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Dipeptidyl peptidase-4 inhibitor-related bullous pemphigoid showing positive autoantibody responses to multiple epitopes

Short title: **DPP-4i-related BP with multiple epitopes**

Rikuma Kitao[a], Takeshi Fukumoto[a], Takashi Hashimoto[b], Kentaro Izumi[c], Haruki Jimbo[a], Chihiro Takemori[a], Chikako Nishigori[a]

[a]*Division of Dermatology, Department of Internal Related, Kobe University Graduate School of Medicine, 7-5-1 Kusunoki-cho, Chuo-ku, Kobe, Hyogo 650-0017, Japan*
[b]*Department of Dermatology, Osaka City University Graduate School of Medicine, 1-4-3 Asahimachi, Abeno-ku, Osaka 545-8585, Japan*
[c]*Department of Dermatology, Hokkaido University Graduate School of Medicine, N15 W7, Kita-ku, Sapporo, Hokkaido 060- 8638, Japan*

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Corresponding author: Takeshi Fukumoto, M.D., Ph.D.
Division of Dermatology, Department of Internal Related, Kobe University Graduate School of Medicine, 7-5-1 Kusunoki-cho, Chuo-ku, Kobe, Hyogo 650-0017, Japan
Tel.: +81-78-382-6134, Fax: +81-78-382-6149, E-mail: fuku@med.kobe-u.ac.jp

Abstract:

Bullous pemphigoid (BP) is an autoimmune blistering disease. Although dipeptidyl peptidase-4 inhibitors (DPP-4i) are used for patients with diabetes mellitus, DPP-4i may cause BP. A 60-year-old man with type II diabetes mellitus presented with tense blisters without erythema that first appeared on his legs and gradually spread to his trunk and arms. This patient had been undergoing hemodialysis and taking teneligliptin (DPP-4i). The initiation of daily oral administration of prednisolone and the discontinuation of DPP-4i improved his symptoms. Detailed analyses revealed multiple potential epitopes, including the noncollagenous 16a (NC16a) and C-terminal domains and LAD-1 regions of BP180. This patient showed a high bullous pemphigoid disease area index, and the reactivities with the multiple epitopes positively correlated with his symptoms, indicating that autoantibodies to these multiple epitopes might be pathogenic. Given the increased use of DPP-4i, the accumulation of similar cases is of significant importance.

Keywords: bullous pemphigoid, dipeptidyl peptidase-4 inhibitor, BP180, LAD-1, hemodialysis

Introduction

Bullous pemphigoid (BP) is an autoimmune blistering disease [1-4]. Although dipeptidyl peptidase-4 inhibitors (DPP-4i) are used in diabetes mellitus patients because of relatively fewer side effects, DPP-4i may cause BP [1-5]. Some DPP-4i-related BP patients are positive for antibodies to noncollagenous 16a (NC16a) domain of BP180, while others are negative [5]. Izumi et al. reported that BP, including DPP-4i-related BP cases, can be classified into four groups based on the results of enzyme-linked immunosorbent assays (ELISAs) of both NC16a domain and full-length BP180 [1]. Herein, we present a rare case of a patient with DPP-4i-related BP who was positive for antibodies against multiple epitopes within BP180. Only a few cases of DPP-4i-related BP were reported to react with multiple epitopes [6, 7].

Case

A 60-year-old man with type II diabetes mellitus presented with tense blisters without erythema that had appeared on his legs and gradually spread to his trunk and arms (Figure 1a,b). Bullous pemphigoid disease area index (BPDAI) was 81 points. He had been undergoing hemodialysis (HD) for 4 years due to diabetic nephropathy and taking teneligliptin (DPP-4i, 20 mg/day) for 41 months. Chemiluminescent enzyme immunoassay (CLEIA) of anti-BP180 NC16a domain showed negative. Skin biopsy showed a subepidermal bulla with eosinophil infiltration (Figure 1c,d). Indirect immunofluorescence (IIF) using normal human skin revealed a linear staining pattern with IgG at the epidermal basement membrane zone (Figure 1e). IIF using 1M NaCl-split normal human skin (ss-IIF) showed IgG reactivity on the epidermal side and a negative response on the dermal side (Figure 1f). Direct immunofluorescence (DIF)

revealed a linear staining pattern with complement C3 and IgG in the epidermal basement membrane zone (Figure 1g,h). The DPP-4i was discontinued, and oral administration of prednisolone (PSL) at 0.5mg/kg/day was initiated. The symptoms improved, and the BPDAI score reduced to five points at 1 week after initiation of treatment with PSL.

Then, we performed further analyses to find out the presence of pathogenic antibodies. IgG autoantibodies (autoAbs) against BP180 NC16a domain and full-length BP180 were positive using ELISA. In addition, we performed immunoblotting (IB) analyses using four different antigen sources for the 3 serum samples collected at 3 different time points (Figure 2). The reactivities in the four IB analyses in general showed positive correlations with clinical symptoms, with some differences among them. Thus, all the 3 serum samples reacted clearly with the full-length BP180 in the epidermal extract and the C-terminal domain recombinant protein (RP), with only slight decrease during the disease course (Figure 2a, c). In contrast, the reactivities with the BP180 NC16a domain RP and LAD-1 in the concentrated culture supernatant of HaCaT cells were in synchrony with disease severity; i.e., they showed strong reaction with the serum sample collected during the active disease stage, but were barely detectable in the serum sample collected at the time of epithelialization (Figure 2b, d).

