Case of bullous pemphigoid coexisting with anti-desmoglein autoantibodies

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ABSTRACT

A 79-year-old Japanese woman had clinical and histopathological features of bullous pemphigoid, while direct immunofluorescence test revealed C3 and IgG depositions in the lower cell surfaces of the epidermis in addition to those in the dermo-epidermal junction. Chemiluminescent enzyme immunoassays were positive for desmoglein-1 and desmoglein-3 antibodies in addition to anti-BP180 antibodies. In an immunoblotting study, antibodies against both 180-kDa bullous pemphigoid antigen and 130-kDa pemphigus vulgaris antigen were detected. Based on these results, bullous pemphigoid coexisting with anti-desmoglein autoantibodies was diagnosed in this case.

Key words : anti-BP180 antibodies, anti-desmoglein antibodies, bullous pemphigoid, immunoblotting, pemphigus.

INTRODUCTION

Bullous pemphigoid (BP) is an autoimmune bullous disease caused by autoantibodies against adhesion molecules in hemidesmosomes. Some reports described patients with BP as having anti-desmoglein (Dsg) antibodies in addition to anti-BP180 antibodies[1,2]. We present a case that showed a BP phenotype but was positive for both anti-BP180 IgG and anti-Dsg IgG in the chemiluminescent enzyme immunoassays (CLEIAs) test and immunoblotting in the early disease stage.

CASE REPORT

A 79-year-old Japanese woman was referred to our clinic because of her erythematous eruptions spreading on her abdomen and lower back. The eruptions started 5 months before and improved somewhat with topical application of corticosteroid ointment. On the day of the consultation, blisters with clear fluid and erosions were found on geographic erythematous lesions that extended symmetrically on the flexor side of her arms, lower back and legs (Fig. 1a,1b). Blisters were also seen on her oral hard palate.

She had had hepatitis C virus infection for 22 years. She took zolpidem tartrate 5 mg/day for insomnia and glucosamine 500 mg/day for arthralgia.

Laboratory examination revealed the following values: white blood cells 8.71 × 10^3 cell/μL (reference range: 3.3-8.6 × 10^3/μL); red blood cells 4.16 × 10^6 cell/μL (4.35-5.55 × 10^6/μL); platelet count 369 × 10^3/μL (158-348 × 10^3/μL),

neutrophils 88% (40-75%), eosinophils 25.6% (0-8.5%), monocytes 4% (2-10%) , lymphocytes 7% (16.5-49.5%); total protein 5.6 g/dL (6.6-8.1 g/dL); albumin 3.0 g/dL (4.1-5.1 g/dL); aspartate transaminase 16 U/L (13-30 U/L); alanine transaminase 37 U/L (10-42 U/L); lactate dehydrogenase 143 U/L (124-222 U/L); creatinine kinase 18U/L (59-248 U/L); total cholesterol 250 mg/dL (150-219 mg/dL); urea nitrogen 25.6 mg/dL (8.0-20.0 mg/dL); creatinine 0.63 mg/dL (0.65-1.07 mg/dL); Na^+ 136mmol/L (138-145 mmol/L); K^+ 4.5 mmol/L (3.6-4.8 mmol/L); and Cl^- 105 mmol/L (101-108 mmol/L). In the CLEIAs, IgG antibodies against the recombinant NC16a domain of BP180 (260 U/mL), Dsg1 (26 U/mL) and Dsg3 (59 U/mL) were detected (Supplementary, Table 1).

Histological examination of the biopsy specimen taken from her left arm revealed a subepidermal blister containing numerous eosinophils (Fig.1c). Direct immunofluorescence (DIF) study revealed linear deposition of C_3 and linear weak deposition of IgG at the dermo-epidermal junction (DEJ) and lower cell surfaces of the epidermis (Fig. 1d, 1e). Immunoblotting performed by using the method described by Hashimoto et al[3] showed two bands with relative molecular masses of 180- and 130-kDa, respectively (Fig. 2, lane 3). The 180-kDa band corresponds to BP180 and the 130-kDa band corresponded to Dsg3 (Fig. 2, lanes 5 and 6). Based on the clinical features, and histopathological and immunofluorescence findings, as well as CLEIAs and immunoblotting test results, BP coexisting with anti-Dsg autoantibodies was diagnosed.

