Hailey-Hailey Disease

Hailey-Hailey disease (HHD), or benign familial chronic pemphigus, typically presents as suprabasal blisters with a perivascular and interstitial lymphocytic infiltrate (Figure 1).[1] Villi, or elongated dermal papillae lined with a single layer of basal cells, protrude into the bullae (Figure 2). In HHD lesions, the epidermis is thickened with scale-crust, and at least the lower half of the epidermis shows acantholysis. Despite the acantholytic changes, a few intact intercellular bridges remain, giving the appearance of a dilapidated brick wall (Figure 2). There may be dyskeratotic cells among the acantholytic cells, though they are scant in many cases. These acantholytic dyskeratotic cells have eosinophilic polygonal-shaped cytoplasm. Hailey-Hailey disease typically does not show adnexal extension of the acantholysis. Direct immunofluorescence is negative in HHD.

Pemphigus vulgaris is an autoimmune intraepidermal bullous disease that presents with suprabasal acantholysis (Figure 3).[2] The epidermis is not thickened and acantholysis is limited to the suprabasal layer. Acantholytic cells with eosinophils and/or neutrophils are found within the bullae. Perivascular and interstitial infiltrates of lymphocytes, eosinophils, and occasionally neutrophils are seen; however, the inflammatory cell infiltrate can vary from extensive to scant. Direct immunofluorescence usually reveals IgG and/or C3 deposition on the surface of the keratinocytes throughout the epidermis.

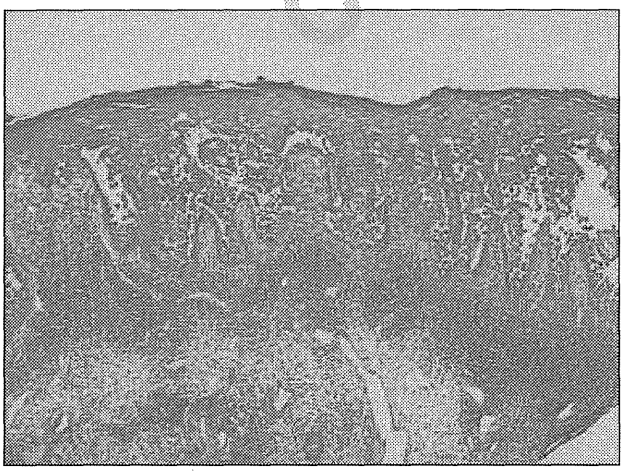

Figure 1. A suprabasal blister with acantholytic changes in the lower half of the epidermis in the setting of Hailey-Hailey disease. A dense perivascular and interstitial lymphocytic infiltrate can be seen in the upper dermis (H&E, original magnification ×40).

Pemphigus foliaceus is another autoimmune intraepidermal bullous disease that is characterized by acantholysis in the granular or upper spinous layers (Figure 4).[3] The epidermis is not thickened. Sometimes acantholytic cells show dyskeratotic change (Figure 4). Some biopsy specimens do not contain the roof of the bullae; therefore, only erosion is seen and the diagnosis may be missed. Moreover, when only the adnexal epithelium shows acantholysis without epidermal involvement, diagnosis can be difficult.[4] Acantholysis is accompanied with a

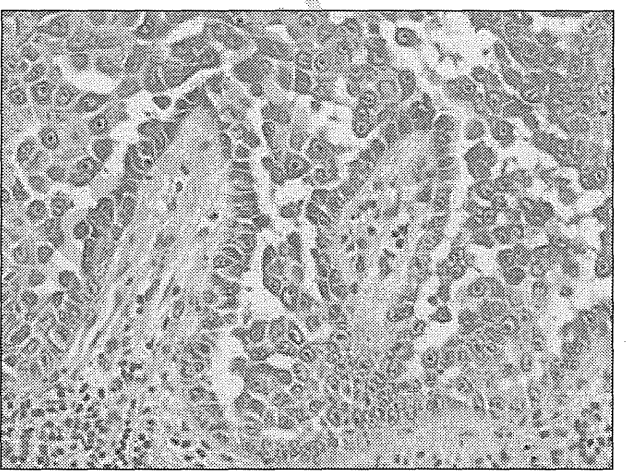

Figure 2. Villi, or protruding dermal papillae lined with a single layer of basal cells, are evident. Above the villi, a few intact intercellular bridges remain, giving the appearance of a dilapidated brick wall (H&E, original magnification ×200).

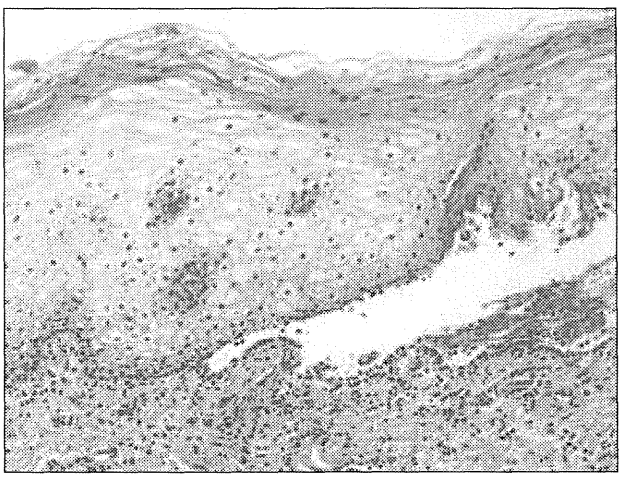

Figure 3. Intraepidermal bulla in pemphigus vulgaris caused by suprabasal acantholysis. A mixed infiltrate of lymphocytes and eosinophils is seen in the upper dermis (H&E, original magnification ×100).

superficial perivascular and interstitial inflammatory cell infiltrate consisting of lymphocytes, eosinophils, and occasionally neutrophils. The amount of inflammatory cell infiltrate may vary. Bullous impetigo and staphylococcal scalded skin syndrome reveal a similar histopathologic pattern. Direct immunofluorescence usually discloses IgG and/or C3 deposition on cell surfaces of keratinocytes in the entire or upper epidermis.

Herpesvirus infection shows ballooning (intracellular edema) of keratinocytes. Eventually acantholysis occurs and intraepidermal bullae are formed. In the bullae, virus-associated acantholytic keratinocytes, some that are multinucleated, can be easily found (Figure 5).[5] These cells are larger than normal keratinocytes and have steel gray nuclei with peripheral accentuation. Some of these cells are necrotic, and the remains of necrotic multinucleated acantholytic cells are easily recognized. Adnexal epithelial cells occasionally are affected by herpesvirus infection; nuclear change is similar to the epidermis. A perivascular and interstitial infiltrate of lymphocytes and neutrophils is seen. Neutrophils accumulate within the old bullae, clinically manifesting as a pustule.

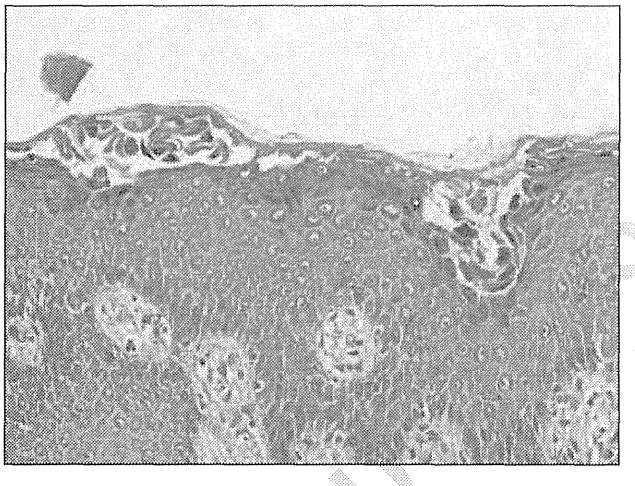

Figure 4. Subcorneal acantholytic cells are evident. Some acantholytic cells are dyskeratotic in pemphigus foliaceus (H&E, original magnification ×200).

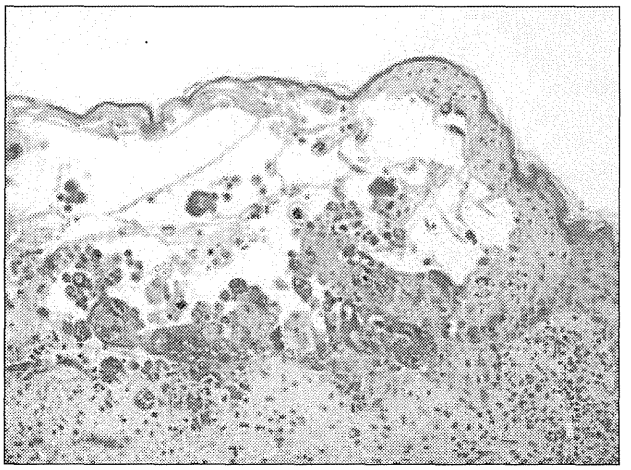

Figure 5. Multinucleated cells with steel gray nuclei are easily found in a blister caused by herpesvirus infection (H&E, original magnification ×100).

