Pemphigus Vulgaris Forming Suprabasal Split Vesicles by Autoantibody-Induced Immune Reaction

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Pemphigus is an autoimmune blistering disease characterized by autoantibodies against epidermal adhesion molecules, desmogleins. Pemphigus vulgaris is most common and shows intraepidermal vesicles caused by the breaking apart of epidermal cells, acantholysis. A 65 years old male patient complained of severe mucosa ulceration on his right mandibular retromolar pad area where traumatic injuries occurred during mastication. He also had multifocal round skin ulcerations, less than 7~8mm in diameter and showed habitual onset and disappeared soon. At this time he was anxious about his oral ulceration with a cancer phobia, thereby a biopsy was made to rule out any malignancy in the ulceration. The histology examination showed multifocal suprabasal splits forming vesicles and erosion. The suprabasal splits were linear and parallel to the basal cell layer. The immunostain of IgK was strongly positive in the vesicular fluid as well as the cell membranes of dissociating keratinocytes, and also positive in many plasma cells infiltrated into the subepithelial zone. TNFα, IL-1, -8, -28 for the pro-inflammatory reaction were weakly expressed, while IL-6 was strongly positive in the acantholytic keratinocytes of vesicle forming area. β-defensin-1, -2, -3 for the innate immunity were diffusely positive in the involved epithelium. The cell survival proteins, pAKT and HSP-70 were diffusely positive in the epithelium, while the apoptosis protein, PARP was consistently positive in some acantholytic keratinocytes. These findings indicated that the vesicle formation occurred by autoantibody reaction without the activation of proinflammatory and cell-mediated immune reactions. The lesion was diagnosed pemphigus vulgaris with abrupt onset of epithelial vesicles at the predisposing areas of traumatic injuries by type II hypersensitive immune reaction.

Key words: Pemphigus vulgaris, Acatholysis, Autoantibody, Apoptosis

I. Introduction

Pemphigus is a disorder with an intraepidermal loss of adhesion and is characterized by fragile blisters and erosions. It is a potentially life-threatening autoimmune disease caused by antibodies directed against desmogleins resulting in the loss of cohesion between keratinocytes in the epidermis, and classified as a type II hypersensitivity reaction. Transudative fluid accumulates in between the keratinocytes and basement membrane, forming a blister. This is a contrasting feature from bullous pemphigoid, where the detachment occurs between the epidermis and dermis (subepidermal bullae). Pemphigus vulgaris is most common and often shows extensive lesions of the oral mucosa, while pemphigus...
foliaceus is the least severe type and commonly restricted to cutaneous involvement with puff pastry-like scale formation.\(^{10,11}\) Paraneoplastic pemphigus is the most severe type and obligatorily associated with malignancies and often presents as hemorrhagic stomatitis with multiforme-like exanthems.\(^{12,13}\) IgA pemphigus typically presents with pustules and annular plaques but not with mucosal involvement.\(^{14,15}\) Pemphigus vegetans is a localized form of pemphigus vulgaris, in which there is a vegetating papillomatous response.\(^{16,17}\) Its diagnosis is achieved via the detection of serum autoantibodies to either desmogleins by enzyme-linked immunosorbent assay (ELISA).\(^{6-8}\) However, the present study included only the histological data but ELISA, because further follow-up examination was not available. The usage of biopsy specimens filed in the Department of Oral Pathology was approved by Life Ethic Committee (KWNUDH-IRB 2009-16-3).

In this study the oral mucosa lesion of pemphigus vulgaris showed the characteristic suprabasal vesicle formation, implying the early onset of acantholysis by autoimmune reaction. Therefore, the vesicle forming lesion was examined by immunohistochemical method to know the pathogenetic mechanism of this pemphigus lesion,

### II. Case Report

A big mucosa ulceration, measuring 15x13x12mm, occurred in the mandibular retromolar pad area, where trauma frequently occurred during the mastication (Fig.1A2). He also showed multifocal skin ulcerations on his face and hand (Fig. 1A1,A3,A4). These round shape ulcerations were not healed easily after topical medication and retained fibrin-like materials, And there appeared multiple vesicles on the outer surface of his finger skin,

In the histological observation the oral mucosa epithelium was thickened and keratinized, and showed multiple linear splits in the suprabasal layer. These multifocal suprabasal splits were parallel to the basal layer, and sometimes they were filled with fluid and became vesicles. However, the basement membrane of basal cell layer was almost intact and tightly attached to the underlying connective tissue.

The immunohistochemical stainings of TNF\(\alpha\) (tumor necrosis factor-alpha), IgK (immunoglobulin kappa), CD3 (cluster of differentiation 3), CD20, CD31, CD68, pAKT (v-Akt murine thymoma viral oncogene homolog), PARP (poly ADP ribose polymerase), HSP-70 (heat shock protein 70), \(\beta\)-defensin-1, -2, -3, and IL-1 (interleukin-1), -6, -8, -28 (Santa Cruz Biotech. Inc., USA; DAKO, Denmark) were performed in the serial microsections in order to know the immunological status of this lesion. The IgK antibody showed the positive reaction in the cell membrane of acantholytic keratinocytes as well as in the cyst fluid of the vesicle (Fig. 1C1-C3). Particularly, the IgK antibody was strongly positive in the plasma cells infiltrated into the subepithelial region of the vesicle lesion (Fig. 1C1).

