, Dsg1 and plakoglobin in the skin of patients with pemphigus foliaceus with IgG to Dsg1 only
In PF skin the IgG deposits were clustered in the lower layers and were mostly smoother in the higher layers, although in incidental biopsies the IgG had an almost completely punctuate appearance (Figure S2b,c). When a blister was present the punctuate deposits were most often located underneath it, although sometimes they were also seen above it (Figure S2d,e). A few biopsies had little IgG deposition and here puncta of IgG were only seen in the lower half of the basal cells (Figure S2a). Compared with mucosal-dominant PV skin the puncta had a coarser appearance. Both Dsg1 and PG heavily colocalized with the IgG clusters (Figure 2a–c). The other desmosomal cadherins remained normally distributed (Figure 2d–l). In areas of heavy PG clustering some concentration of other plaque molecules was visible. Figure 2m–r shows that DP and PP3 to some extent follow their binding partner PG. Adherens junction proteins E–cadherin and β–catenin did not aggregate (Figure S1m–r).
Figure 1 – Distribution of IgG and desmosomal adhesion molecules in healthy skin of patients with pemphigus vulgaris (PV) with IgG antibodies to desmoglein (Dsg)3. Double immunofluorescence staining (c, f, i, l, o are overlays from the reds and greens in the panels to the left of them) shows a punctate epidermal distribution of Dsg3 (Dsg3–G194) in the skin of a patient with PV (d, g, j, m) that contrasts with the Dsg3 distribution in normal human skin (a). The Dsg3 dots colocalize with the IgG (k) that is deposited in the same PV skin. Other cadherins such as Dsg1 (Dsg1–P23) (e) do not concentrate in these puncta and remain distributed as in normal human skin (b). Also desmocollin (Dsc)3 (U114) (h) does not follow the Dsg3. Some plakoglobin (PG–15F11) (n) colocalizes with the Dsg3 puncta. All images have the same magnification. The white bar is 40 μm.
Figure S1 – Distribution of desmosomal plaque and adherens junction molecules in healthy skin of patients with pemphigus vulgaris (PV) and pemphigus foliaceus (PF). In PV skin no colocalization with the Dsg3 dots (Dsg3–G194) (a, d, g, j) is seen for other desmosomal plaque molecules proteins desmoplakin (Dp2.15) (b), plakophilin–3 (PKP3–270.6.2) (e), plectin (HD121) (h) or with adherens junction molecules as shown here for ß–catenin (9G2) (k). Also in PF skin the adherens junctions molecules E–cadherin (NCH–38) (n) and ß–catenin (9G2) (q) do not colocalize with the puncta represented here by the deposited IgG (m, p). Images c, f, i, l, o, r are overlays from the reds and greens left of them.
Figure S2 – IgG deposition patterns in skin of a patient with pemphigus foliaceus (PF). Immunofluorescence demonstrated different IgG deposition patterns in the skin of a patient with PF. In skin with little IgG deposition (a) punctate aggregation was already present, but close to the basement membrane zone (BMZ) (white arrows). Other samples showed more intense IgG deposition that could be punctate in lower cell layers and smoother in upper cell layers (b, d) or punctate all over (c, e). This was observed in both non-lesional (b, c) and lesional skin (d, e).
Figure 2 – Distribution of desmosomal adhesion and plaque molecules in skin of patients with pemphigus foliaceus (PF).

