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The IgG Lupus Band Deposition Pattern of Pemphigus Erythematosus: Its Association with the Desmoglein 1 Ectodomain as Revealed by Three Cases

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Abstract

Background Pemphigus foliaceus (PF) is an autoimmune skin disease characterized by subcorneal blistering and IgG antibodies directed against desmoglein 1 (Dsg1). In skin these antibodies deposit intraepidermally. On rare occasions an additional 'lupus band' of granular depositions of IgG and complement is seen along the epidermal basal membrane zone (BMZ). This combined pattern has in the past been connected with a variant of PF named pemphigus erythematosus (PE).

Observations We describe three PF cases that had received phototherapy after having been misdiagnosed for psoriasis. This resulted in a flare-up of skin lesions. Direct immunofluorescence of skin biopsies that were taken several weeks later demonstrated both intraepidermal and granular BMZ depositions. The BMZ depositions consisted of IgG, complement and the ectodomain of Dsg1, and were located at the level of the lamina densa.

Conclusions It is likely that high doses of UV-light induce the cleaving-off of the Dsg1 ectodomain. In PF patients the circulating anti-Dsg1 antibodies precipitate this cleaved-off ectodomain along the BMZ, resulting in a 'lupus band'-like appearance. In PE a similar mechanism may be active which might explain the so-called 'lupus-band' phenomenon.
Introduction
Pemphigus foliaceus (PF) is an autoimmune skin disease characterized by subcorneal blistering, and intraepidermal deposition of IgG antibodies that bind the desmosomal cadherin desmoglein 1 (Dsg1). Occasionally additional deposition of IgG is present along the epidermal basement membrane zone (BMZ), and this combined pattern has in the past been correlated with pemphigus erythematosus (PE). Based on its typical clinical manifestations of a lupus-like butterfly rash or severe seborrheic dermatitis, Senear and Usher initially suggested that PE was a condition where pemphigus vulgaris (PV) was combined with lupus erythematosus (LE), later named Senear-Usher syndrome. When insights into the differences between PV and PF crystallized, PE was not classified with PV anymore but instead considered an early or not-generalized form of PF. When immunofluorescence became a diagnostic tool the association with LE revived. Chorzelski et al described a so-called ‘lupus-band’ deposition in sun-exposed skin areas of PE patients together with anti-nuclear antibodies (ANA) as in LE. Later papers however showed less clinicopathological concurrency with LE as ANA antibodies appeared often absent, and the overall significance of this became disputed as it emerged that ANA antibodies were also present in a high percentage of the normal population. Although it is clear that in occasional cases LE can present simultaneously with pemphigus, the gross of the PE patients do not by far meet the criteria for SLE as published by the American College of Rheumatology (ARA). Therefore, what is called PE today should be separated from the sparse cases of actual concurrent LE and PV/PF. The basic teaching books nowadays consider PE to be a localized form of PF. The diagnostic criteria for PE do remain somewhat obscure. Clinically, PE is suspected in non-generalized disease with either symmetric distribution in the face or seborrheic areas. Histologically, PE and PF are both characterized by subcorneal blistering, and in both circulating autoantibodies against Dsg1 are present. In contrast to PF, however, biopsies of PE patients often reveal the ‘lupus-band’ phenomenon in which a coarse granular deposition of IgG and complement is found along the BMZ in addition to the typical intercellular substance (ICS) deposition. This band is present in a high percentage of patient biopsies (up to 60%), and likely reflects a unique immunopathological aspect of PE. As in LE, the mechanism of this BMZ deposition in PE remains unclear.