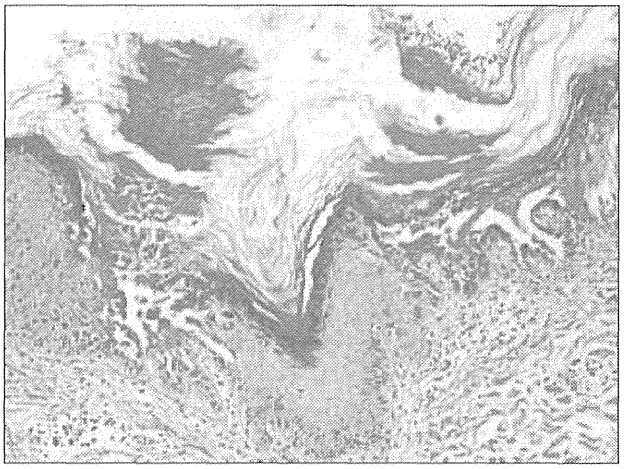

Figure 6. Narrow foci of suprabasal clefts are seen intermittently in Darier disease. Above the suprabasal clefts, acantholytic changes with occasional acantholytic dyskeratotic cells throughout the epidermis are seen with columns of parakeratosis. Villi also are seen, similar to Hailey-Hailey disease (H&E, original magnification ×100).

Darier disease is characterized by suprabasal clefts and acantholysis above the basal layer (Figure 6).[6] Similar to HHD, villi protrude within the clefts (Figure 6). Conspicuous columns of parakeratosis above the acantholytic epidermis often are observed. Dyskeratotic cells exist among acantholytic keratinocytes in the granular layer and parakeratotic column, which are known as corps ronds and crops grains, respectively. A scant to moderate lymphocytic infiltrate is found in the upper dermis.

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Molluscum Contagiosum With CD30+ Cell Infiltration in a Patient With Mycosis Fungoides

To the Editor:

CD30+ cells are occasionally present around molluscum contagiosum (MC).[1] In these settings, T-cell clonality has not been detected, although CD30+ cells do not always belong to T-cells. We report a case of multiple MC with CD30+ cell infiltrate, developing in a patient with mycosis fungoides (MF). MC lesions were not located on MF lesions, however, T-cell clonality was shown.

A 60-year-old Japanese male without any particular family history admitted to our hospital for the treatment of widespread skin lesions. Physical examination revealed erythematous skin lesions on the trunk and extremities (Fig. 1A), and biopsy from the thigh revealed lymphocytic infiltrate in the epidermis and within the dermal papillae with scant spongiosis and patchy lichenoid infiltrate of lymphocytes with wiry bundles of collagen in the papillary dermis (Figs. 1B, C). Immunohistochemistry revealed that the lymphocytes in the upper dermis were positive for CD3 and CD4, and relatively weakly positive for CD8. In contrast, these cells were negative for CD20 and CD30. The patient was diagnosed as MF (patch stage, T2N0M0B0, stage IB). Narrow band UVB therapy (NB-UVB) with topical steroid was successful.

The authors declare no conflicts of interest.

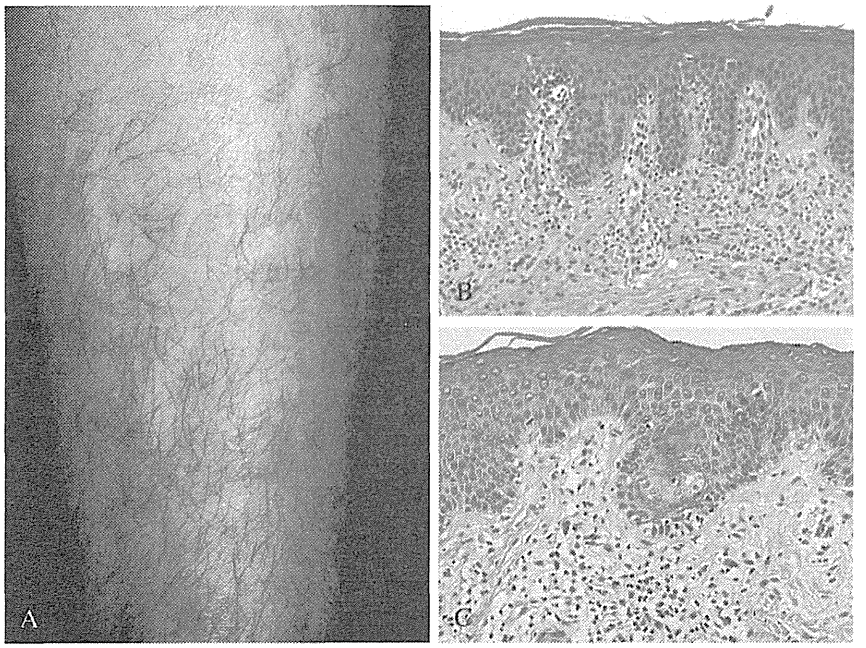

FIGURE 1. A, Skin lesions on the thigh. B and C, Hematoxylin–eosin staining.

However, after discontinuation of NB-UVB, the skin lesions exacerbated.

Two years later, the patient showed relatively ill-demarcated red to brown scaly macules of MF on the entire body. In addition, multiple pink salmon-colored papules of 5 mm in size developed on the trunk and extremities, sparing MF lesions (Fig. 2A). Some papules were umbilicated (Fig. 2B). Laboratory tests revealed no abnormal findings, except for slightly elevated level of soluble IL-2 receptor (673 U/mL).

Histopathology of biopsy from MF macule revealed diffuse infiltrates of lymphocytes without apparent atypia in the upper dermis, with foci of accumulated lymphocytes within the epidermis. These lymphocytes were positive for CD3 and CD4, but negative for CD20 or CD30. Occasional $CD8^+$ lymphocytes were also found.

Histopathology of biopsy from papules revealed tumorous islands of proliferating keratinocytes with eosinophilic molluscum inclusion bodies in the cytoplasm, surrounded by inflammatory infiltrates in the superficial dermis (Fig. 2C). Some of the inflammatory cells showed large cytoplasm with irregular-shaped nuclei (Fig. 2D). From these findings, the papules were diagnosed as MC.

Immunohistochemistry revealed that the infiltrating cells around MC lesions were positive for CD3. The $CD3^+$ cells were relatively weakly positive for CD4 (Fig. 2E), but strongly positive for CD8 (Fig. 2F). Intriguingly, $CD3^+$ large cells were positive for CD30 (Fig. 2G, H). Infiltrating cells were almost negative for CD20.

DNA extracted from the paraffin block of the initial MF and MC showed the same T-cell receptor γ gene clonality performed by polymerase chain reaction analysis (Fig. 2I).[2] Monoclonality in MC lesion was considered to be derived from $CD4^+$ cells, $CD30^+$ cells, or both.

All MC lesions were manually removed, and MF was treated again with NB-UVB with topical steroid with slight improvement. MC occurs not only in infants or sexually active adults, but also in immunosuppressive or immune-deficient patients.[3] In addition, a patient with MF, in whom MC developed only on MF lesions, was previously reported.[4] In that case, Th2 predominance in MF was speculated to cause MC virus (MCV) infection. Similarly, Th2 dominant condition in our patient, evidenced by the exacerbation of MF, might also cause the susceptibility to MCV infection.

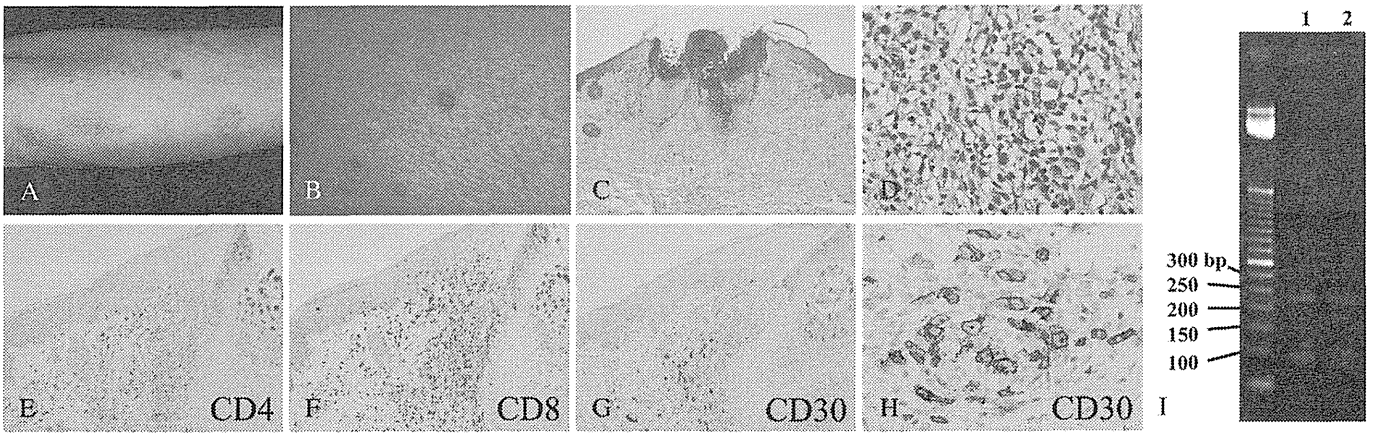

FIGURE 2. A, Skin lesions on the right forearm. B, Close-up view of MC. C–H, The results of the studies for MC lesion. C and D, Hematoxylin–eosin staining. E–H, Immunohistochemistry for (E) CD4, (F) CD8, and (G, H) CD30. I, TCR γ polymerase chain reaction (PCR) products. A 3% of agarose gel showed typical PCR products of ~230 bp using the inner Vγ1-8 and Jγ consensus primer pair in lane 1 and lane 2. Bands at ~100 bp in both lanes were artifacts and nonspecific. Lane 1, initial MF lesion. Lane 2, MC lesion.

In the previous study, most of the lymphocytes around MC lesions in the patients with no significant medical history were positive for CD3 and CD8. $CD30^+$ cells were also present, but in small amount. DNA extracted from the paraffin block showed no evidence of T-cell receptor γ gene.[1] $CD8^+$ Th1-T-lymphocytes were considered to seem to clear viral infections.[4] In our patient, the smaller $CD8^+$ T-lymphocytes around the MC lesion were considered to be inflammatory Th1-T-lymphocyts, but not MF tumor cells.