Double immunofluorescence staining of PF patient skin reveals a dotted epidermal distribution of desmoglein (Dsg)1 (27B2) (a, d, g, j, m, n). The distribution of the plaque molecule plakoglobin (PG) (PG–15F11) (b) follows that of Dsg1 and is present in the same dots. The other cadherins desmocollin (Dsc)1 (U100) (e), Dsc3 (U114) (h) and Dsg3 (Dsg3–G194) (k) do not follow the rearranged Dsg1. The plaque proteins desmoplakin (DP2.15) (n) and plakophilin–3 (PKP3–270.6.2) (q) partly concentrate at the same locations as PG and Dsg. Images c, f, i, l, o, r are overlays from the reds and greens in the panels to the left of them. All images have the same magnification. The white bar is 40 μm.
Clustering of IgG, Dsg1, Dsg3, and plakoglobin in the skin of patients with mucocutaneous pemphigus vulgaris with IgG to Dsg1 and Dsg3
As expected both types of clusters were present in skin of patients with IgG to both Dsg1 and 3. Although some aggregates were dominantly of the IgG/Dsg3/PG type and some of the IgG/Dsg1/PG type, they were usually concentrated at the same positions. The degree of clustering of the individual desmogleins could differ widely between patients. Patients with relatively high circulating anti-Dsg1 IgG as shown by ELISA values demonstrated heavier Dsg1 clustering, whereas anti-Dsg3 clustering dominated in patients with higher anti-Dsg3 ELISA values (Figure 3a–d). This was reflected in the degree of PG clustering. PG was severely more reallocated by anti-Dsg1 than by anti-Dsg3 clustering (Figure 3e–f shows Dsg3 vs. PG).

Intercellular widening of the lower layers in the non-lesional skin of patients with pemphigus foliaceus and mucocutaneous pemphigus vulgaris, but not in the skin of those with mucosal-dominant pemphigus vulgaris
To investigate if clustering of Dsg leads to altered cellular morphology we stained our biopsy sections with haematoxylin and eosin (H&E). Skin with clustered Dsg3 did not show any abnormalities (Figure 4a). In PF skin with profound Dsg1 clustering, the keratinocytes exhibited subtle widening of the ICS, especially between basal cells and to a lesser degree between the suprabasal cells (Figure 4b). In lesional skin widening also extended to higher layers. The intercellular widening roughly correlated with the degree of Dsg1 clustering. Biopsies without visible widening had little IgG deposition and minimal Dsg1 clustering. Intercellular widening was also present in the uninvolved skin of patients with mucocutaneous PV with additional antibodies to Dsg1, and whose skin demonstrated substantial Dsg1/PG aggregation (Figure 4c), but this was not seen in the skin of those with mucosal-dominant PV who lacked anti-Dsg1 antibodies. Additional electron microscopy confirmed these observations. Intercellular widening was present in the basal cell layer of a N+ PF skin biopsy, but not in mucosal-dominant PV skin although the corresponding immunofluorescence freeze biopsy demonstrated clustered Dsg3 (Figure 4d,e). Moreover the desmosomes in mucosal-dominant PV skin were of normal size and number (see the insert in Figure 4e). Therefore, clustered Dsg does not represent piled up floating desmosomes, which we initially thought, but represents complexes of IgG–bound non-junctional Dsg.
Figure 3 – Distribution of desmoglein (Dsg)1, Dsg3 and plakoglobin (PG) in skin of patients with IgG to both Dsg1 and Dsg3. Both Dsg1 (27B2) (b, d) and Dsg3 (Dsg3–194) (a, c, e, g) become rearranged. The extent to which individual cadherins become concentrated in puncta differs between patients and seems to correlate with their respective anti-Dsg titres. Images a, b and e, f are from a patient with ELISA index values of 249 for Dsg1 and 126 for Dsg3 and images c, d, g, h from a patient with index values of respectively 88 and 301. PG (15F11) (f, h) did not follow the distribution of Dsg3 (e, g), but clustered with similar intensity as Dsg1 [compare image (b) with (f) and (d) with (h)]. All images were obtained through double staining and were photographed in separate channels, thus reds and greens represent the same section. The white bar is 40 μm.
Figure 4 – Intercellular widening of the lower layers in skin of patients with antibodies to desmoglein (Dsg)1. Haematoxylin and eosin–stained sections of non-lesional skin of a patient with mucosal-dominant pemphigus vulgaris (PV) with IgG to Dsg3 only (a), of non-lesional skin of a patient with pemphigus foliaceus (PF) (b) and of non-lesional skin of a patient with mucocutaneous PV with IgG antibodies to both Dsg1 and Dsg3 (c). Subtle intercellular widening of the intercellular space is seen in the skin of the patients with PF and mucocutaneous PV, but not in the skin of the patient with mucosal-dominant PV. Electron microscopy demonstrates intercellular widening in N+ non-lesional PF skin (d) but not in non-lesional mucosal-dominant PV skin (e). Despite Dsg3 depletion the desmosomes in non-lesional mucosal-dominant PV skin are of normal size [higher magnification insert in (e), the bar is 500 nm]. The asterisk denotes the dermal compartment.
Figure S4 – The rearrangement of Dsg and plakoglobin (PG) into clusters is induced by IgG from a patient with pemphigus. When biopsies of normal human skin were incubated with pemphigus foliaceus (PF) IgG (b, d, f) the IgG (b) became bound in the epidermis in a similar granular pattern to that observed in patient's skin. Both Dsg1 (Dsg1–P23) (d) and plakoglobin (PG) (15F11) (f) rearranged into these same clusters. When incubated with normal human IgG (a, c, e) the IgG (a) was not bound in the epidermis and redistribution of Dsg1 (c) or PG (e) did not occur. All images have the same magnification. The white bar is 40 μm.
Figure S3 – IgG and Fab fragments both induce the typical pemphigus blisters. Normal human skin biopsies incubated with pemphigus IgG or Fab fragments demonstrated blistering identical to that observed in pemphigus patient skin. Shown are the typical pemphigus vulgaris (PV) suprabasal blister that was induced with PV IgG (b) and the subcorneal pemphigus foliaceus (PF) blister that was induced here with PF Fab fragments (d). Control incubations with normal human (NH) IgG (a) or normal human Fab fragments (c) did not show blistering or acantholysis.