On day 11, oral administration of prednisolone 50 mg/day was started, and

the bullous lesions disappeared on day 22. Then, the prednisolone dosage was tapered to 12.5 mg/day, without recurrence of the bullous lesions. During the 5 months' follow-up, serum levels of the IgG autoantibodies against the BP180, Dsg1 and Dsg3 decreased (Supplementary, Table 1). After 2 years, no bands were detected by immunoblotting with her serum (Fig. 2, lane 4), while the CLEIA index for anti-BP180 IgG was still positive (159 U/mL).

DISCUSSION

Considering the tense blistering, and histological and immunofluorescence findings, BP was diagnosed in our case. However, our case was positive for anti-Dsg antibodies and oral mucous membrane involvement, which could be caused by the anti-Dsg antibodies, suggesting the diagnosis of overlapping BP and pemphigus. The positive CLEIA index for anti-Dsg3 antibodies was confirmed by immunoblotting (as shown in Fig. 2), but the test failed to detect anti-Dsg1 antibodies. This is due to the low titer of anti-Dsg1 antibodies (26 U/mL), suggesting non-pathogenic anti-Dsg1 antibodies. Considering that we detected anti-Dsg3 antibodies by using the CLEIA and immunoblotting, the anti-Dsg3 antibodies were likely pathogenic for inducing the oral mucous blistering because the indirect immunofluorescent study revealed IgG deposition in both the DEJ and lower cell surfaces of the epidermis (Fig. 1e). In addition, the mucous membrane involvement was disappeared after the prednisolone treatment, in parallel with the decrease in the levels of the anti-Dsg3 antibodies (Supplementary, Table 1).

Some reports described BP with positive IgG deposition in both the DEJ and the cell surfaces of epidermis similar to that in our case as shown in Table 1. In these reported cases BP was diagnosed in 4 cases[2,4,5,9], and BP and pemphigus were diagnosed in 9 cases[1,6-8,10-14] according to their histological findings; 4 BP cases had sub-epidermal cleft but 9 BP and pemphigus cases mostly had acantholysis in addition to sub-epidermal cleft. These reports indicate that diagnosis of BP or BP coexisting pemphigus can be given according to its histological findings though they have combined autoantibodies against DEJ and cell surface adhesion molecules. We made a diagnosis of BP coexisting with anti-desmoglein autoantibodies in this case, because the histological examination revealed subepidermal blisters, although the patient had mucous involvement and anti-Dsg3 antibodies throughout the disease course. Interestingly, apart from BP cases showed in Table 1, cases of pemphigoid vegetans[15], pemphigoid gestationis[16,17], and mucous member pemphigoid[18] were also reported having pemphigus autoantibodies. In addition, some cases of pemphigus[19,20] were reported to have anti-DEJ antibodies. In the most of these cases, diagnosis was given according to their histological findings.

In conclusion, we report a case of BP coexisting with anti-Dsg autoantibodies that shows subepidermal blistering with positive for both anti-BP180 antibodies and anti-Dsg antibodies.

CONFLICT OF INTEREST: None.

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LEGENGS OF FIGURES

Figure 1. Clinical appearance, and pathological and immunofluorescence findings of the patient. (a) Edematous erythema on the trunk and upper arms. (b) Tense blisters on the erythematous plagues. (c) Pathological finding of the blister. Subepidermal bulla containing eosinophils was seen. (d) C3 deposition on DEJ and lower cell surfaces of the epidermis found in the direct immunofluorescence method. (e) IgG deposition on DEJ and lower cell surfaces of the epidermis found in the direct immunofluorescence method.

Figure 2. Immunoblotting of epidermal extract incubated with serum of the patient and specific antibodies. An extract of normal human epidermis was separated by 7.5% sodium dodecyl sulfate-polyacrylamide gel electrophoresis, transferred to nitrocellulose membrane, and incubated with BP180-specific monoclonal antibody to C-terminus of BP180 (ab184996, Abcam) (Lane 1), BP patient control serum (Lane 2), serum from the patient before treatment (Lane 3), serum of the patient after treatment (Lane 4), Dsg3-specific monoclonal antibody (ab128927, Abcam) (Lane 5), pemphigus vulgaris patient control serum (Lane 6), Dsg1-specific monoclonal antibody (ab124798, Abcam) (Lane 7), pemphigus foliaceus patient control serum (Lane 8) and serum from a