Intriguingly, the same T-cell clonality was detected in MC lesion and the initial MF lesion in the present case. The number of $CD4^+$ MF cells in the MC lesion might be large enough to show T-cell clonality, although no MC developed in MF lesion. The $CD30^+$ larger cells infiltrated around the MC lesion showed irregularly-shaped nuclei, suggesting that these cells were MF tumor cells. MF cells are generally $CD4^+$ Th2-T-lymphocytes, and CD30 is considered to be expressed in Th2-T-lymphocytes.[5] Thus, some $CD4^+$ cells were considered to be transformed to $CD30^+$ cells. It is speculated that MCV infection first accumulated $CD4^+$ MF tumor cells, and then transformed some of them to $CD30^+$ cells. We do not know whether monoclonality was expressed in $CD4^+$ cells, $CD30^+$ cells, or both.

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Locations of acantholysis in pemphigus vulgaris and pemphigus foliaceus

Background: Acantholysis in pemphigus vulgaris (PV) and pemphigus foliaceus (PF) occurs predominantly in the suprabasal area and the granular layer, respectively. However, acantholysis can occasionally be observed in unusual locations.
Methods: We retrospectively studied the acantholysis locations in 35 PV and 27 PF cases, and analyzed them using the index value of Desmoglein (Dsg) 1 and Dsg3 by enzyme-linked immunosorbent assay, clinical data, and inflammatory cells. We also analyzed the relationship between clinical subtype and various parameters.
Results: In PV, acantholysis was noted in the suprabasal area in 3 cases, in the lower half of the epidermis in 19 cases, and throughout the epidermis in 13 cases. In PF, acantholysis was observed in the granular layer in 6 cases, in the upper half of the epidermis in 14 cases, and throughout the epidermis in 7 cases. Mean index value of Dsg1 in PV patients with acantholysis throughout the epidermis was 2-fold higher than other PV patients. Neutrophils tended to infiltrate the dermis and epidermis more in PF than in PV.
Conclusions: Higher Dsg1 index values seem to correlate with acantholysis in the upper part of the epidermis in PV. Neutrophils may play some role in unusual acantholysis locations in PF.

Keywords: Acantholysis, Desmoglein1, Desmoglein3, Pemphigus foliaceus, Pemphigus vulgaris

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Pemphigus is a potentially life-threatening autoimmune bullous disease in which desmoglein (Dsg) 1 and/or Dsg3 are targeted by autoantibodies. Pemphigus vulgaris (PV) and pemphigus foliaceus (PF) are two major classic forms of this disease. After enzyme-linked immunosorbent assays (ELISAs) for anti-Dsg1 and Dsg3 autoantibodies became commercially available – i.e. about a decade ago, diagnostic criteria using ELISA results have been commonly used for pemphigus. PV is diagnosed when anti-Dsg3 autoantibodies are present regardless of the presence or absence of anti-Dsg1 autoantibodies, whereas PF is diagnosed in the presence of anti-Dsg1 autoantibodies without anti-Dsg3 autoantibodies.[1]

Two clinical subtypes are well known in PV. One type affects mainly mucous membranes with minimal skin lesions (mucosal dominant type), whereas the other type affects mucous

membranes as well as skin (mucocutaneous type). Serum samples from mucosal dominant PV patients do not contain anti-Dsg1 antibodies but do contain anti-Dsg3 antibodies, whereas samples from mucocutaneous PV patients contain both anti-Dsg1 and Dsg3 antibodies.[2] Clinical and serological correlation is well explained by the hypothesis advocated by Mahoney et al.[3] Skin expresses Dsg1 throughout the epidermis and Dsg3 in the lower epidermis, whereas mucous membranes express Dsg1 in the upper mucous epithelium and Dsg3 throughout the mucous epithelium. The presence of anti-Dsg3 antibodies only is not sufficient to cause acantholysis in the skin because Dsg1 can compensate for the loss of Dsg3 function in cell-cell adhesion (called as 'the desmoglein compensation hypothesis'); however, they are enough to induce acantholysis in the mucous membranes in the suprabasal area. In PF, which usually affects skin while sparing the mucous membranes, Dsg3 in the mucous membranes compensate for the loss of function of Dsg1, resulting in acantholytic change in the upper layer of the epidermis in the skin, but no acantholysis in the mucous membranes.

Rare clinical types have also been reported. In 2005, four cases of PV presenting with only skin lesions without mucosal involvement were reported as cutaneous type of PV.[4] In 2012, we reported two cases of PF presenting with only mucosal lesions without skin involvement, and three cases of PF displaying mucosal as well as skin lesions.[5] Thus, it seems better to recognize three subtypes, namely mucosal dominant, mucocutaneous and cutaneous, in both PV and PF.

The predominant location of acantholysis in PV is the suprabasal area regardless of the subtype.[6] In contrast, the predominant location of acantholysis in PF is the upper layer of the epidermis.[6] These predominant locations of acantholysis in PV and PF can be useful to differentiate these two diseases in cases where ELISA for anti-Dsg1 and Dsg3 are not available. However, PV and PF occasionally show acantholysis in the upper layer and suprabasal area of the epidermis, respectively.[6] The main aim of the present study was to investigate the distribution of acantholysis in 35 PV and 27 PF cases. To elucidate the relationship between the location of acantholysis and anti-Dsg1 and Dsg3 autoantibodies, we also performed ELISA using sera taken at the same time when the biopsies were taken. In addition, we studied the relationship between the location of acantholysis and various parameters as well as the relationship between clinical types and various parameters.

Patients and methods

Patients

All patients with PV or PF, who visited the Department of Dermatology, Kurume University School of Medicine between 1996 and 2013, were enrolled in this study. The diagnoses of PV or PF had been made based not only on clinical manifestations with bullae or erosions, but on histopathological findings of acantholytic change, direct immunofluorescence showing IgG and/or C3 deposition to cell surfaces of keratinocytes, and positive index values of Dsg1 and/or Dsg3 by ELISA in all cases. The initial sera from patients, who visited earlier than July 1, 2003, the exact date when ELISA became commercially available in Japan, had been kept in our department, and were investigated using ELISA for Dsg1 and Dsg3 in the latter half of 2003 to confirm the diagnoses. This study was approved by the Ethical Committee of Kurume University.

Methods

Paraffin-embedded biopsy specimens of the enrolled patients were retrieved from the archives of the Department of Dermatology, Kurume University School of Medicine. One dermatopathologist (CO) and one dermatologist (NI), who was the specialist for autoimmune bullous diseases, reviewed the histopathology slides of all cases to confirm the location of the acantholysis, the predominant inflammatory cells, the presence or absence of acanthosis, dyskeratotic cells, and adnexal involvement. Clinical data regarding age, sex, clinical subtypes of pemphigus, and Dsg1 and Dsg3 ELISA index values at the same time when the biopsy specimens were taken (before the initiation of the treatment) were collected.

Statistical analysis

To investigate the relationship of the acantholysis location in PV and various parameters including the Dsg1 and Dsg3 ELISA index values, we subdivided the PV patients into two groups. One group showed acantholysis in the suprabasal area or in the lower half of the epidermis (50% group), and the other group throughout the epidermis (100% group). We did this because the number of the patients was small, and acantholysis within the lower half of the epidermis suggested PV rather than PF histopathologically.

For similar reasons, we subdivided the PF cases into two groups. One group showed acantholysis in the granular layer or subcorneal area, or in the lower half of the epidermis (50% group), and the other group throughout the epidermis (100% group). Fisher's exact test and Wilcoxon rank-sum test were performed using Stata 13 software (Stata Corp, College Station, TX, USA). All p values were 2-sided; a p value less than 0.05 was considered to indicate statistical significance.

Results

General features in PV and PF

PV group

There were 17 male patients and 18 female patients (Table 1). The clinical types of mucosal dominant, mucocutaneous and cutaneous groups included 3 , 28 and 4 cases, respectively. Acantholysis was found in the suprabasal area in 3 cases, in the lower half of the epidermis in 19 cases, and throughout the epidermis in 13 cases, respectively. Three PV cases exhibited acantholysis predominantly in the upper part of the epidermis rather than the lower part (Fig. 1), and another case showed acantholytic bullae both in the suprabasal area and in the upper epidermis. Direct immunofluorescence showed exaggerated IgG and C3 deposition in the lower half of the epidermis in one case with IgG deposition and two cases with C3 deposition throughout the epidermis. The Dsg1 index values by ELISA ranged from 5 to 2437.6