Figure 5 – IgG but not Fab fragments induce clustering. In skin that was incubated with pemphigus foliaceus (PF) IgG the plakoglobin (PG) (15F11) aggregated (a), while in contrast in skin that was incubated with Fab fragments prepared from the same IgG the PG did not become affected (b). Nevertheless the Fab fragments induced blistering at the subcorneal level. Both images have the same magnification. The white bar is 40 μm.
Native pemphigus IgG, but not its Fab fragments, induce the typical desmoglein clustering in skin. 

*In vitro* incubation of normal human skin with purified native PV–IgG and PF–IgG induced respectively suprabasal and subcorneal blistering. The IgG became bound in the epidermis in the same punctate pattern as in biopsies from patients (Figure S4b). Likewise, the targeted Dsg clustered, and PG co–clustered with the IgG (Figure S4d,f). Typical pemphigus blistering was also reached when the Fab fragments of these sera were used (Figure S3a–d). However, Fab fragments bound smoothly around the cells and clustering of Dsg or PG was not observed (Figure 5). The clustering of Dsg therefore results from an intrinsic characteristic of the IgG. The difference between IgG and Fab fragments is that the IgG is bivalent and able to crosslink desmogleins, in contrast to the monovalent Fab fragments. Clustering of IgG therefore is an indicator for the targeted Dsg, but not necessary for the pathogenesis of acantholysis.

**Discussion**

Here we show that clustering of IgG in the skin of patients with pemphigus is explained by the complex formation of non-junctional Dsg to which the IgG is directed. The *in vitro* experiments showed that this clustering is caused by crosslinking of Dsg by bivalent IgG. The ability of IgG but not of Fab fragments to crosslink Dsg has been reported before.\(^{20}\) The clusters do not result from acantholysis nor do they indicate active acantholysis. Instead they reveal that the targeted non-junctional Dsg becomes sequestered from desmosomal components. This fits the present experimental data that predict that IgG–bound soluble Dsg is not incorporated into desmosomes anymore, but leads to the disturbed assembly and desmosomal depletion of Dsg.

