Acantholysis in pemphigus
van der Wier, Gerda

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Smaller desmosomes are seen in the skin of pemphigus patients with anti–desmoglein 1 antibodies but not in patients with anti–desmoglein 3 antibodies

G. van der Wier, H.H. Pas, D. Kramer, G.F.H. Diercks and M.F. Jonkman

Centre for Blistering Diseases, Department of Dermatology, University of Groningen, University Medical Centre Groningen, Groningen, The Netherlands

Abstract

Background Pemphigus is an autoimmune blistering disease due to autoantibodies targeting desmoglein (Dsg)1 and/or Dsg3 present in mucocutaneous desmosomes. The binding of pathogenic autoantibodies impairs desmosome function leading to acantholysis, however the mechanism behind this is unclear.

Objectives In this study we will investigate the individual influence of Dsg1 and Dsg3 autoantibodies on desmosome size and number in skin of patients with pemphigus foliaceus (PF), mucosal–dominant pemphigus vulgaris (PV), and mucocutaneous PV.

Patients/ Methods IgG, Dsg1 and Dsg3 patterns were investigated by direct immunofluorescence (DIF) microscopy. The size and number of desmosomes were determined by electron microscopy.

Results In Nikolsky negative (N–) PF skin there was minimal clustering of Dsg1 in the basal layer, while in Nikolsky positive (N+) PF skin Dsg1 clustering spread up to the suprabasal layers. N+ PF skin had smaller and less desmosomes than N– PF skin, or control skin. In mucosal-dominant PV skin Dsg3 was clustered and strongly reduced, but the desmosomes remained of normal size and number. In skin of mucocutaneous PV patients with autoantibodies against both Dsg1 and Dsg3 a reduction of desmosome size but not in number was found.

Conclusions In conclusion, targeting Dsg1, and not Dsg3, reduces desmosome size in skin of pemphigus patients.

Introduction

Pemphigus is a chronic mucocutaneous autoimmune blistering disease caused by autoantibodies directed against the desmosomal cadherins, desmoglein (Dsg)1 and/or Dsg3. In patients with pemphigus foliaceus (PF), there is superficial blistering of the skin but not of mucous membranes, with autoantibodies directed against Dsg1. In mucosal–dominant pemphigus vulgaris (PV), mucous membranes are involved but not the skin, with autoantibodies directed against Dsg3. Mucocutaneous PV is characterized by suprabasal blistering of both the skin and mucous membranes, with autoantibodies directed against both Dsg1 and Dsg3.

Desmosomes are intercellular adhering discoid junctions serving to attach neighboring cells to each other. Their diameter varies between 0.2 and 0.7 μm in the epidermis. During epidermal differentiation, smaller, less well–organized desmosomes in the basal cells are replaced by larger, more electron–dense structures in the upper layers.1–4

Desmosomes are composed of members of at least three protein families. Desmosomal cadherins (desmogleins and desmocollins) constitute the transmembrane adhesive interface, whereas armadillo and plakin family proteins build up the cytoplasmic plaques. The cytoplasmic tail of
the transmembrane desmogleins and desmocollins interacts with plakoglobin (PG), which in turn binds to desmoplakin. Desmoplakin anchors to the intermediate filaments. The interactions are stabilized laterally by plakophilin.\textsuperscript{2,5}

In pemphigus, both anti–Dsg1 and anti–Dsg3 antibodies can cause acantholysis, although the actual pathomechanism is unknown. Current theories include steric hindrance, desmosomal nonassembly and disassembly, or cell signalling.

We recently described the ultrastructure of the epidermis in PF patients.\textsuperscript{6} We found no abnormalities in the desmosomes or in the intercellular distance in Nikolsky–negative (N-) PF skin, whereas in Nikolsky–positive (N+) PF skin we observed intercellular widening between the desmosomes and a slight reduction in the size and number of desmosomes in the lower epidermal layers but not in the higher ones. Full acantholysis was only observed in lesional PF skin due to a severe reduction in the size and number of desmosomes in the higher epidermal layers.\textsuperscript{6} In the present study, we performed morphometric studies on the skin of PF, mucosal-dominant PV, and mucocutaneous PV patients to determine the influence of Dsg1 and Dsg3 autoantibodies on the number and length of desmosomes. We correlated the morphometric data to the immunofluorescence staining pattern of the most important immunological effectors in pemphigus: IgG, Dsg1, and Dsg3.