Table 1. General features of PV and PF cases

	PV (n = 35)	PF (n = 27)	p
Gender, male/female	17/18	14/13	0.7978
Age, mean ± SD (yrs)	54.8 ± 12.7	57.6 ± 18.1	0.1303
Clinical type			
Mucosal dominant, n (%)	3 (8.6)	1 (3.7)	
Mucocutaneous, n (%)	28 (80.0)	2 (7.4)	
Cutaneous, n (%)	4 (11.4)	24 (88.9)	
Acantholysis location			
<10%†, n (%)	3 (8.6)	6 (22.2)	
Up to 50%‡, n (%)	19 (54.3)	14 (51.9)	
Throughout the epidermis, n (%)	13 (37.1)	7 (25.9)	
IgG deposition on ICS in DIF			
Up to 50%‡, n (%)	2 (5.7)	0 (0.0)	
Throughout the epidermis, n (%)	33 (94.3)	27 (100.0)	
C3 deposition on ICS in DIF			
Up to 50%‡, n (%)	10 (28.6)	0 (0.0)	
Throughout the epidermis, n (%)	25 (71.4)	22 (81.5)	
Dsg1 index value, mean (95% CI)	315.4 (120.9–509.8)	565.2 (315.3–815.1)	0.0395*
Dsg3 index value, mean (95% CI)	820.7 (178.3–1183.0)	negative	–
Predominant inflammatory cells in the epidermis			
Eosinophils, n (%)	8 (22.9)	2 (7.4)	
Neutrophils, n (%)	9 (25.7)	16 (59.3)	
Eosinophils and neutrophils, n (%)	9 (25.7)	4 (14.8)	
Lymphocytes or none, n (%)	9 (25.7)	5 (18.5)	
Eosinophils with or without neutrophils, n (%)	17 (48.6)	6 (22.2)	0.0386*
Neutrophils with or without eosinophils, n (%)	18 (51.4)	20 (74.1)	0.1139
Predominant inflammatory cells in the dermis			
Eosinophils, n (%)	17 (48.6)	5 (18.5)	
Neutrophils, n (%)	5 (14.3)	17 (70.0)	
Eosinophils and neutrophils, n (%)	1 (2.9)	3 (11.1)	
Lymphocytes or none, n (%)	12 (34.3)	2 (7.4)	
Eosinophils with or without neutrophils, n (%)	18 (51.4)	8 (29.6)	0.1203
Neutrophils with or without eosinophils, n (%)	6 (17.1)	20 (74.1)	<0.0001*
Neutrophils in the vessels, n (%)	25 (71.4)	18 (66.7)	0.7837
Acanthosis, n (%)	21 (60.0)	21 (77.8)	0.1758
Acantholytic dyskeratotic cells, n (%)	29 (82.9)	25 (92.6)	0.4474
Non-acantholytic dyskeratotic cells, n (%)	12 (34.3)	14 (51.9)	0.2000
Adnexal involvement, n (%)	15 (42.9)	5 (18.5)	0.0567

PV, pemphigus vulgaris; PF, pemphigus foliaceus; ICS, intercellular surfaces; DIF, direct immunofluorescence; Dsg, desmoglein.

†'<10%' means suprabasal area in PV and granular layer and subcorneal area in PF.

‡'up to 50%' means lower half of the epidermis in PV and upper half of the epidermis in PF.

*statistically significant.

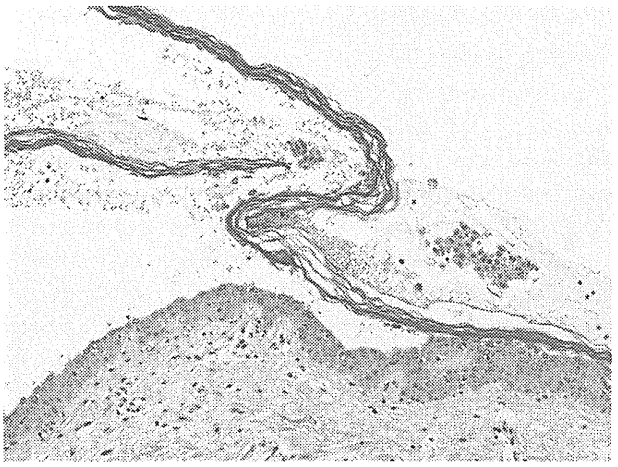

Fig. 1. Acantholysis in the upper part of the epidermis in PV (hematoxylin and eosin (HE), original magnification ×100).

(cut-off value <20), and the mean was 315.4. The Dsg3 index values by ELISA ranged from 42.8 to 5346.4 (cut-off value <20), and the mean was 820.7. Eosinophils were the predominant inflammatory cells in the dermis. Neutrophils were seen within the vessels in the upper dermis (Fig. 2a) in 25 cases. Acanthosis was found in 21 cases. Acantholytic dyskeratotic cells were found (Fig. 2b) in 29 cases, and non-acantholytic dyskeratotic cells were found (Fig. 2c) in 12 cases. The adnexal epithelium such as follicles, sebaceous glands, eccrine ducts and apocrine glands (Fig. 2d) was involved in 15 cases. A dilated or distorted configuration was seen in three cases.

PF group

There were 14 male patients and 13 female patients (Table 1). The clinical types of mucosal dominant, mucocutaneous and cutaneous groups included 1, 2, and 24 cases, respectively. Acantholysis were found in the granular layer of the subcorneal area in 6 cases, in the upper half of the epidermis in 14 cases, and throughout the epidermis in 7 cases, respectively. Two PF cases exhibited acantholytic bullae both in the suprabasal area (Fig. 3) and in the upper epidermis. Direct immunofluorescence showed exaggerated IgG deposition in the upper half of the epidermis in four cases. In contrast, in one case with IgG and C3 depositions and two cases with C3 deposition throughout the epidermis, IgG and C3 deposition was exaggerated in the lower half of the epidermis, respectively. The former case showed acantholysis in the upper half of the epidermis and the latter two cases showed it throughout the epidermis, respectively. The Dsg1 index values by ELISA ranged from 21.45 to 2286.0, and the mean was 565.2. The Dsg3 index values by ELISA were all less than 20. Neutrophils were predominant inflammatory cells in the dermis and the epidermis. Neutrophils were seen within the vessels in the upper dermis

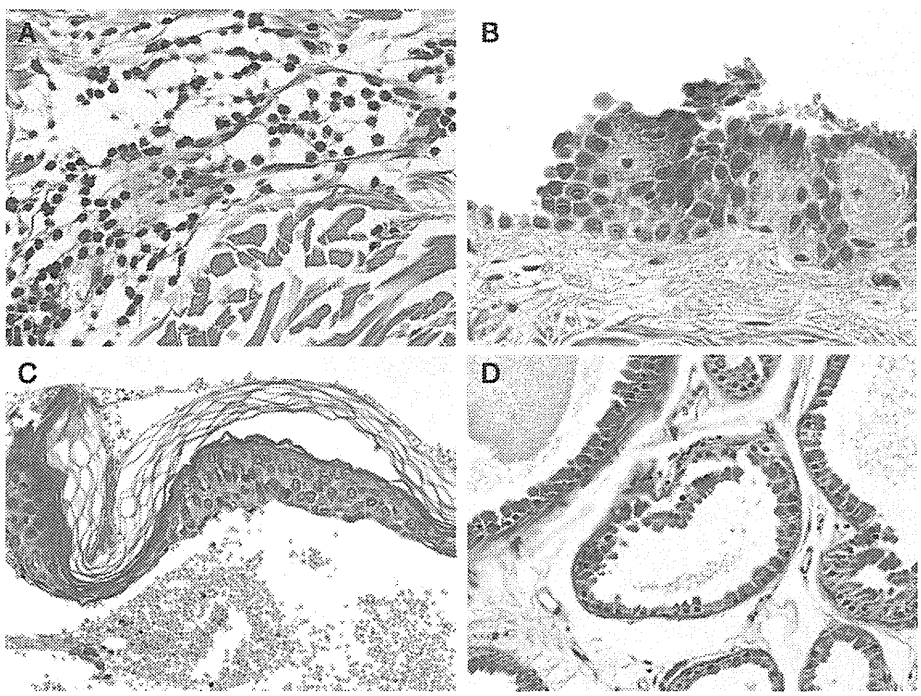

Fig. 2. Histopathology in PV (A) Neutrophils within the vessel (HE, ×400). (B) Acantholytic dyskeratotic cells in suprabasal area (HE, ×400). (C) Dyskeratotic cells in the non-acantholytic epithelium of the lid of the bulla (HE, ×200). (D) Acantholysis in an apocrine gland (HE, ×200).

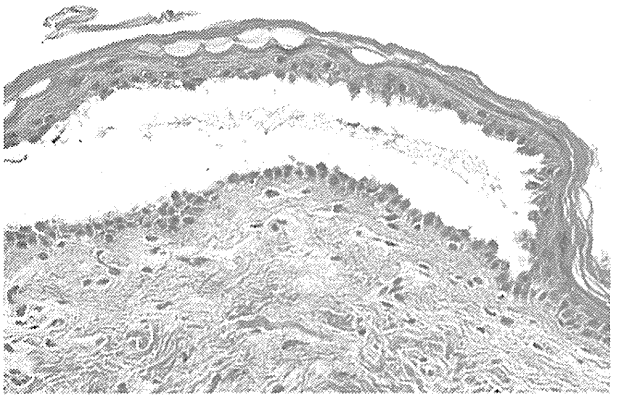

Fig. 3. Acantholytic bulla located in the lower part of the epidermis in PF (HE, ×200).

in 18 cases. Acanthosis was found in 21 cases. Acantholytic dyskeratotic cells were found in 25 cases, and non-acantholytic dyskeratotic cells were found in 14 cases. Adnexal epithelium was involved in five cases. A distorted configuration was seen in one case.

Comparison of PV and PF

The mean Dsg1 index value was significantly higher in the PF group than that in the PV group ($p = 0.0395$) (Table 1). Eosinophils were present significantly more commonly in the epidermis in PV ($p = 0.0386$), and neutrophils were present significantly more commonly in the dermis in PF than PV ($p < 0.0001$).