healthy adult control (Lane 9). The 130 kDa-band in lane 1 might be soluble form of BP180, which appears as proteolytic processing of full-length form, as described in the literature (describe the paper PMID: 15161638). The serum of the BP patient control reacted only with BP180 (180kDa) (Lane 2), the pemphigus vulgaris patient serum reacted with the 130 kDa protein (Lane 6) and the pemphigus foliaceus serum demonstrated a 160 kDa band indicating antibodies against Dsg1 (Lane 8). The patient serum before treatment showed 180 kDa band corresponding to BP180 and 130 kDa band corresponding to Dsg3 (Lane 3). The patient serum after treatment showed no band (Lane 4).

Figure 1.

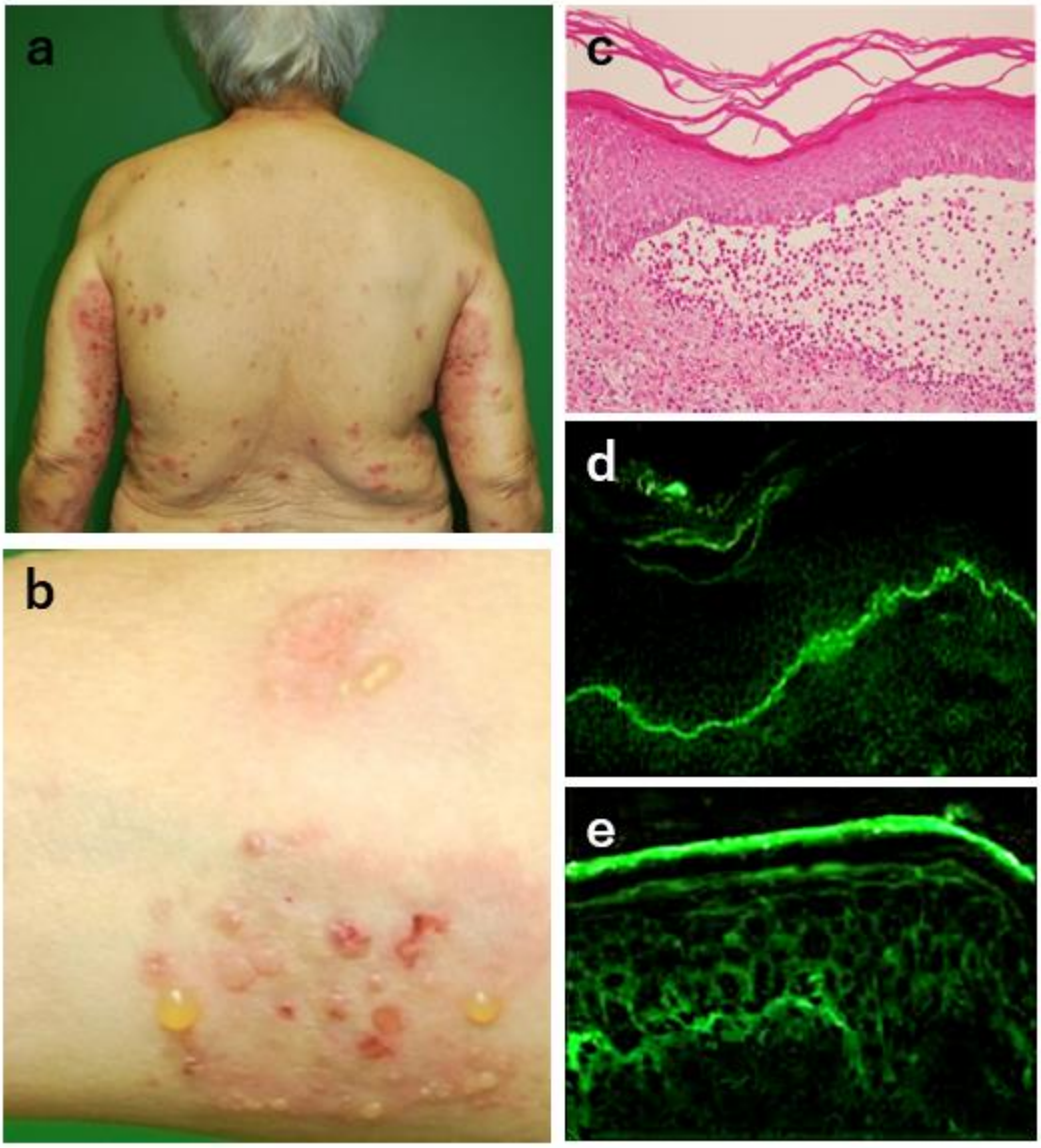

Figure 2.