**Materials and methods**

Skin biopsies of two human controls, and eight pemphigus patients were studied. The skin biopsies were taken from N- or N+ non–lesional skin from four PF patients (two N- and two N+), two mucosal-dominant PV patients (both N-), and two mucocutaneous PV patients (one N- and one N+). The diagnosis of PF, mucosal-dominant PV, or mucocutaneous PV was confirmed by histology, direct immunofluorescence (DIF) and indirect immunofluorescence (IIF) of the skin and serum, and by enzyme-linked immunosorbent assay (ELISA) that demonstrated exclusively anti–Dsg1 antibodies in the case of PF, anti–Dsg3 in the case of mucosal-dominant PV, and both Dsg1 and Dsg3 antibodies in the case of mucocutaneous PV. Two biopsies were taken from each patient at the same location. One biopsy was used for immunofluorescence staining and the other for electron microscopy. The biopsies used for immunofluorescence staining were taken as a part of the standard patient care. For the biopsies used for electron microscopy informed consent was documented in the patient files. Rubbing for the Nikolsky sign was performed juxtaposed to the biopsy site at a distance of 2 cm, so that the biopsy site, which appeared normal, remained undisturbed by the rubbing.
Results
In control skin samples, IgG was absent (Figure 1a and d) and Dsg1 and Dsg3 were distributed smoothly over the cell membranes (Figure 1b and e). Morphometric analysis showed that the average size of the desmosomes in the different layers was comparable, with 0.25 μm in the basal layer, 0.31 μm in the spinous layer, and 0.30 μm in the granular layer (Figure 2 and Figure S1 a–c). The number of desmosomes increased from the basal layer upward: 0.15 per μm² in the basal layer, 0.26 per μm² in the spinous layer, and 0.48 per μm² in the granular layer.

Figure 1 – Distribution of desmoglein (Dsg)1 and Dsg3 in the skin of pemphigus patients. In normal human skin (NHS), mucosal–dominant pemphigus vulgaris (mdPV) skin, and Nikolsky–negative (N- mucocutaneous PV (mcPV)) skin, there is an even distribution of Dsg1 around the cells (b, t, z). In N- pemphigus foliaceus (PF) skin, clustering of Dsg1 starts at the basal layer (h). In Nikolsky–positive (N+) PF skin, clustering of Dsg1 is present at the level of the basal, spinous, and granular layers (n). In N+ mcPV skin, clustering of Dsg1 is present throughout the layers (a, f). Dsg3 has a smooth staining pattern in NHS and PF skin (e, k, q), whereas in mdPV (w) and mcPV skin (ac, ai) Dsg3 shows heavy clustering and a reduced level of expression. Scale bar = 40 μm.
In N- PF skin samples, we saw limited IgG deposition on the epithelial cell surface (ECS) in the basal layer (Figure 1g and j). There was also some limited clustering of IgG and Dsg1 in the basal cell layer (Figure 1g–i). The desmosomal size (Figure 2 and Figure S1 d–f) and the number of desmosomes per $\mu m^2$ in the different layers were comparable to the control samples.

In the N+ PF skin, there was clustered deposition of IgG and Dsg1 at the basal and spinous layers (Figure 1m–o). Dsg3 kept its smooth distribution as in the control samples and N- PF samples (Figure 1q). The desmosomes in the N+ PF skin were significantly smaller in the basal and spinous layers, measuring on average 0.18 $\mu m$ and 0.20 $\mu m$, respectively, compared with those in control and N- PF skin, (Figure 2 and Figure S1 g and h). The number of desmosomes in the spinous layer was reduced to 0.13 per $\mu m^2$. 

**Figure 2** – Desmosome length in the skin of pemphigus patients. Desmosomes at the level of the basal layer and the spinous layer in Nikolsky–positive pemphigus foliaceus (N+ PF) and Nikolsky–positive mucocutaneous pemphigus vulgaris (N+ mcPV) skin are smaller than those in normal human skin (NHS). mdPV, mucosal–dominant pemphigus vulgaris; N-, Nikolsky negative.
Immunofluorescence staining of mucosal-dominant PV skin that was not affected showed transsepidermal IgG deposition in a clustered ECS pattern (Figure 1s and v). Dsg3 followed the pattern of the IgG and was also strongly reduced compared with normal skin (Figure 1w), whereas Dsg1 had a smooth distribution (Figure 1t). However, the size and number of desmosomes over the different layers were comparable to control skin samples (Figure 2 and Figure S1 j–l).