Characteristic features in different acantholysis locations

Pemphigus vulgaris

The comparison of the features between PV patients with acantholysis in the lower half of the epidermis (50% group) and throughout the epidermis (100% group) are provided in Table 2. Although no statistical differences were noted in the Dsg1 and Dsg3 indices between two groups, the mean Dsg1 index value of the 100% group was more than twofold of that in the 50% group. The Dsg3 index values tended to be higher in the 100% group than in the 50% group. In each group, there were three patients whose sera showed negative Dsg1 index values. The mean Dsg3/Dsg1 index value ratio was 8.5 in the 50% group, and 113.4 in the 100% group. However, no statistical significance was found. Twenty-one patients in the 50% group and six patients in the 100% group showed higher indices of Dsg3 than those of Dsg1. There were significantly more patients with higher index values of Dsg3 than those of Dsg1 in the 50% group than that in the 100% group ($p = 0.0017$).

Patients in the 100% group were significantly older than those in the 50% group ($p = 0.0035$). The distribution of clinical subtypes between the two groups was significantly different ($p = 0.0168$). Patients in the 50% group had the typical clinical types of PV such as mucosal dominant or mucocutaneous type, whereas four

Table 2. Comparison of features between PV cases with different acantholysis locations

	50% group (n = 22)	100% group (n = 13)	p
Acantholysis location	lower half epidermis	throughout the epidermis	
Dsg1 index value, mean (95% CI)	223.3 (−1.2–447.7)	471.3 (80.8–861.7)	0.4222
Dsg3 index value, mean (95% CI)	726.7 (411.2–1042.2)	979.8 (413.5–1880.7)	0.7200
Dsg1 negative, n (%)	3 (13.6%)	3 (23.1%)	0.6485
Dsg3/Dsg1 index value ratio, mean (95% CI)	8.5 (3.7–13.3)	113.4 (−66.8–293.5)	0.4029
Dsg3/Dsg1 index value >1, n (%)	21 (95.5%)	6 (46.2%)	0.0017*
Age, mean ± SD (yrs)	50.4 ± 11.5	62.2 ± 11.3	0.0035*
Male, n (%)	12 (54.6)	5 (38.5)	0.4887
Clinical type			0.0168*
Mucosal dominant, n (%)	2 (9.1)	1 (7.7)	
Mucocutaneous, n (%)	20 (90.9)	8 (61.5)	
Cutaneous, n (%)	0 (0.0)	4 (30.8)	
Predominant inflammatory cells in the epidermis			
Eosinophils with or without neutrophils, n (%)	13 (59.1)	4 (30.8)	0.1642
Neutrophils with or without eosinophils, n (%)	12 (54.6)	6 (46.2)	0.7332
Predominant inflammatory cells in the dermis			
Eosinophils with or without neutrophils, n (%)	14 (63.6)	4 (30.8)	0.0858
Neutrophils with or without eosinophils, n (%)	2 (9.1)	4 (30.8)	0.1662
Neutrophils in the vessels, n (%)	17 (77.3)	8 (61.5)	0.4437

PV, pemphigus vulgaris; Dsg, desmoglein.
*statistically significant.

Table 3. Comparison of features between PV cases with acantholysis mainly in the upper epidermis and other PV cases

	Upper group (n=3)	Others (n=32)	p
Dsg1 index value, mean (95% CI)	1045.5 (−1240.0−3330.9)	246.93 (70−423.7)	0.0554
Dsg3 index value, mean (95% CI)	71.6 (2.8−140.4)	890.9 (502.5−1279.4)	0.0215*
Dsg1 negative, n (%)	0 (0.0)	6 (18.8)	1.0000
Dsg3/Dsg1 index value ratio, mean (95% CI)	0.2 (−0.26−0.57)	51.9 (−17.6−121.4)	0.0073*
Dsg3/Dsg1 index value > 1, n (%)	0 (0.0)	27 (84.4)	0.0086*
Age, mean ± SD (yrs)	69.3 ± 12.7	53.4 ± 12.0	0.0552
Male, n (%)	1 (33.3)	16 (50.0)	1.0000
Predominant inflammatory cells in the epidermis			
Eosinophils with or without neutrophils, n (%)	1 (33.3)	16 (50.0)	1.0000
Neutrophils with or without eosinophils, n (%)	1 (33.3)	17 (53.1)	0.6026
Predominant inflammatory cells in the dermis			
Eosinophils with or without neutrophils, n (%)	1 (33.3)	17 (53.1)	0.6026
Neutrophils with or without eosinophils, n (%)	1 (33.3)	5 (15.6)	0.4417
Neutrophilss in the vessels, n (%)	2 (66.7)	23 (71.9)	1.0000

PV, pemphigus vulgaris; Dsg, desmoglein.
*statistically significant.

Table 4. Comparison of features between PF cases with differing acantholysis location

	50% group (n=20)	100% group (n=7)	p
Acantholysis location	Upper half epidermis	Throughout the epidermis	
Dsg1 index value, mean (95% CI)	617.9 (307.4−928.3)	414.8 (−92.8−922.3)	0.5613
Age, mean ± SD (yrs)	57.3 ± 18.3	58.3 ± 19.0	0.9558
Male, n (%)	9 (45.0)	5 (71.4)	0.3845
Clinical type			0.0120*
Mucosal dominant, n (%)	0 (0.0)	1 (14.3)	
Mucocutaneous, n (%)	0 (0.0)	2 (28.6)	
Cutaneous, n (%)	20 (100.0)	4 (57.1)	
Predominant inflammatory cells in the epidermis			
Eosinophils with or without neutrophils, n (%)	6 (30.0)	0 (0.0)	0.1548
Neutrophils with or without eosinophils, n (%)	14 (70.0)	6 (85.7)	0.6334
Predominant inflammatory cells in the dermis			
Eosinophils with or without neutrophils, n (%)	8 (40.0)	0 (0.0)	0.0681
Neutrophils with or without eosinophils, n (%)	14 (70.0)	6 (85.7)	0.6334
Neutrophils in the vessels, n (%)	14 (70.0)	4 (57.1)	0.6527

PF, pemphigus foliaceus; Dsg, desmoglein.
*statistically significant.

(30.8%) patients had the cutaneous type in the 100% group.

Although neutrophilic infiltrate in the dermis in the 50% group was seen in only two cases, neutrophils in the vessels in the 50% group were found in 17 cases, and almost as frequent as that in the 100% group.

Because the three PV cases that had acantholysis predominantly in the upper part of the epidermis rather than the lower part histopathologically mimicked PF and were extremely confusing, we compared these rare cases (upper group) with other PV cases (Table 3). Although no statistical difference was found, the mean Dsg1 index value in the upper group was four times higher than that in the others. The mean Dsg3 index value in the upper group was significantly lower than that in the others ($p=0.0215$). The mean Dsg3/Dsg1 index value ratio was 0.2 in the upper group, whereas it was 51.9 in the others, which was statistically higher than that in the upper group ($p=0.0073$). Significantly more patients in the others group had higher indices of Dsg3 than of Dsg1 ($p=0.0086$).

Pemphigus foliaceus

Comparison of features between the PF patients with acantholysis in the upper half of the epidermis (50% group) and throughout the epidermis (100% group) are provided in Table 4. There was

Table 5. Comparison of features between PV cases with typical clinical manifestations and other PV cases

	Typical group (n=31)	Cutaneous group (n=4)	p
Dsg1 index value, mean (95% CI)	216.8 (49.2–384.5)	1079.1 (–218.3–2376.5)	0.0148*
Dsg3 index value, mean (95% CI)	915.2 (516.7–1313.7)	88.5 (34.0–142.9)	0.0314*
Dsg1 negative, n (%)	6 (19.4)	0 (0.0)	1.0000
Dsg3/Dsg1 index value ratio, mean (95% CI)	53.5 (–18.3-125.3)	0.2 (–0.1-0.4)	0.0022*
Dsg3/Dsg1 index value > 1, n (%)	27 (77.1)	0 (0.0)	0.0013*
Age, mean ± SD (yrs)	53.0 ± 11.5	68.5 ± 14.5	0.0404*
Male, n (%)	15 (48.4)	2 (50.0)	1.0000
Acantholysis			0.0285*
Suprabasal, n (%)	3 (9.7)	0 (0.0)	
Lower half, n (%)	19 (61.3)	0 (0.0)	
Throughout the epidermis, n (%)	9 (29.0)	4 (100.0)	
Predominant inflammatory cells in the epidermis			
Eosinophils with or without neutrophils, n (%)	15 (48.4)	2 (50.0)	1.0000
Neutrophils with or without eosinophils, n (%)	17 (54.8)	1 (25.0)	0.3377
Predominant inflammatory cells in the dermis			
Eosinophils with or without neutrophils, n (%)	16 (51.6)	2 (50.0)	1.0000
Neutrophils with or without eosinophils, n (%)	6 (19.4)	0 (0.0)	1.0000
Neutrophils in the vessels, n (%)	23 (74.2)	2 (50.0)	0.5607

PV, pemphigus vulgaris; Dsg, desmoglein.
*statistically significant.