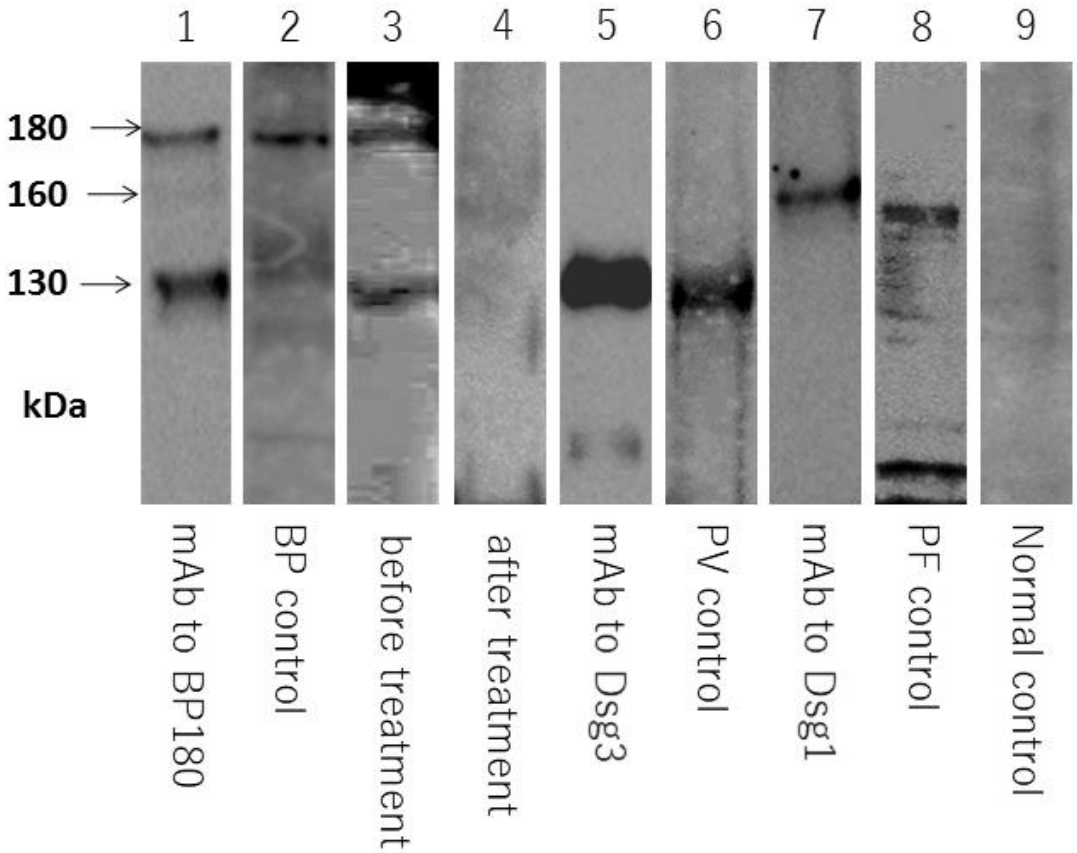
1
2
3
4
5
6
7
8
9
180
160
130
kDa
mAb to BP180
BP control
before treatment
after treatment
mAb to Dsg3
PV control
mAb to Dsg1
PF control
Normal control

Table 1. Reported cases with antibodies against both dermo-epidermal junction and cell surfaces of epidermis or keratinocyte

