

Immunolocalization of Desmoglein in Autoimmune Pemphigus

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Background: Pemphigus foliaceus(PF) and pemphigus vulgaris(PV) have different target antigens which belong to the desmoglein(DG) subfamily of the desmosomal cadherins; DG in the case of PF and PV antigen(PVA) in the case of PV.

Objective: Because DG is also a normal major component of the intercellular adhesive core, we investigated the immunohistochemical distribution of DG in PF to compare and contrast the findings with those of PV.

Methods: Immunohistochemical analysis using streptavidin-biotin complex method with anti-DG monoclonal antibody was done in six cases of PF and six cases of PV, as well as two samples of normal skin as control.

Results: Both disorders showed abnormal intense diffuse cytoplasmic staining patterns in the lesional skin. Contrary to PF, showing complete loss of normal, rim-like, membranous staining, two of six cases of PV showed the relatively well preserved normal staining patterns in the upper epidermis.

Conclusion: The differences in the expression of DG in the lesional skins between PF and PV suggest that PVA is distinct from DG, although an overlapping of antigens between PF and PV could exist. (Ann Dermatol 6:(1) 31~36, 1994)

Key Words: Desmoglein, Pemphigus foliaceus, Pemphigus vulgaris, Pemphigus vulgaris antigen

Pemphigus is a group of autoimmune blistering diseases in which there is a loss of adhesion between keratinocytes as a consequence of autoantibodies binding to the cell surface¹. There are two distinctive subsets of pemphigus; the pemphigus vulgaris(PV) type that includes pemphigus vegetans and the pemphigus foliaceus(PF) type with pemphigus erythematosus as a subtype. Although both types have acantholytic bullae in the epidermis, PV is distinct from PF because of frequent mucosal involvement and suprabasal acantholysis.

In contrast, PF is characterized by crusted scales on the face and upper trunk with a histologic feature of subcorneal acantholysis. Although histologic studies can usually distinguish PF from PV, immunofluorescence studies frequently can not.

Previous studies using electron microscope showed that the earliest changes in PF may involve alterations in the desmosome-tonofilament complex, whereas in PV the earliest change is the loss of cell to cell contact between desmosomes^{2,3}. Despite the paucity of reports, it has been stated that desmosomal proteins and glycoproteins were intact in pemphigus, contrary to the genodermatoses such as Darier's disease and Hailey-Hailey disease showing intense diffuse cytoplasmic staining of desmosomal components in the lesional skins as well as acantholytic cells.^{4,6}

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Although the precise molecular characterization of antigenic moieties recognized by each pemphigus autoantibodies remains obscure, several observations and studies have demonstrated that desmoglein(DG), a major adhesive component of desmosome, and PV antigen(PVA) are the target antigens of PF and PV, respectively.^{7,8} Recently, sequence analysis revealed that DG and PVA are both in the cadherin family of Ca⁺⁺-dependent cell adhesion molecules⁹ but are more closely related to each other than to typical cadherins or to desmocollins.⁸ This close relation, but distinct nature, of DG and PVA led us to investigate the immunohistochemical distribution of desmosomal component, DG in PF and PV, which might provide insights into the pathophysiology of these autoimmune blistering diseases and contribute to our understandings of the differences between them.

MATERIALS AND METHODS

Materials

Punch biopsies of typical lesions were obtained from twelve pemphigus patients who had characteristic acantholytic bullae on histology, intercellular IgG deposits detected by direct immunofluorescence test, and circulating antibodies against the intercellular space by indirect immunofluorescence test with the use of normal human skin section as a substrate. Among these patients, six were considered to have PV showing suprabasal blister with acantholysis and remaining six had PF exhibiting subcorneal blister with acantholysis. Two samples of normal skin were used as control. These specimens were fixed in 10% formalin and processed into paraffin using routine techniques.

Methods

Immunohistochemical staining was performed on paraffin-embedded sections by using the routine streptavidin-biotin complex method. Desmosomal component was labelled using the mouse monoclonal anti-desmoglein antibody 32-2B¹⁰, which was applied at a dilution of 1:200. Tissue samples of normal skin were used as positive controls, and omission of the primary antibody served as a negative control. In the following protocol, sections were rinsed twice with Tris-buffered saline(TBS)(145mMol/L NaCl, 20mMol/L Tris,

pH 7.6) after each change of solution, up to dehydration steps; all incubations were done at room temperature(25°C), and all stated concentrations are final.