Table 6. Comparison of features between mucosal dominant PV cases and mucocutaneous PV cases (n=31)

	Mucosal dominant group (n=3)	Mucocutaneous group (n=28)	p
Dsg1 index value, mean (95% CI)	65.9 (–196.1-327.9)	233.0 (47.7–418.3)	0.1812
Dsg3 index value, mean (95% CI)	1057.4 (–1041–3155.3)	900.0 (465–1334.5)	0.7892
Dsg1 negative, n (%)	2 (66.7)	4 (14.3)	0.0879
Dsg3/Dsg1 index value ratio, mean (95% CI)	106.0 (–294.0–506.0)	47.9 (–29.9–125.8)	0.0712
Dsg3/Dsg1 index value > 1, n (%)	3 (100.0)	24 (85.7)	1.0000
Age, mean ± SD (yrs)	52.0 ± 6.0	53.1 ± 12.0	0.9201
Male, n (%)	1 (33.3)	14 (50.0)	1.0000
Predominant inflammatory cells in the epidermis			
Eosinophils with or without neutrophils, n (%)	0 (0.0)	15 (53.6)	0.2258
Neutrophils with or without eosinophils, n (%)	1 (33.3)	16 (57.1)	0.5764
Predominant inflammatory cells in the dermis			
Eosinophils with or without neutrophils, n (%)	0 (0.0)	16 (57.1)	0.1012
Neutrophils with or without eosinophils, n (%)	1 (33.3)	5 (17.9)	0.4883
Neutrophils in the vessels, n (%)	3 (100.0)	20 (71.4)	0.5497

PV, pemphigus vulgaris; Dsg, desmoglein.

no significant difference in the Dsg1 index value between the two groups.

The distribution of clinical subtypes between the two groups was significantly different (p=0.0120). The patients in the 50% group had the typical clinical types of PF such as the cutaneous type, whereas three (42.9%) patients had mucosal dominant or mucocutaneous type in the 100% group.

It was remarkable that no case in the 100% group had eosinophils as the predominant inflammatory cells in the epidermis and dermis. Neutrophils were the more predominant inflammatory cells in both groups.

Characteristic features in different clinical types

Pemphigus vulgaris

The comparison of features between 31 PV patients with typical clinical types such as mucosal dominant and mucocutaneous types (typical group) and 4 PV patients with a rare clinical type (cutaneous group) are provided in Table 5. The mean Dsg1 index value was significantly higher in the cutaneous group than that in the typical group (p=0.0148). In contrast, the mean Dsg3 index value was significantly higher in typical group than in the cutaneous group (p=0.0314). Accordingly, the mean

Table 7. Comparison of features between PF cases with typical clinical manifestations and other PF cases

	Typical group (n=24)	Mucosa involved group (n=3)	p
Dsg1 index value, mean (95% CI)	614.4 (338.8–890.1)	171.3 (–233.9–576.5)	0.2472
Age, mean ± SD (yrs)	57.0 ± 19.1	62.0 ± 5.0	0.8772
Male, n (%)	12 (50.0)	2 (66.7)	1.0000
Acantholysis			0.0188*
Granular layer or subcorneal, n (%)	6 (25.0)	0 (0.0)	
Upper half, n (%)	14 (58.3)	0 (0.0)	
Throughout the epidermis, n (%)	4 (16.7)	3 (100.0)	
Predominant inflammatory cells in the epidermis			
Eosinophils with or without neutrophils, n (%)	6 (25.0)	0 (0.0)	1.0000
Neutrophils with or without eosinophils, n (%)	17 (70.8)	3 (100.0)	0.5453
Predominant inflammatory cells in the dermis			
Eosinophils with or without neutrophils, n (%)	8 (33.3)	0 (0.0)	0.5323
Neutrophils with or without eosinophils, n (%)	17 (70.8)	3 (100.0)	0.5453
Neutrophils in the vessels, n (%)	17 (70.8)	1 (33.3)	0.2503

PV, pemphigus vulgaris; Dsg, desmoglein.
*statistically significant.

Dsg3/Dsg1 index value ratio was significantly higher in the typical group than in the cutaneous group (p=0.0022), and significantly more patients in the typical group demonstrated a higher Dsg3 index value than Dsg1 index value in the typical group than in the cutaneous group (p=0.0013). Patients in the cutaneous group were significantly older than those in the typical group (p=0.0404). The distribution of acantholysis location between the two groups was significantly different ($P=0.0285$). All patients in cutaneous group showed acantholysis throughout the epidermis, whereas only nine (29.0%) patients showed the same pattern of acantholysis in the typical group.

We also compared mucosal-dominant PV and mucocutaneous PV (Table 6). Although the mean Dsg1 index value was more than three times higher in the mucocutaneous group than that in the mucosal dominant group, there was no statistical difference. The mean Dsg3 index value was slightly higher in the mucosal dominant group than that in the mucocutaneous group. Two of three PV patients with the mucosal-dominant type showed a positive Dsg1 index value. The mean Dsg3/Dsg1 index value ratio was more than twice in the mucosal dominant group than that in the mucocutaneous group.

Pemphigus foliaceus

The comparison of the features between 24 PV patients with the typical clinical types such as cutaneous type (typical group) and 3 PV patients that had the rare clinical type such as mucosal dominant and mucocutaneous type (mucosa involved group) are provided in Table 7. The mean Dsg1 index value was more than three times higher in the typical group than that in the mucosa involved group; however, no statistical difference was found. The distribution of the acantholysis locations between the two groups was significantly different (p=0.0188). All patients in the mucosa-involved group showed acantholysis throughout the epidermis, while only four (16.7%) patients showed the same pattern of acantholysis in the typical group. It was remarkable that no case in the mucosa-involved group had eosinophils as the predominant inflammatory cells in the epidermis and dermis.

Discussion

Acantholytic changes in the suprabasal area and in the granular layer or subcorneal area are histopathologic hallmarks of PV and PF, respectively. PV affects skin as well as mucous membranes; the typical skin lesions are the flaccid bullae or erosions anywhere on the skin surface, and typical mucosal lesions are painful erosions anywhere in the oropharyngeal cavity.[7] In contrast, PF affects mostly the skin; the typical skin lesions are scaly, crusted erosions, and flaccid bullae are rarely seen.[7] Therefore, the clinical manifestation and biopsy specimen are enough to make a diagnosis of PV or PF in some cases, resulting in the avoidance of ELISA for Dsg1 and Dsg3. However, some complicated or rare cases,[4,5,8] which do not fit the typical PV or PF histopathologic paradigm, exist.

In the present study, patients with rare clinical subtypes such as cutaneous PV and mucosal

dominant or mucocutaneous PF represented around 11% among all PV and all PF cases, respectively. Typical acantholysis locations were seen in 8.6% of PV and 22.2% of PF. In addition, 62.9% of PV and 74.1% of PF showed acantholysis in the lower half and upper half of the epidermis. It is not difficult to diagnose PV unless acantholysis is seen in the upper half of the epidermis, or to diagnose PF unless acantholysis is seen in the lower half of the epidermis. Thus, 62.9% of PV and 74.1% of PF could be correctly diagnosed histopathologically without difficulty. In contrast, more than a quarter of PV and PF cases showed acantholysis throughout the epidermis, and histopathological correct diagnoses were difficult to make. Among them, there were three paradoxical PV cases, which showed acantholysis mostly in the upper part of the epidermis. Other complicated cases were one PV case and two PF cases that exhibited acantholytic bullae both in the suprabasal area and in the upper epidermis. In these cases, the Dsg1 and Dsg3 index values were useful for reaching the correct diagnoses. It is important to be aware that the location of acantholysis in some pemphigus cases can be misleading.

In PV, the mean Dsg1 index value of the 100% group was more than twofold of that in the 50% group. In addition, more than half of the patients in the 100% group showed a higher Dsg1 index value than Dsg3 index value, which was in contrast to only one patient in the 50% group with the same finding. Moreover, three PV cases with acantholysis mainly in the upper part of the epidermis (upper group) had a more than four times higher mean Dsg1 index value than that in the other PV cases, and the mean Dsg3/Dsg1 index value ratio in the upper group was significantly lower than that in the others group ($p=0.0073$). Thus, acantholysis in the upper half of the epidermis in PV can be considered to be closely related to a higher Dsg1 index value. In accordance with this result, cutaneous type PV, which is reminiscent of PF, was found only in the 100% group. The cutaneous type PV group also showed a significantly higher mean Dsg1 index value than that in the typical clinical type group ($p=0.0148$). Intriguingly, the mean Dsg3 index value was significantly higher in the typical group than that in the cutaneous group ($p=0.0314$).

The comparison between the mucosal dominant PV group and the mucocutaneous PV group was also interesting. Although no statistical differences were found, the mean Dsg1 index value was more than three times higher in the mucocutaneous group than that in the mucosal dominant group; the mean Dsg3 index value was slightly higher than that in the mucocutaneous group. These results raised the possibility that higher Dsg1 index values can lead to more frequent skin involvement in typical PV.

In PV, patients in the 100% group and cutaneous group were significantly older than those in the 50% group ($p=0.0035$) and the typical group ($p=0.0404$), respectively. Moreover, the difference between the age of patients in the upper group and that in the others was marginally significant ($p=0.0552$). These results suggested that the older PV patients were more likely to have acantholysis in the upper part of the epidermis and to be affected in the skin rather than in the mucous membranes. In other words, the PV in the older patients tended to simulate PF. It was not clear whether this result correlated with the result that the mean age of PF patients was slightly older than that of PV patients. However, older individuals tended to have PF rather than PV, when taking into consideration some reports from different countries that described that PF patients were older than PV patients.[9–11] In those reports, the mean onset age of PV ranged from 43 to 50.5 years old, and that of PF ranged from 52 to 63 years old.