Reference	Diagnosis	Clinical feature	Histopathology	**DIF	***IIF	Other tests
1991, Korman NJ, et al.[1]	*BP and pemphigus	Faccid blisters	Sub-epidermal cleft. Acantholysis.	CS	DEJ+CS	**Immunoprecipitation:** BP230, Dsg 1
1994, Hashimoto T, et al.[2]	BP	Tense blisters	Sub-epidermal cleft	DEJ+CS	DEJ+CS	**Immunoblotting:** BP230, BP180, ****Dsc 1 and Dsc 2 **Immunoelectron microscopy:** IgG deposition on hemidesmosome
1994, Ninomiya J, et al.[4]	BP	Tense blisters Mucosal erosion	Sub-epidermal cleft	DEJ+CS	DEJ+CS	**Immunoblotting:** 190 kDa band
1995, Takahashi H, et al.[5]	BP	Tense blisters	Sub-epidermal cleft	DEJ+CS	DEJ+CS	**Immunoblotting:** BP230, *****Dsg3
1995, Matsubara K, et al.[6]	BP and Pemphigus	Tense blisters	Sub-epidermal cleft and mild acantholytic	DEJ+CS	CS	**Immunoblotting :**180 kDa band
1995, Ishiko A, et al.[7]	BP and Pemphigus	Tense blisters	Sub-epidermal cleft and acantholytic	DEJ+CS	DEJ+CS	**Immunoblotting:** BP230, BP180, Dsg1 **Immunoelectron microscopy:** Autoantibodies deposition on desmosome and hemidesmosomes
1995, Kore-eda S, et al.[8]	BP and Pemphigus	Tense blisters Mucosal erosion	Sub-epidermal cleft and intraepidermal blisters	DEJ+CS	DEJ+CS	**Electron microscopy:** acantholysis **Immunoelectron microscopy:** IgG deposition on desmosome and lamina lucida
2004, Takahashi H, et al.[9]	BP	Tense blisters	Sub-epidermal cleft	DEJ+CS	DEJ+CS	**ELISA:** BP180, Dsg3 **Immunoblotting:** BP180 **Immunoprecipitation:** Dsg3
2006, Tabuchi K, et al.[10]	BP and pemphigus	Mucosa erosions	Not in detail (pemphigoid pattern)	DEJ+CS	Not performed	**ELISA:** BP180, Dsg1

2009, Ando S, et al.[11]	BP and Pemphigus	Tense blisters Mucosal erosions	Sub-epidermal cleft and acantholytic	DEJ+CS	DEJ+CS	**Immunoblotting:** BP180 **ELISA:** Dsg1 and BP180
2010, Rossi A, et al.[12]	BP and pemphigus	Flaccid blisters	Sub-epidermal cleft and acantholytic	DEJ+CS	DEJ+CS	**ELISA:** BP180, Dsg1
2010, Shiohara J, et al.[13]	BP and pemphigus	Both tense and flaccid blisters	Sub-epidermal cleft and acantholysis	DEJ+CS	DEJ+CS	**ELISA:** BP180, Dsg1 **Immunoblotting:** BP230 **Immunoprecipitation:** BP180
2013, Ohata C, et al.[14]	BP and pemphigus	Mucosa erosions and small blisters	Sub-epidermal cleft and intraepidermal blisters	DEJ+CS	CS	**ELISA:** BP180, Dsg1, Dsg3, Dsc2, Dsc3 **Immunoblotting:** BP180

***BP:** Bullous pemphigoid. ****DIF:** Direct immunofluorescence, **DEJ**: Dermo-epidermal junction. **CS**: cell surfaces of epidermis or keratinocyte. *****IIF:** Indirect immunofluorescence. ******Dsc:** Desmocollin. *******Dsg:** Desmoglein.

Supplementary

Table 1 CLEIA index of anti-BP180, anti-Dsg1, and anti-Dsg3 IgG

Day	*BP180 (U/mL)	**Dsg1 (U/mL)	***Dsg3 (U/mL)	Prednisolone (mg/day)
Day 1	260	26	59	
Day 11				50
Day 17	420	13	32	50
Day 22				40
Day 29				35
Day 35				30
Day 37	76	5	10	30
Day 42				25
Day 43	66	5	9	25
Day 48				22.5
Day 56				20
Day 63	36	5	5	20
Day 77	72	3	3	17.5
Day 99	46	3	3	17.5
Day 112	43	3	3	15
Day 140	45	3	3	15
Day 168	38	3	3	12.5

*BP180: positive is defined as higher than 9 U/mL

**Dsg1 and ***Dsg3: positive is defined as higher than 20 U/mL