Following dewaxing and re-hydration through graded alcohols to water, the tissue sections were immersed in 0.5% hydrogen peroxide in methanol for 15 minutes to block endogenous peroxidase. Sections were washed with water, immersed in TBS for 5 minutes and then covered with normal rabbit serum diluted 1:5 with TBS. After 10 minutes, the excess rabbit serum was removed and replaced by primary antibody. Following 60-minute incubation, the secondary antibody, a biotinylated rabbit anti-mouse antibody(Dako corp., Santa Barbara, CA, U.S.A.), was applied at a dilution of 1:500 for 30 minutes. The peroxidase reaction was carried out using a streptavidin-biotin peroxidase complex(Dako Ltd., Copenhagen, Denmark) for 30 minutes. Finally, the reaction was developed using diaminobenzidine(80mg diaminobenzidine in 100ml of a 0.68g/dl solution of imidazole) with 1% hydrogen peroxide. After 1-2 minute incubation in this solution, sections were thoroughly washed, counter-stained with Mayer's hematoxylin, and mounted. Trypsination was not used in the present study because preliminary experiments indicated that the pre-incubation of the sections with trypsin for 5 or 10 minutes did not improved the quality of the staining.

RESULTS

Normal skin

There was no immunostaining in the bases of the basal cells where they were in contact with the basement membrane zone. Elsewhere in the epidermis there was fine stippled membranous staining of the periphery of epidermal keratinocytes from basal cell layer up to the granular cell layer. Staining was not detected in the stratum corneum(Fig. 1).

PF

There was a diffuse cytoplasmic or patchy perinuclear staining in the lesional skin and the majority of the acantholytic cells. This abnormal patchy staining was found on the floor as well as the roof of the bulla. It is noteworthy that the fine stippled membranous patterns of normal skin were com-



Fig. 1. Immunohistochemical staining of normal skin using anti-desmoglein antibody(32-2B) on paraffin-embedded section showing fine stippled, membranous staining pattern at the periphery of keratinocytes. Note there is no immunostaining in the region of hemidesmosomes and stratum corneum.

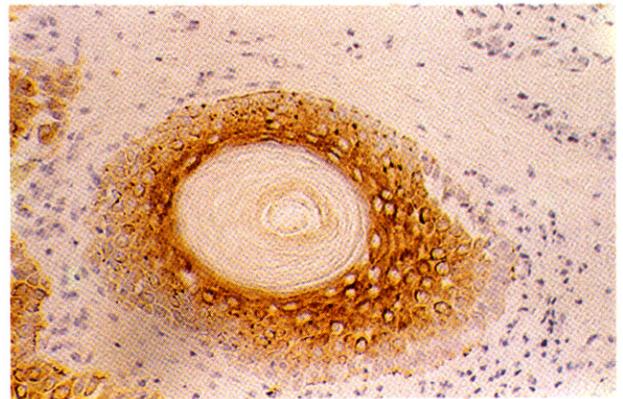
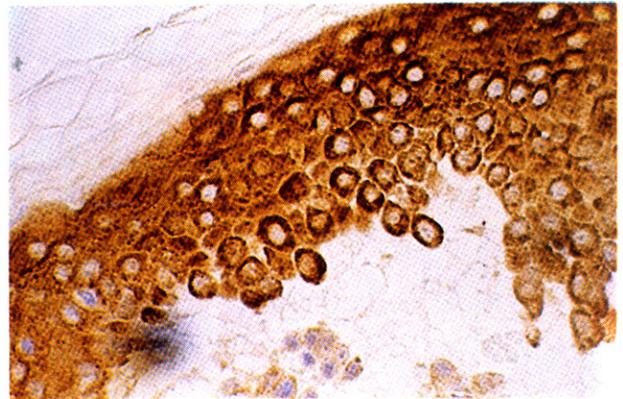
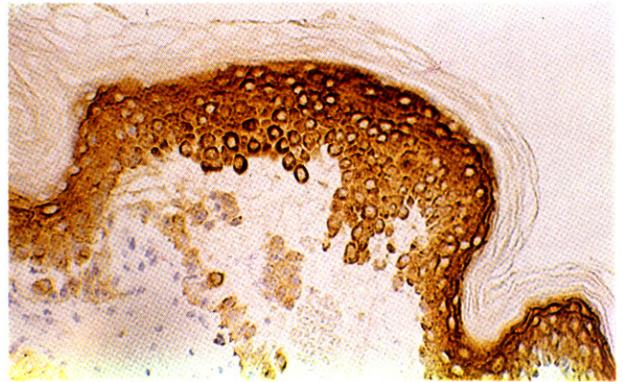


Fig. 3. Overview (A) and detail (B) of immunohistochemical staining of pemphigus vulgaris showing diffuse cytoplasmic or patchy perinuclear staining mainly on the roof of the bulla. Note there is a complete loss of normal membranous staining pattern. This patchy pericytoplasmic staining was also observed around the cells of hair follicles(C).