In the immunoreactions of CD3 and CD20 for the activated T and B cells, respectively, both CD3 and CD20 were weakly positive in the lymphocytes (Fig. 1E,G), thus implicating that the T-cell mediated immune reaction was relatively low. Nevertheless, the immunostains of CD31 and CD68, the biomarkers of PECAM-1 (platelet/Endothelial Cell Adhesion Molecule 1) and macrophages, respectively, were strongly positive in the acantholytic keratinocytes and macrophages in the vesicle lesions (Fig. 1F,H). And the proinflammatory biomarkers of TNF\(\alpha\), IL-1, IL-8, and IL-28 were sparsely positive in the vesicle lesions (Fig. 1D,I,K,L), while IL-6 which is known to target the dying and dead cells was strongly positive in the acantholytic cells (Fig. 1J).
Fig. 1, Pemphigus vulgaris. A: Multiple vesicles and ulcerations (arrows) in skin. B: Histology, H&E stain. C-R: Immunohistochemistry, using each antibody (lower left corner) without background staining. S: Negative control, Ve: vesicle, arrow: positive reaction.
In order to know the innate immune response against the autoantibody-induced hypersensitivity the immunostains of β-defensin-1, -2, -3 showed increased positive reaction in the acantholytic keratinocytes of the vesicle lesion (Fig. 1M,N,O). On the other hand, the biomarkers of cellular stress and survival, HSP-70 and pAKT, respectively, were strongly positive in the acantholytic keratinocytes (Fig. 1P,R). And more, the biomarker of cellular apoptosis, PARP became positive in some acantholytic keratinocytes (Fig. 1Q).

Taken together, the present case was diagnosed pemphigus vulgaris, and subsequently the clinical application of topical steroid therapy was effective to reduce the symptoms of this blister forming lesion.

### III. Discussion

Pemphigus is an autoimmune disease destroying the intercellular bridges of keratinocytes, desmosomes, by disabling desmoglein-1 and -3 proteins. The constellation of immunological reactions including cellular and antibody-dependent events may affect the progress of the autoimmune disease and also have implications for the pathogenetic mechanism of vesicle formation in pemphigus.

Although the present study had only one case of pemphigus vulgaris, the biopsy specimen showed the vesicle formation by suprabasal splits of oral mucosa epithelium. From the histological observation we thought that this early phase pemphigus lesion might disclose an important mechanism of its autoimmune reaction.

First of all, the immunostains of IgK, using antibody against the light chain (kappa chain) of immunoglobulin, showed clear positive reaction in some cell membrane of acantholytic keratinocytes and the cyst fluid of vesicles, and together with the strong reaction in the antibody-producing plasma cells infiltrated into the subepithelial region. Therefore, it was assumed that the suprabasal splits and vesicle formation were closely related to the autoantibody-induced immune reaction.

Interestingly, even though the active autoimmune reaction in the pemphigus lesion, there appeared only weak expression of TNFα, a key molecule of inflammatory cytokines, in the vesicle forming lesion. And the epitopes of CD3 and CD20 were not actively expressed in the T and B cells infiltrated into the lesion, respectively. These findings may imply that the autoimmune reaction recruiting T and B cells was not activated in the present oral mucosa lesion of pemphigus, but rather the pathogenetic autoantibody made by the previous cell-mediated sensitization in the other place may be causative. Therefore, it was presumed that in the present case the pro-inflammatory cytokine recruiting cell-mediated immune reaction was rarely observed, while the autoantibody-induced type II hypersensitive immune reaction was frequently observed in the oral mucosa epithelium.

Around the vesicle forming area many macrophages and epithelial cells were positive for IL-6, but rarely positive for IL-1, IL-8, and IL-28, which are the cytokines initiating the cellular inflammatory reaction and chemotaxis, respectively. As IL-6 was known to target the dying and dead cells to remove them via C-reactive protein-induced phagocytosis, the overproduction of IL-6 in the pemphigus vulgaris might be relevant to the acantholytic keratinocytes which were subsequently dead and replaced by vesicles.

Generally the oral epithelium involved with the pemphigus became hyperkeratotic and edematous in part. This pathological damage may affect the cellular status of whole epithelial cells. Therefore, we also explored the cellular responses to the autoimmune reaction by
immunohistochemical method. Particularly, the acantholytic epithelium near the autoimmune reaction was strongly positive for HSP-70, implicating the stressful condition. And more some acantholytic keratinocytes were strongly positive for PARP, which is the biomarker of cellular apoptosis. These findings might indicate that the acantholytic keratinocytes were damaged by the autoimmune reaction and disappeared through the programmed cell death.

On the other hand, the remaining epithelium adjacent to the vesicle forming area was still active with the overexpression of pAKT and β-defensin-1, 2 and -3, which are the biomarkers of cell survival and innate immunity, respectively. The activation of pAKT pathways by the autoimmune reaction in the pemphigus vulgaris may have a potential to induce the prolonged survival of acantholytic keratinocytes, resulted in the papillomatous growth and vegetation likely pemphigus vegetans, a variant of pemphigus vulgaris. However, the overexpression of β-defensins was another characteristic in the pemphigus vulgaris, and it was supposed that the acantholytic keratinocytes were sensitive to the autoimmune reaction in order to protect themselves from chemical irritations as well as pathogenic microbes.

In conclusion, the present case of pemphigus vulgaris produced suprabasal split vesicles by autoantibody-induced type II hypersensitive reaction, which was absent of the proinflammatory and cell-mediated immune reaction, The acantholytic keratinocytes in the vesicle forming area were much damaged and underwent through the cellular apoptosis, together with the cytotoxic influence of IL-6, and anti-BP180 antibodies: a case report and brief review of cases with coexistence of pemphigus vulgaris and bullous pemphigoid, J Biol Regul Homeost Agents 2009;23:197-201.


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IV. Reference