In the skin of our patients with anti–Dsg3 antibodies the Dsg3 was present in a punctate pattern and at a reduced level. This decrease is in line with the observation that less Dsg3 than normal can be extracted from pemphigus skin.\(^{21}\) Also in cultured cells PV–IgG initiates a decrease of Dsg3. First non-junctional Dsg3 disappears and next, as no replacement Dsg3 is now available to the desmosomes, desmosomal Dsg3 also disappears, leaving Dsg3–depleted desmosomes.\(^{13,14,21–23}\) This suggests that Dsg1 can fully compensate for Dsg3–depletion and preserves the size and number of the desmosomes in the basal cells of mucosal-dominant PV skin. Immunoelectron microscopy demonstrated that newly formed half–desmosomes were depleted of Dsg3, but not of Dsc3 by mucosal-dominant PV serum.\(^{24}\) Our data from the skin of patients favour this depletion hypothesis, as Dsg3 did not anymore colocalize with the other desmosomal cadherins. Importantly, however, this disorganization and depletion happens in skin
that, despite being loaded with IgG, does not show any signs of acantholysis or pre–acantholysis. Our patients with pure mucosal-dominant PV had oral lesions, thus their IgG was pathogenic. At the same time they had healthy nonblistering skin that was not susceptible to minor trauma or friction and Nikolsky's sign could not be evoked. This contrasts with experiments with cell monolayers that demonstrated decreased mechanical–stress resistance after Dsg3 depletion, which suggested that Dsg3–depleted desmosomes would result in fragile N+ skin.14,25 Our study of patients’ skin instead demonstrates that epidermis is able to withstand the pathogenic effects of the anti–Dsg3 antibodies and compensates for Dsg3 depletion.

In PF skin, Dsg1 clusters instead of Dsg3. From the biopsies a picture emerges of an aggregation process that starts low in the basal layer, close to the basal membrane zone and that, when more IgG becomes available, also spreads to the layers above. That the basal layer shows the earliest change is not surprising as that layer contacts the IgG that enters from the dermis first. Other cadherins do not reallocate and this suggests, in analogy with Dsg3 depletion in PV, that in PF desmosomes become depleted of Dsg1. PF blisters never occur in the lower layers, thus sufficient compensation is present to prevent the lower cells from becoming acantholytic, although intercellular widening is present, as will be discussed below. Further electron microscopy studies are necessary to answer the question whether PF desmosomes in basal cells become hypoplastic.

A striking difference between PV and PF skin is the extent to which PG coclusters. Where in mucosal-dominant PV skin PG largely maintains its normal distribution it becomes markedly disturbed in PF skin and is seen as large dots colocalizing with Dsg1. The reason for this is not clear, but could be that PG is more tightly bound to Dsg1 than to Dsg3. The actual strength of binding of both cadherins with PG is unknown, but stoichiometric analysis of PG–cadherin complexes revealed that Dsg3 binds one molecule of PG where Dsg1 binds two.26 An alternative explanation involves the phosphorylation state of Dsg. Dsg3 becomes phosphorylated when IgG binds to it after which PG dissociates from Dsg3.27,28 The sequestering of IgGbound Dsg3 therefore may have little effect on PG. For Dsg1 it is currently unknown if binding of IgG results in similar phosphorylation and dissociation, but our patient skin data suggest that this might not be the case in view of the coclustered Dsg1 and PG.

The intercellular widening in the lower layers correlates with clustering of Dsg1. Widening is also seen when the expression level of Dsg1 is lower than normal. Haploinsufficiency of DSG1 in palmoplantar keratoderma gives a similar widening of intercellular spaces.29 Furthermore silencing of the Dsg1 expression in human raft cultures also induces intercellular widening.30 Dsg1 therefore might have an important role in keratinocyte cell–cell apposition in the lower epidermis.
It is without doubt, however, that in PF skin widening is present in layers where no acantholysis
takes place. Ultrastructural studies of PF skin are in line with our observations. Wilgram et al.\textsuperscript{31}
observed widening between basal cells that they described as ‘early acantholysis’. Injecting
IgG from endemic PF patients into mice results in early widening of interdesmosomal areas of
the basal and spinous layer.\textsuperscript{32} In this respect it is interesting that interdesmosomal widening is
also seen in PV skin, and after injecting PV serum into mice.\textsuperscript{33,34} Based on these observations
intercellular widening is considered a pre–acantholytic event in PV, which also has initiated
alternative theories involving basal cell shrinkage as a cause for acantholysis.\textsuperscript{35} It is, however,
important to realize that these ultrastructural studies date back to the time when the antibody
specificity of patients was not known and, considering the fact that they probably were patients
with mucocutaneous PV having both anti–Dsg3 and anti–Dsg1 antibodies, it is conceivable that
the early widening in mucocutaneous PV is caused by the concomitant anti–Dsg1 antibodies. A
definitive answer can only be provided by repeating the electron microscopy studies with a series
of defined patient samples.