In N- mucocutaneous PV skin, IgG and Dsg3 were clustered throughout the layers of the epidermis (Figure 1ab–ad), whereas Dsg1 was still distributed smoothly (Figure 1z). As in mucosal-dominant PV skin, the desmosomal size (Figure 2 and Figure S1 m–o) and number remained normal. In the basal layer, the desmosomal size was reduced, but this was not statistically significant compared with that in normal skin samples.

In N+ mucocutaneous PV skin, however, Dsg1 co-clustered with IgG and Dsg3 (Figure 1ae–aj). Now the desmosomal size was significantly reduced compared with control skin samples at both the basal and spinous layers, where the desmosomes measured only 0.17 μm (Figure 2 and Figure S1 p and q). The number of desmosomes was not changed.
Figure S1 – Electron microscopy of pemphigus patient skin samples. Small desmosomes are present in Nikolsky-positive (N+), pemphigus foliaceus (PF) and N+ mucocutaneous PV (mcPV) skin at the level of the basal and spinous layers (g, h, p and q). The desmosomes are normal in size at the level of the granular layer in N+ PF and N+ mcPV skin (i and r), and in all other biopsies. Scale bar = 200 nm.
Discussion

Previously, we showed that in the skin of pemphigus patients, IgG binding induced clustering of the Dsg autoantigens and that this can be used as an *in vivo* marker of antigen binding. In a subsequent study, we observed that, to a limited extent, the desmosomes were smaller in the lower but not in the higher epidermal layers of N+ PF skin. The reduction of desmosome size extends to the higher layers and advances further in the higher layers of lesional PF skin, where spontaneous acantholysis occurred.

With this morphometric study, we have shown that desmosomal size is decreased in the lower layers of N+ PF skin and N+ mucocutaneous PV skin, but not in mucosal-dominant PV skin. In N+ PF and mucosal-dominant PV skin samples, there was clustering of Dsg1 and Dsg3, respectively, whereas in mucocutaneous PV skin both Dsg1 and Dsg3 were clustered. Dsg clustering is therefore an antigen marker of IgG binding, but is not necessarily pathogenic, as Dsg3 clustering was also observed in clinically unaffected mucosal-dominant PV skin, in which the desmosome size remained normal.

We therefore believe that the effects of Dsg1 antibodies on the epidermis are as follows: IgG directed against Dsg1 enters the epidermis from the dermis. First it contacts the basal layer. It binds to non–desmosomal Dsg1, where it assembles in large clusters, most likely by extensive cross-linking. As a first effect of the depletion of non-desmosomal Dsg1, intercellular widening occurs. Then, when the desmosomes also become depleted of Dsg1, they shrink in size and number. When the amount of anti-Dsg1 IgG increases, the IgG will spread further upward into the higher layers, also leading to intercellular widening and desmosomal reduction there. Finally, when the IgG reaches the layers where Dsg3 is absent and cannot compensate for the loss of Dsg1, desmosomes will no longer be able to form stable structures and will melt away with subcorneal acantholysis as the final result.

The effects of anti–Dsg3 antibodies on the epidermis differ from those of anti–Dsg1 antibodies. IgG directed against Dsg3 spreads through the epidermis and leads to clustering and depletion of Dsg3 throughout the Dsg3–expressing layers. This does not, however, lead to intercellular widening or a reduction in the size and number of the desmosomes. Apparently, loss of Dsg3 is less devastating than loss of Dsg1 to the desmosomes as they retain their normal shape. This fits with observations in patients with mucosal-dominant PV, who have blistering of the mucous membranes but a perfectly healthy and strong skin. Although their skin is loaded with anti–cell surface IgG deposits, it does not blister, even when it is firmly rubbed to elicit the Nikolsky sign. Next, when antibodies directed against Dsg1 are also present in addition to antibodies against Dsg3 (as in mucocutaneous PV), the depletion of Dsg1 will affect the desmosomes, which then
start to shrink. As the desmosomes can no longer compensate for the loss of both Dsg1 and Dsg3, they will melt away in the lower layers, which eventually leads to suprabasal acantholysis. We therefore conclude that Dsg1, but not Dgs3, is necessary for preserving the normal size and number of desmosomes in the human epidermis and that loss of Dsg1 is conditional for developing cutaneous acantholysis in pemphigus.
References