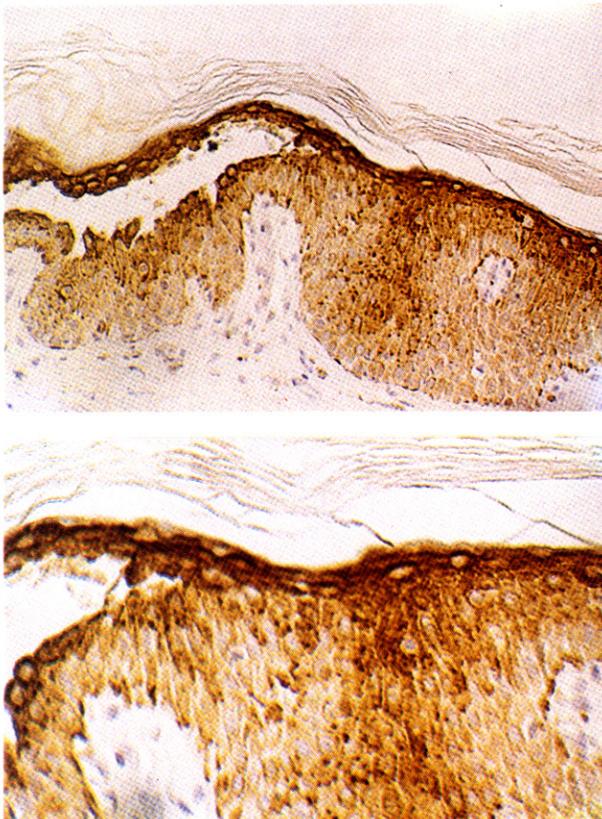


Fig. 2. Overview (A) and detail (B) of immunohistochemical staining of pemphigus foliaceus showing diffuse cytoplasmic or patchy perinuclear staining on the floor as well as the roof of the bulla. Note there is complete loss of normal membranous staining pattern.

pletely lost in the majority of the cases(Fig. 2A,B).

PV

The findings were similar to those of PF, al-

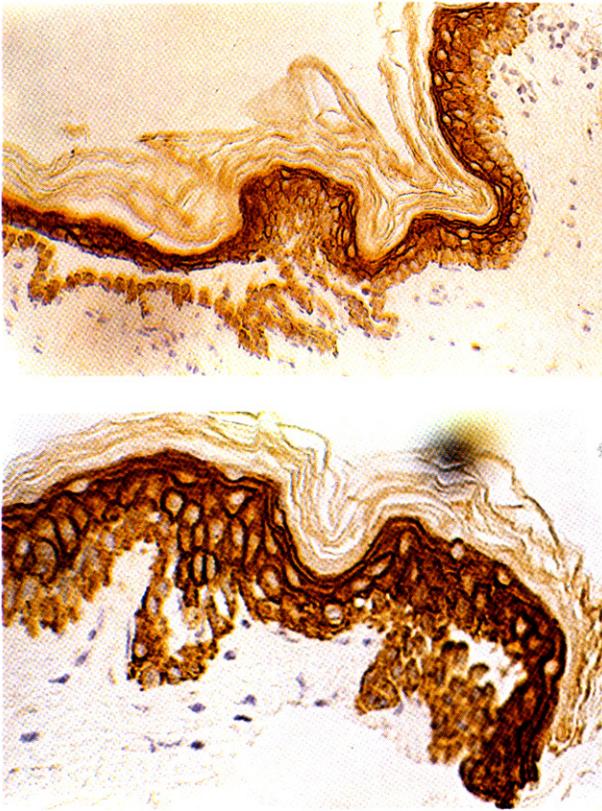


Fig. 4. Overview (A) and detail (B) of immunohistochemical staining of pemphigus vulgaris showing diffuse cytoplasmic staining with relatively preserved normal membranous pattern in the upper dermis.

though the abnormal staining was found mainly on the roof of the bulla and acantholytic cells (Fig. 3A,B). And this patchy pericytoplasmic staining was also observed around the cells of hair follicles (Fig. 3C). Contrary to PF, in two of six cases, it was possible to detect the relatively preserved normal membranous staining pattern in the upper epidermis (Fig. 4A,B).

DISCUSSION

Keratinocyte adhesion is mediated by special junctions, the desmosomes, along with other adhesion molecules on the cell surface. Desmosomes, which are associated with keratin filaments, are thought to be the major adhering junctions responsible for the attachment of cells¹¹. In addition to desmosomes, adherens junctions, which are associated with actin filaments, are intercellular and cell-matrix junctions that mediate adhesion of

cells to each other or to matrix structures¹².