In contrast to PV, various acantholysis locations and clinical subtypes in PF were not explained by the Dsgs index values, because Dsg1 was the only Dsg that was detected in PF. However, the mean Dsg1 index values in the 50% group and typical group were higher than that in the 100% group and in the mucosa-involved group, respectively. These results do not seem to suggest that anti-Dsg1 autoantibodies caused acantholysis in the lower half of the epidermis and in mucosal epithelium. Instead, the possibility of the existence of antibodies to non-Dsg molecules in some cases of PF was inferred.[5] Another possibility was the influence of infiltrating inflammatory cells. The present study demonstrated a closer association with neutrophils in PF than in PV. In fact, neutrophils more predominantly infiltrated the epidermis and dermis than eosinophils in PF, while eosinophils and neutrophils infiltrated almost equally throughout the epidermis, and more eosinophils infiltrated the dermis than the neutrophils in PV. Because neutrophils release proteolytic enzymes, which can cause acantholysis,[12] relatively frequent neutrophilic infiltrate in the epidermis in PF may play some role in various acantholysis locations. Although neutrophilic infiltrate in PV was relatively less

frequent, neutrophils in the vessels were found as often as in PF. Because neutrophils in the vessels represent an earlier stage before infiltration to the dermis and/or epidermis, neutrophils may play an important role not only in PF but also in PV.

Acantholytic dyskeratotic cells are well-known in PF, while their existence is not stressed in PV.[6] In the present study, acantholytic dyskeratotic cells were seen in more than 60% of both PV and PF. Moreover, non-acantholytic dyskeratotic cells were seen in more than 80% of PV and PF. Acantholytic dyskeratosis may be related to acanthosis, considering some acantholytic diseases including Darier's disease, Hailey-Hailey disease, Grover's disease and epidermal nevus, all of which are usually acanthotic.[13,14]

The extension of acantholysis to the adnexal structures are highlighted in PV, but not in PF.[6] Although less frequent in PF, adnexal involvement was seen in both PV and PF in the present study. Acantholytic dyskeratotic cells were also found in the adnexal structures. We have reported on PF with acantholysis in dilated follicular infundibulum.[15] In the present study, three PV cases and one PF case showed dilated or distorted folliculo-sebaceous units with acantholysis. These five cases raise the possibility that acantholysis in adnexal structures may cause dilation or distortion of adnexa.

Direct immunofluorescence is not used as a tool for distinction between PV and PF.[6] In both PV and PF, IgG and C3 tend to deposit throughout the epidermis. The correlation between direct immunofluorescence and the acantholysis location was difficult to evaluate based on the present study.

In summary, the present study revealed a close association between the Dsg1 index value and acantholysis in the upper part of the epidermis in PV. The Dsg1 index value did not seem to correlate with acantholysis in the lower part of the epidermis. Because neutrophils were predominant in the dermis and epidermis in PF, the unusual acantholysis location in PF may be related to neutrophils. Because the number of patients in this study was not large, further investigation may be necessary to fully confirm our results.

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LETTER TO THE EDITOR

Frequent office visits for injections may reduce adalimumab survival rate in patients with psoriasis

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Adherence to biological treatment is influenced by drug efficacy, adverse events, and patients' satisfaction with treatment. Administration intervals and route may also affect treatment persistence (how long patients stay on treatment). Adalimumab is administered subcutaneously every other week, and injections at hospitals are widely used, requiring frequent visits that may be a burden for patients. We retrospectively analyzed 36 consecutive patients treated with adalimumab for moderate to severe psoriasis to determine persistence with the treatment regimen.Patients were enrolled between February 2010 and April 2013 and were followed until August 2013. Patients for whom adalimumab had been initiated in other hospitals and who were subsequently treated in our department were excluded from the study. We collected information on gender, age, disease type, duration of the disease prior to baseline, Psoriasis Area and Severity Index (PASI) at baseline, side effects, presence or absence of self-injection, and comorbidities relevant to psoriasis. Patients were divided into two groups on the basis of adherence to adalimumab at the end of the observational period: the retention group and the drop-out group. All patients were initiated with hospital-based

Table 1. Summary of characteristics of the study population.

Characteristic	All patients ($n=36$)	Retention group ($n=16$)	Drop-out group ($n=20$)	p
Males/females, *n*	29/7	13/3	16/4	1.0000[c]
Age, mean ± SD (years)	54.4 ± 13.9	53.9 ± 15.6	54.9 ± 12.7	0.8477[d]
Psoriasis vulgaris/psoriatic arthritis, *n*	33/3	14/2	19/1	0.5742[c]
Disease duration, mean (range, median) (years)	11.8 (0.5–37, 10)	12.5 (0.5–25, 10)	11.3 (0.5–37, 10)	0.4819[e]
PASI at the baseline, mean (range, median)	18.6 (0–72, 17.0)	23.1 (0–72, 21.1)	15.1 (1.6–42.3, 14.0)	0.2651[e]
Adverse events, *n*	6 (16.7%)	2 (12.5%) • Temporal worsening of psoriasis • tinea corporis	4 (20.0%) • Rash on the whole body at first injection[a] • actinic keratosis[a] • prostatic cancer[a] • herpes zoster[b]	0.6722[c]
Self-injection with/without, *n*	14 (38.9%)	9 (56.3%)	5 (25.0%)	0.0874[c]
Relevant comorbidities, *n*				
Hypertension	5	2	3	1.0000[c]
Dyslipidemia	1	1	0	0.4595[c]
Diabetes mellitus	5	3	2	0.6486[c]
Adhere to adalimumab/drop-out from adalimumab over observational period, *n*	16/20	–	–	

SD, standard deviation; PASI, Psoriasis Area and Severity Index. Adalimumab due to adverse event, but later discontinued for patient-related reason. Statistical analysis was performed using JMP 10.0.2 (SAS Institute Inc., Cary, NC). $p<0.05$ was considered significant.
[a]Discontinued adalimumab due to adverse events.
[b]Did not discontinue.
[c]Fisher's exact test; [d]*t*-test; [e]Wilcoxon rank sum test.

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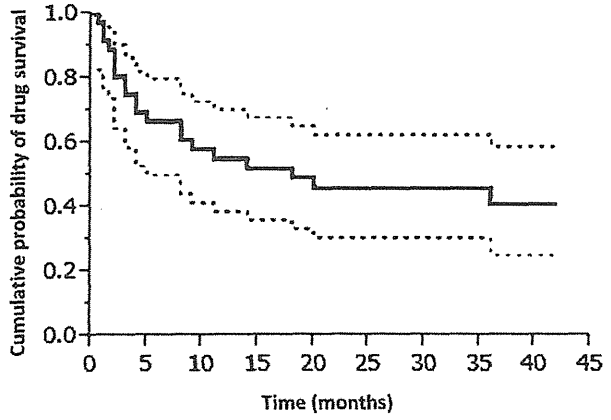

Figure 1. Drug survival rate curve for adalimumab, analyzed by the Kaplan–Meier method. Dotted lines show 95% confident intervals. For the multivariate analysis using the Cox proportional hazard model, the data were stratified by gender, age, and PASI score at baseline. $p<0.05$ was considered significant.

Table 2. Adalimumab drug survival rate.

Study	*n*	Study duration (months)	12 Months	24 Months	36 Months
The present study (Japan)	36	38	53.6%	46.0%	40.9%
Gniadecki et al. (1) (Denmark)	347	33	70%[a]	50%[a]	40%[a]
Esposito et al. (2) (Italy)	92	60	80%[a]	60%[a]	47%[a]
Lopez-Ferrer et al. (3)[d] (Spain)	119	62	65%[b,a] 78%[c,a]	48%[b,a] 78%[c,a]	46%[b,a] 78%[c,a]
Umezawa et al. (4) (Japan)	59	12	79.7%	–	–

[a]Numerical value was read in the Kaplan–Meier curve.
[b]Biologic-exposed group.
[c]Biologic-naïve group.
[d]Multivariate analysis revealed that lengthening of injection intervals was the significant variables ($p=0.015$) for drug survival.

administration of the adalimumab, but during the follow-up period, some patients switched to home injections when they preferred self-injections regardless of the degree of the improvement or when they agreed to our recommendation for self-injection because of the achievement of PASI 75. Approval for this retrospective study was obtained from Kurume University review boards.

The general characteristics of the retention group and drop-out group were compared, and no statistical differences were found (Table 1). The mean probability of drug survival was 20.0 months (median, 18 months; Figure 1). Multivariate analysis using the Cox proportional hazard model disclosed that age, PASI at baseline, and gender were not significant predictors for drug survival rate.

Persistence to adalimumab treatment was less than that reported in several previous studies (Table 2) (1–4). This may be because of the burden of frequent visits for injection. No precise description of self-injection was available in many studies regarding drug survival rates of adalimumab as well as other biological agents (1–3,5). However, introduction of an autoinjection pen for adalimumab was reported to increase the percentage of patients self-administering medication from 51 to 84% and decrease the percentage of patients attending primary care for injection from 33 to 2% in Spanish study population including 55 patients (rheumatoid arthritis 29, psoriatic arthritis 17, ankylosing spondykitis 9) (6). The high rate of self-injection in Spanish study population was in contrast to low rate in the present study. It is conceivable that greater use of self-injection may improve persistence to adalimumab.

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Declaration of interest

Authors do not have any conflict of interest

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B-cell activating factor detected on both naïve and memory B cells in bullous pemphigoid

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Abstract: B-cell activating factor (BAFF), an important immune regulatory cytokine, is involved in development of autoimmune diseases. Although BAFF is expressed in various cells, including dendritic cells (DCs) and monocytes, BAFF expression on B cells has not been well documented. In the present study, BAFF molecules on DCs and naïve and memory B cells in autoimmune bullous diseases, including pemphigus vulgaris, pemphigus foliaceus and bullous pemphigoid (BP), were analysed by flow cytometry. Compared with healthy controls (HC), BAFF expression on naïve and memory B cells increased significantly in BP. No difference in BAFF receptor expression in naïve and memory B cells was shown among all study groups. Furthermore, BAFF expression in both naïve and memory B cells of BP, but not HC, was detected by confocal microscopic analysis. These results implied that BAFF expressed by B cells may play a pathogenic role in autoimmune bullous diseases, particularly BP.