We have recently investigated the ICS deposition in PF and have shown that the IgG deposits in a punctate pattern in the skin, especially in the lower layers. This granular deposition is caused by IgG induced clustering of Dsg1 and plakoglobin (PG). Here we have extended this study to three UV-irradiated PF patients who had skin biopsies that displayed the combined ICS and BMZ deposition.
Report of cases

Case 1
An 80-year-old woman was admitted to our hospital in February 1993, with a 3-year history of generalized progressive erythematous-squamous skin lesions with pustules and flaccid blisters. This had initially been diagnosed elsewhere as psoriasis pustulosa complicated by secondary infection with Staphylococcus aureus. The patient had received several therapies including methotrexate, systemic erythromycin, acitretin and cyclosporine. Due to methotrexate related hepatotoxicity and insufficient effectivity of the other therapies, the patient switched over to a twice-weekly regimen of psoralen-UVA (PUVA) therapy with 40mg methoxsalen in January 1993. During PUVA therapy, the skin lesions worsened and therapy was stopped after 3 weeks. Physical examination at this time, revealed suberythroderma, consisting of confluent and scattered red macules with scales and purulent crusts. In the face a malar distribution was present. In addition multiple erosions and flaccid blisters were seen and Nikolsky’s sign was positive. The mucous membranes were not involved. Histopathology of a skin biopsy revealed an intraepidermal cleft just below the granular layer and slight dermal inflammation. Direct immunofluorescence microscopy (IF) of lesional and non-lesional skin showed intra-epidermal ICS depositions with a smooth staining pattern in the higher spinous layers and a coarse granular pattern in the lower spinous layers, as well as granular IgA and fibrin depositions in subepidermal vessel walls. In addition, non-lesional skin revealed coarse granular IgG and C3c depositions along the BMZ. Indirect IF on monkey esophagus revealed circulating anti-ICS IgG with a titer of >1:320. Retrospective analysis by ELISA revealed the presence of anti-Dsg1, but no anti-Dsg3, antibodies. Blood tests were negative for antinuclear, anti-ENA, anti-dsDNA, anti-SSA, anti-smooth muscle and anti-striated muscle antibodies.

Case 2
A 76-year-old woman with a 2-month history of generalized cutaneous blistering was admitted to our hospital in May 1997. The patient reported that, 6 months earlier, she had developed itching plaques all over her body and scalp, with exception of her legs. This was diagnosed elsewhere as psoriasis vulgaris, and the patient was treated with UVB-therapy, starting 4 months before. Two months after the start of UVB therapy, she developed painful blisters on trunk and face, with a burning sensation resembling that of sunburn. UVB therapy was stopped, but the blistering progressed. Physical examination, two months after the last UVB treatment, revealed multiple crusts on scalp, face and lips, without involvement of oral mucosa. In addition, significant erosive lesions were present on neck, arms and legs, and on the trunk erythema with crusts. The legs and dorsal trunk had a positive Nikolsky’s sign. The overall presentation resembled that of toxic epidermal necrolysis, staphylococcal scalded skin syndrome, or pemphigus foliaceus. A skin biopsy taken from the upper leg 4 months after start of UVB therapy, showed ulcerative and erosive inflammation and secondary impetigo with beginning re-epithelialization. Direct IF of non-lesional and perilesional skin revealed smooth intraepidermal ICS IgG depositions in the higher spinous layers and a coarse granular depositions in the lower layers, with weaker C3c depositions. In addition, granular IgG, IgM and C3c depositions were present along the BMZ in the non-lesional and perilesional skin. Indirect IF on monkey esophagus showed circulating anti-
ICS antibodies with IgG titers of 1:640. Indirect IF on rat bladder urothelium was negative. Anti-Dsg1 antibodies but no anti-Dsg3 were detected by ELISA, retrospectively. Circulating antinuclear antibodies were detected at titers of 1:20, falling below the cut-off range, and thus valued as negative.