Biochemically, desmosomes are composed of transmembrane desmosomal glycoprotein and interacting desmosomal plaque protein components which have been characterized mostly from bovine epidermal sources. The major components are DG (Mr 160kd, also known as desmoglein), DCI and DCII (Mr 120kd and Mr 110kd, desmocollins I and II), DPI and DPII (Mr 250kd and Mr 210kd, desmoplakins I and II), and plakoglobin (Mr 85kd). The glycoproteins DCI and DCII exhibit close molecular homology, as do the plaque proteins DPI and DPII¹³.

Desmosomes have a well defined ultrastructure, which consist of two components, an intercellular adhesive component, often referred to as desmoglea, and a intracytoplasmic plaque component. Ultrastructural immunohistochemical studies have revealed DCI and DCII located principally in the extracellular space, DG spanning the extracellular space and the cytoplasmic plaque, plakoglobin in the plaque, and DPI and DPII predominantly outside the plaque on its cytoplasmic face, in the region where cytokeratin intermediate filament converge¹⁴.

We have demonstrated striking alterations in the pattern of staining for DG in PF and PV. In PF, normal rim-like membranous staining patterns were completely lost in association with diffuse cytoplasmic staining, suggesting desmosomal breakdown. The most likely explanation for cytoplasmic desmosomal staining is that it represents internalizations of desmosomal halves associated with loss of intercellular adhesion. In cultured keratinocytes, loss of adhesion gives rise to intense diffuse cytoplasmic staining for desmosomal components¹⁵ similar to the pattern seen in the present study.

In most of the cases of PV, the same abnormal staining patterns were observed, although the intensity of staining was somewhat decreased. The reason why this abnormal staining is also observed in the lesional skin of PV is not clear, since previous studies have shown that PVA is different from DG. Immunoblotting and immunoprecipitation studies have demonstrated that PV antigen is a complex of 130kd and 85kd polypeptides, and PF antigen is a complex of 160kd and 85kd polypeptides, in which the 85kd polypeptide is plakoglobin¹⁶, a component of both desmosome and adherens junc-

tion¹⁷. As mentioned previously, adherens junctions are another membrane specialization which reflect non-desmosomal contact sites.

These junctions superficially resemble desmosomes in their structural appearance and share a common cytoplasmic component, plakoglobin¹⁷. In addition, cadherins, Ca⁺⁺-dependent cell adhesion molecules, are known to be the major adhesive components of adherens junction like DG in desmosomal structure. A comparison of amino acid sequences of desmocollins, DG, and cadherins shows that although these intercellular junctional adhesion molecules share a consensus sequence in their adhesive domains that defines them as a family, several features, including the divergence in the sequence of their cytoplasmic tails, divide them into three distinct subtypes¹⁸.

Among them, desmocollins show little resemblance to the cytoplasmic tail of either DG or cadherins⁸. Recently, sequence analysis revealed that PVA does indeed belong to the DG subfamily of the cadherin superfamily of cell adhesion molecules⁹.

It is interesting that some cases of PV showed the complete loss of normal, rim-like, membranous staining patterns, as encountered in PF, while the others showed relatively well preserved normal staining patterns, suggesting that transmembrane glycoprotein DG is not affected by PV autoantibodies primarily. These findings are in keeping with the results of Eyre and Stanley¹⁹ who reported that two thirds of PV sera precipitate the PF complex in addition to the PV complex, whereas the PF sera do not precipitate the PV complex by immunoprecipitation technique. Furthermore, Hashimoto et al.²⁰ displayed that 4 out of 16 PV sera reacted with 150kd polypeptide of PF antigen in desmosomal preparations, suggesting an overlapping of antigens between PF and PV.

It is noteworthy that the intensity of diffuse cytoplasmic or patchy perinuclear stain was strong in the upper epidermis regardless of types of pemphigus, although DG is the major intercellular adhesive component distributed throughout the epidermal layer, as seen in the normal skin. The reason for these findings is unclear, but there are several possible explanations. It might be that desmosomes contribute more to cell adhesion in the superficial epidermis compared with other, non-desmosomal, cell adhesion systems that might

contribute more in the deeper epidermis.

An alternative explanation is that the number and composition of desmosomes changes with keratinocyte differentiation. Konohana et al.²¹ demonstrated an increase in the ratio of the DG relative to the desmoplakins in the upper epidermis using the electrophoretic patterns of the whole epidermal extracts from 6 serial layers of the epidermis. Although the precise pathophysiologic mechanism leading to acantholysis has not been determined, these findings might partly explain the reason for the subcorneal location of blister in PF rather than a suprabasal location. Further work is needed in this important area of investigation to clarify the relationship of these various desmosomal components.

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