How do our findings on patient skin agree with current hypotheses on PV acantholysis? In
view of the sequestering and decreased Dsg3 level in uninvolved skin, we do not doubt that
the selective depletion of Dsg3 from desmosomes that has been repeatedly demonstrated in
experimental cell systems, is also present in patients’ skin. The data therefore do not favour
steric hindrance within desmosomes where no sequestering is expected, but do not exclude the
possibility of steric hindrance in non-desmosomal Dsg. They also question the cell–signalling
hypothesis. If PV–IgG induces acantholysis by activating the p38MAPK pathway then basal and
suprabasal skin cells of anti–Dsg3 mucosal-dominant PV patients should become acantholytic,
which contrasts with our observations. Nevertheless, cell signalling might have a place in the
sequelae after intercellular widening has occurred from IgG binding. In the disassembly depletion
hypothesis it is suggested that the antibodies interfere with desmosomal Dsg leading to internal-
ization of Dsg and dismantling of the desmosome. Although this mechanism is probably present
it is not crucial in our vision, but rather secondary to the nonassembly depletion. Moreover,
under normal homeostatic conditions desmosomes are also subject to dynamic turnover and
it is questionable if IgG binding makes a difference here.\textsuperscript{36,37} Knowing the binding of IgG to
soluble Dsg pools found by Aoyama et al.\textsuperscript{14} and the early non-desmosomal widening between
epidermal cells in pemphigus,\textsuperscript{38} we favour from our patient data the nonassembly Dsg depletion
hypothesis of desmosomes as also suggested by Payne’s group.\textsuperscript{22} We however expand this
model with Dsg1 depletion based on our observations that Dsg3–depleted patient skin is sturdy,
N- and ultrastructurally undisturbed. The compensatory mechanisms in skin are evidently of
such strength that they prevent skin from becoming N+. Based on our observations on PF skin, which suggest that anti–Dsg1 antibodies deplete desmosomes of Dsg1, we hypothesize that in mucocutaneous PV skin concomitant Dsg1 depletion will further weaken desmosomes leading to N+ and finally to acantholytic skin (Figure 6). Such a view is in line with desmosomes being dynamic structures that are continuously renewed, partly by the formation of completely new desmosomes, partly by replacement of molecules in existing desmosomes.36,37 Newly synthesized cadherins are transported from the Golgi to the membrane and form floating patches destined for incorporation into desmosomes. Frustration of this process by pathogenic IgG will rapidly lead to desmosomes becoming depleted of essential cadherins.
Figure 6 – Conceptual model of pemphigus IgG–induced desmosomal depletion of desmogleins. (a) Under normal homeostatic conditions desmosomal proteins are continuously renewed. Freshly synthesized desmogleins (Dsg1 in yellow, Dsg3 in blue) are transported from the Golgi apparatus to the membrane where they become present as non-junctional Dsg. It is still unknown whether this non-junctional Dsg is also involved in intercellular binding. Desmogleins are in continuous turnover in the desmosome and will be discarded by cellular uptake to be destroyed or recycled (right side desmosome), and replenished by fresh non-junctional Dsg (left side desmosome). (b) Selective depletion of the desmosome occurs when patients have IgG (red) to one Dsg isoform. This Dsg now becomes crosslinked by the IgG before entering the desmosome and will assemble in clusters outside the desmosomes. This leaves desmosomes that are depleted of one Dsg isoform but that are still able to resist acantholysis. (c) When both Dsg isoforms become crosslinked into clusters (red and green IgG) then desmosomes will not be able to renew themselves properly any more and, as they are subject to the same protein turnover as normal, will melt away because of lack of fresh replacement molecules. Without desmosomes, cells do not have sufficient adhesive properties and acantholysis will occur.
References