Case 3
A 68-year-old male was referred to our hospital in April 2005 with a 2-year history of red scaly skin lesions starting in the medial corner of the right eyelid, and progressing to his chest and back. This was initially diagnosed elsewhere as psoriasis. The patient was subsequently treated with methotrexate. In addition, PUVA therapy was started 5 weeks later. The patient reported that 24 hours after the first PUVA treatment, generalized itching developed, followed by blistering on the whole body including scalp and extremities. Physical examination revealed facial malar erythema and erythematous confluent macules with central erosions, excoriations, crusts, and flaccid blisters on the scalp, trunk and extremities. Mucous membranes were not involved. Nikolsky’s sign was positive at blister margins, but negative on non-lesional skin. Skin biopsies taken from the upper leg and back, 5 weeks after start of PUVA therapy, showed a globally intact epidermis with what looked like a remainder of a blister in the corneal layer and subepidermal neutrophilic infiltrates surrounding the blood vessels. Direct IF of perilesional skin showed intraepidermal intercellular IgG, IgA and C3c depositions, with smooth staining of IgG in the upper and granular staining in the lower layers. In addition, granular IgG, IgA and C3c deposits were present along the BMZ. Indirect IF on monkey esophagus revealed the presence of circulating anti-ICS antibodies, and anti-Dsg1 but no anti-Dsg3 IgG antibodies were detected by ELISA. Blood tests were negative for antinuclear antibodies.

Despite a malar distribution of skin lesions in two of the three patients, and the relation with UV-exposure in all three, the patients did not otherwise fulfill the ARA criteria for LE. Instead the immunopathological findings fitted the diagnosis PF, and the malar involvement and presence of BMZ depositions were typical for PE.

Results
A total of nine skin biopsies had been stored from the three cases. The skin biopsies had all been taken from UV-exposed sites, since the whole body of the patients had received UV-therapy. All nine biopsies showed intra-epidermal deposition of IgG, and six had additional BMZ deposition of IgG and complement C3c (Figure 2A shows biopsies of all three patients). These BMZ deposits were not found in 51 biopsies of 14 other PF patients that had not received phototherapy (not shown).

As Dsg1 is the autoantigen in PF we stained our biopsies with anti-Dsg1 monoclonal Dsg1-P23. The staining overlapped with the IgG and C3c depositions, thus Dsg1 was not only present in the intraepidermal deposits but also in the BMZ deposits (Figure 2B). As controls we stained four biopsies of different LE patients with the anti-Dsg1 monoclonal. LE patients also have BMZ depositions of IgG (lupus band) but ANA antibodies instead of anti-Dsg1 antibodies. As expected
Dsg1 was absent from the BMZ depositions in the LE biopsies, thus the presence of Dsg1 in such band is exclusive to PF (Figure 2C).
To confirm our observation we stained with three other anti-Dsg1 monoclonal antibodies. The Dsg ectodomain specific monoclonal antibodies (Dsg1-P23, Dsg1-P124) colocalized with the BMZ IgG, whereas the Dsg endodomain antibodies (27B2 and DG3.10) did not, demonstrating the absence of the Dsg1-endomain in the lower BMZ deposits (Figure 3A and 3B). The somewhat higher clusters do contain both the Dsg1 endomain and ectodomain (figure 3B, yellow clusters). These clusters are part of the normal intraepidermal granular IgG depositions in PF skin that consist of IgG, full-length Dsg1 and PG 17, and may look like BMZ deposits, but are not, since they are located in the basal cells (yellow dots in figure 3B, right panel). To investigate if PG was also present in the BMZ deposits we double stained for PG and the Dsg1-ectodomain. PG appeared to be only present in the epidermal deposits (Figure 3C, left) but not in the BMZ deposits that contained the Dsg1-ectodomain. The BMZ deposits did furthermore not contain other desmosomal cadherins, Dsg3, Dsc1 and 3 (not shown).
The absence of the cytoplasmic protein PG and the endodomain of Dsg1 in the BMZ deposits suggested that the deposits were located outside the cells and were not connected with the cell membrane of the basal keratinocytes. To more precisely determine the location of the deposits, we performed double stainings with monoclonals to the Dsg1 ectodomain and the adhesion molecules type XVII collagen, laminin-332 and type VII collagen that map to different levels of the BMZ. Most of the deposits colocalized with type VII collagen with some found above or beneath the type VII collagen, indicating that the deposits are scattered around the lamina densa with some lying in the sublamina densa zone (Figure 3D).