Key words: B-cell activating factor – B-cell activating factor receptor – bullous pemphigoid – dendritic cells – naïve and memory B cells

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Background

B-cell activating factor (BAFF), an important cytokine for B cells, regulates immune responses. BAFF is expressed on surface of dendritic cells (DCs), monocytes, neutrophils, stromal cells, epithelial cells, activated T cells and malignant B cells (1). However, BAFF expression on B cells has not been well addressed. Most of expressed BAFF is cleaved from cell surface and is secreted as soluble active homotrimer (2). Mainly by binding to BAFF receptor (BAFF-R), BAFF acts as rheostat for B-cell selection. BAFF at physiological concentration leads to deletion of autoreactive B cells, whereas BAFF at higher concentration results in survival of autoreactive B cells, which may cause development of autoimmune diseases (3–5). Increased serum levels of BAFF have been reported in a number of autoimmune diseases, including systemic lupus erythematosus, rheumatoid arthritis, Sjögren's syndrome and systemic sclerosis, as well as psoriasis vulgaris and bullous pemphigoid (BP; 6–11).

Questions addressed

Questions, which were addressed in this study for the first time, were whether BAFF is expressed in naïve and memory B cells in various autoimmune bullous diseases and whether BAFF and BAFF-R expression on naïve and memory B cells is different among the diseases.

Experimental design

Leucocytes were first isolated by Ficoll–Hypaque gradient centrifugation from 13 pemphigus vulgaris (PV) patients, 10 pemphigus foliaceus (PF) patients, 11 BP patients and 7 healthy controls (HC).

Dendritic cells and naïve and memory B cells were analysed by flow cytometry using EPICS Altra (Becqman Coulter, Brea, CA, USA). Conventional DCs were gated as FITC-CD14$^{(-)}$ (Beckman Coulter), FITC-CD16 $^{(-)}$ (Beckman Coulter) and PC5-ILT3$^{(+)}$ (Immunotech, Marseille, France) cells (Fig. 1a). Naïve B cells were gated as FITC-CD19$^{(+)}$ (Beckman Coulter) and PC5-CD27$^{(-)}$ (Beckman Coulter) cells, and memory B cells were gated as FITC-CD19$^{(+)}$ (Beckman Coulter) and PC5-CD27$^{(+)}$ (Beckman Coulter) cells (Fig. 2a). In conventional DCs (Fig. 1a) and naïve and memory B cells (Fig. 2a), expression of BAFF and BAFF-R was analysed by PE-CD257 (Beckman Coulter) and PE-CD268 (Beckman Coulter), respectively. Expression levels of target proteins were evaluated by mean fluorescence intensity (MFI). Statistical analysis was performed using Student's two-tailed *t*-test. A probability value < 0.05 was considered to be statistically significant.

Confocal microscopic analysis was employed to evaluate the BAFF expression in B cells of BP and HC. Naïve and memory B cells were isolated from two BP patients and two HC by relative cell isolation kits (MACS, Auburn, AL, USA). Cells were then attached to the glass slides and fixed by 4% paraformaldehyde solution containing 0.2% triton-X100. After blocking with 5% BSA in PBS, the cells were stained with 50-fold diluted mouse anti-BAFF monoclonal antibody (Santa Cruz Biotechnology, Heidelberg, Germany), followed by incubation with 500-fold diluted of Alexa488-conjugated second antibody (Invitrogen) and 1000-fold diluted DAPI (Invitrogen). Finally, BAFF expression was determined by confocal microscopic analysis.

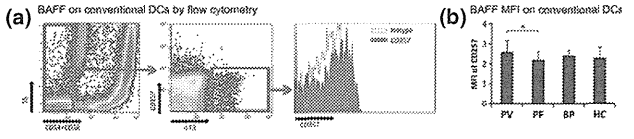

Figure 1. B-cell activating factor (BAFF) expression on conventional dendritic cells (DCs). (a) Conventional DCs was gated as $CD14^{(-)}$ $CD16^{(-)}$ $ILT3^{(+)}$ leucocytes. Expression of BAFF was analysed by CD257 in conventional DCs. (b) BAFF MIF on conventional DCs was shown for indicated diseases. * $P < 0.05$.

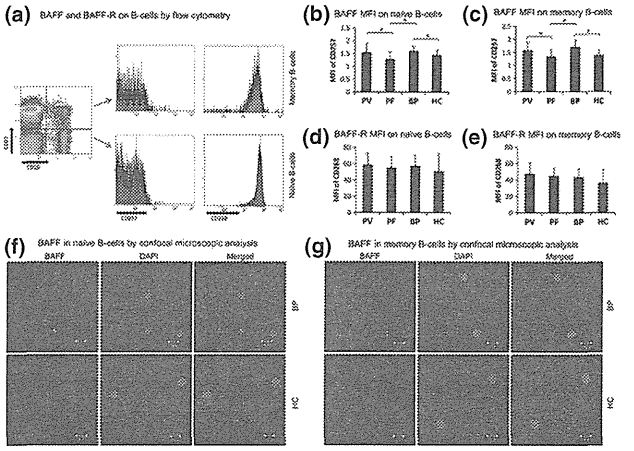

Figure 2. (a–e) Results of flow cytometric analysis of B-cell activating factor (BAFF) and BAFF-R expression on naïve and memory B cells. (a) Naïve and memory B cells were gated as $CD19^{(+)}$ $CD27^{(-)}$ and $CD19^{(+)}$ $CD27^{(+)}$ leucocytes, respectively. Expression of BAFF and BAFF-R was analysed as CD257 and CD268 in both naïve and memory B cells, respectively. BAFF mean fluorescence intensity (MFI) (b, c) and BAFF-R MFI (d, e) on both naïve and memory B cells were shown for indicated diseases. *$P < 0.05$. (e, f) Results of confocal microscopic analysis of BAFF. (e) BAFF expression in naïve B cells isolated from bullous pemphigoid (BP) and healthy controls (HC). (f) BAFF expression in memory B cells isolated from BP and HC.

Results

BAFF MFI on conventional DCs in PV, PF and BP were not different from HC, while that in PV was significantly higher than PF ($P < 0.05$; Fig. 1b). BP, but not PV and PF, showed significantly higher BAFF MFI on both naïve and memory B cells than HC ($P < 0.05$; Fig. 2b,c). BP ($P < 0.05$) and PV ($P < 0.05$) also showed higher BAFF MFI on both naïve and memory B cells than PF (Fig. 2b,c). In contrast, BAFF-R MFI on naïve and memory B cells showed no difference among PV, PF, BP and HC (Fig. 2d,e). There was no difference in percentages of BAFF or BAFF-R positive cells for DCs and naïve B cells and memory B cells among PV, PF, BP and HC (data not shown).

In addition, confocal microscopic analysis detected relatively weak BAFF expression in both naïve and memory B cells from BP, but not from HC (Fig. 2f,g). The positive staining was observed mainly in cytoplasma, while cell surface staining was not prominent.

Conclusions

In the present study, we first performed flow cytometric analysis. This study showed significantly higher BAFF level in both naïve and memory B cells, but not in conventional DCs, in BP patients, when compared to HC. Additional confocal microscopic analysis also showed that BAFF expression was specifically found intracellularly in B cells from BP patients, but not from HC. To the best of our knowledge, this is the first report of BAFF expression in naïve and memory B cells in autoimmune diseases. Malignant B cells are known to express BAFF, which acts as essential autocrine survival factor (1,12). Therefore, besides malignant B cells, non-malignant B cells in BP also express BAFF.

Abnormal production of BAFF may disturb immune tolerance by allowing survival of autoreactive B cells, and trigger autoimmune diseases (3–5). Therefore, BAFF expressed in B cells may play a pathogenic role in BP, although possible contribution of T cells in BP was also reported (13). BAFF expression was reported to be induced by some known factors, such as NF-κB and interferon-γ (14,15). However, factors, which induced BAFF expression in B cells of BP in our study, were unknown.

BAFF-R level on B cells can be a biomarker for activity in autoimmune diseases (16). Decreased expression of BAFF-R on naïve and memory B cells has been demonstrated in several autoimmune diseases, including Sjögren's syndrome and systemic lupus erythematosus. In our study, BAFF-R levels on naïve and memory B cells among PV, PF, BP and HC were similar, suggesting that BAFF-R levels were not relevant in these autoimmune bullous diseases. To the best of our knowledge, this is also the first report of BAFF-R expression on naïve and memory B cells in PV, PF and BP.

BAFF engages three receptors, transmembrane activator and calcium-modulator and cytophilin ligand interactor (TACI), B-cell maturation antigen (BCMA) and BAFF-R (4). Therefore, it is possible that BAFF binds to TACI and BCMA, in addition to BAFF-R.

In conclusion, the results in this study indicated BAFF expressed in B cells may play a role in the pathogenesis at least in BP.

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Author contribution

Hua Qian, Masahiro Kusuhara, Xiaoguang Li, Atsunari Tsuchisaka, Norito Ishii, Hiroshi Koga, Taihei Hayakawa, Koji Ohara, Tadashi Karashima, Bungo Ohyama performed the researches. Hua Qian, Masahiro Kusuhara and Xiaoguang Li, Daisuke Tsuruta and Takashi Hashimoto wrote the paper. Daisuke Tsuruta, Chika Ohata, Minao Furumura contributed essential reagents and samples. Masahiro Kusuhara originally designed this study. Takashi Hashimoto revised the manuscript.

Conflict of interests

The authors have declared no conflicting interests.

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