Figure 1
(A) Extensive lesions in patient # 2, 2 months after ending UVB therapy. (B) The typical PE facial butterfly eruption of patient #1.
Figure 2 Immunofluorescence analysis of skin biopsies. (A) ‘Lupus band’ IgG deposition in skin of the three PF patients. (B) The deposited IgG (left, green) colocalizes with Dsg1 (middle, red) in PF patient skin but not in (C) SLE patient skin. All images have the same magnification. The white bar is 40 micrometers.
Figure 3 Immunofluorescence analysis of the deposits.

(A) The Dsg1 endodomain (left, green) is absent in the BMZ deposition. (B) Detail of (A) showing that the lower BMZ deposits contain the Dsg1 ectodomain (red) only, while the higher deposits in the basal cells contain both the endo- and ectodomain (yellow). (C) Also plakoglobin (left, green) is absent in the BMZ deposition. (D) The Dsg1 ectodomain (green) is found on and around type VII collagen. The images in A, C and D have the same magnification, the white bar in D is 40 micrometers. The white bar in B is 10 micrometers.
Discussion

Previous case reports have demonstrated that UV irradiation can induce skin lesions in PE and PF\textsuperscript{19-21}. Although BMZ deposition of complement is often seen in PF, BMZ deposition of IgG is uncommon. The three cases described here, suggest that UV-light can induce a ‘lupus band’ in PF patients.

In PE, the BMZ IgG deposition was previously shown to be related with UV-exposure. Giannetti demonstrated for PE patients that BMZ depositions were specific to sun-exposed areas. He investigated biopsies taken from lesional skin of both face and back of five patients. Where in four of five facial biopsies BMZ IgG deposition was present, no depositions were seen in any biopsies of the back\textsuperscript{7}.

The granular BMZ depositions in our UV-irradiated patients skin consist of the Dsg1 ectodomain, IgG and complement. The mechanism involved in the shedding of this Dsg1 ectodomain might include apoptosis. Dsg1 is reported to be a target of the effector caspase-3 in UV-induced apoptosis of keratinocytes\textsuperscript{22}. This apoptotic proteolysis also involves additional metalloproteinase dependent shedding of the 75 kDa Dsg1 ectodomain fragment. This fragment contains the extracellular (EC) 1 and EC2 domains that harbor the epitopes recognized by the pathogenic autoantibodies in PF\textsuperscript{23}. Therefore when these fragments diffuse out into the dermal compartment they can form immune complexes with the circulating anti-Dsg1 antibodies and deposit.

The data obtained from our three UV-irradiated patients may well provide the first clue for unraveling the mechanism behind BMZ deposition in PE since its first demonstration by Chorzelski et al. in 1968. We hypothesize that in PE also a UV-driven mechanism is active that releases Dsg1 fragments from the cell membrane, thus forming deposits along the BMZ. As this does not happen in ‘ordinary’ PF, PE patients somehow are predisposed for developing such pathophysiology. In this way, PE parallels discoid and subacute cutaneous lupus erythematosus (CDLE, SCLE) where the lupus band is also induced by UV-radiation in sun-protected non-lesional skin\textsuperscript{24}. PE and both LE forms are autoimmune diseases but with different autoantibodies, respectively anti-Dsg1 and ANA. In CDLE and SCLE characterization of the antigen involved in the lupus band so far has been unsuccessful, but the favored view is that the IgG reacts with nuclear and cytoplasmic antigens that are slowly released from damaged, possibly apoptotic, keratinocytes\textsuperscript{25}. Similarly, in PE, the IgG might react with the Dsg1 fragments.

Considering the data presented in this study, we conclude that PE is a subform of PF, immunologically characterized by unique, Dsg1 ectodomain containing granular epidermal BMZ immune deposits. In PE, an additional pathomechanism specific to PF is active that is evoked by UV radiation. Therefore, PE should be considered a photosensitive subform of PF, and not be confused with a combination of LE and pemphigus.
References

2. Dsg1 ectodomain in the lupus band of PE