Discussion

DPP-4i-related BP tends to manifest with tense blisters without erythema as 'non-inflammatory' BP, and our patient’s symptoms were comparable with those in the common type of DPP-4i-related BP [5]. He presented with symptoms 41 months after initiating treatment with DPP-4i. The onset was significantly delayed compared with

those of previously reported DPP-4i-related BP cases [2, 3]. 15.6% of teneligliptin (DPP-4i) is reportedly removed by HD [8]. Therefore, the delayed time of onset in this case might be due to the removal of DPP-4i by HD. However, no studies have investigated the time of onset of DPP-4i-related BP patients undergoing HD. Therefore, further studies are required to confirm the correlation between the time of onset of DPP-4i-related BP and HD.

From detailed analyses, we inferred that autoAbs in this case reacted with multiple epitopes on the NC16a and C-terminal domains and LAD-1 region of BP180. Epitopes within the C-terminal domain and LAD-1 of BP180 are known targets for autoAbs in anti-BP180-type mucous membrane pemphigoid and linear IgA bullous dermatosis, respectively. However, these epitopes are different from those in typical cases of common BP and DPP-4i-related BP [3].

Based on the previously proposed classification of BP [1], this case was classified into the “Full with NC16a-BP” group. Most patients classified into this group had shown high BPDAI scores with severe urticarial erythema, presenting as “inflammatory” BP [1], whereas our patient showed a high BPDAI score without erythema, presenting as “non-inflammatory” BP. Furthermore, a previous report showed that the presence of autoAbs recognizing epitopes within both the NC16a and C-terminal domains increased the morbidity of mucosal lesions [9], whereas our patient had no mucosal lesions. In terms of response to treatment, he showed a remarkable response to PSL.

To investigate further the association between the autoAbs to multiple epitopes and the symptoms in our patient, we performed four different IB analyses for three sera taken at three-time points (Figure 2). The differences in the changes in reactivity in the

four IB analyses among the 3 samples of the patient's serum might indicate that epitopes within the BP180 NC16a domain and LAD-1 region were more pathogenic than those in the BP180 C-terminal domain. Interestingly, a few cases were reported to react with multiple epitopes within BP180, particularly LAD-1 [6], although the changes in reactivity to multiple epitopes during disease activity have rarely been reported.

On the other hand, Izumi et al. reported that non-inflammatory-type BP targeted the non-NC16a domain within BP180 [1]. In addition, in this case, reactivity of the patient serum taken at the disease stage before treatment was very strong for the C-terminal domain and LAD-1 region of BP180, but weak for the NC16a domain. Considering all the findings mentioned above, we think that the most pathogenic epitopes in this case resided within the LAD-1 region.

A discrepancy in the results between CLEIA and ELISA was observed in this case. Mai et al. reported a similar discrepancy between CLEIA and ELISA for desmogleins and attributed it to the different incubation times between the two systems [10]. Although further studies are required to confirm this, we believe that the cause of the discrepancy in our study might be the same.

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Figure legends

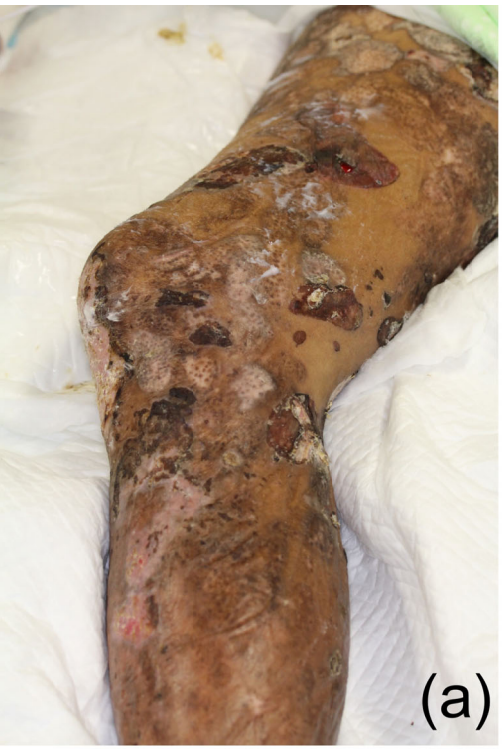
Figure 1. Clinical and histopathological features of the patient

(a and b) Tense blisters without erythema on the right leg (a) and generalized skin lesions with new and old blisters on the patient's body (b). **(c and d)** Histopathological analysis for the biopsy taken from the skin with blisters on left abdomen showing subepidermal bulla with eosinophil infiltration (Hematoxylin and eosin; H&E staining, original magnifications × 20 [c] and × 200 [d]). **(e)** Indirect immunofluorescence (IIF) using normal human skin showing a linear staining pattern with IgG in the epidermal basement membrane zone (original magnifications × 200). **(f)** IIF using 1M NaCl-split normal human skin (ss-IIF) showing IgG reactivity only on the epidermal side (original magnifications × 200). **(g)** Direct immunofluorescence (DIF) showing a linear IgG deposit at the epidermal basement membrane zone (original magnifications × 200). **(h)** DIF showing a linear complement C3 deposit at the epidermal basement membrane zone (original magnifications × 200).

Figure 2. Autoantibody responses immunoblotting (IB) analyses of different antigen sources

1: Serum 1, 2, and 3 were obtained before oral administration of prednisolone (PSL) [bullous pemphigoid disease area index (BPDAI) 81 points], at the disease point when epithelization was completed [BPDAI 0 points, Day 95 after PSL treatment initiation], and 1 month after epithelialization was completed [BPDAI 0 points, Day 123 after PSL treatment initiation], respectively. (a) IB using normal human epidermal extract showing a positive reaction to BP230 and BP180 with slight reduction with disease course. (b) Relatively weak reactivity with recombinant protein (RP) of BP180-NC16a domain was

shown by serum 1 with reduction with disease course. (c) Strong reactivity with BP180 C-terminal domain RP was shown by serum 1 with slight reduction with disease course. (d) Considerably strong reactivity with LAD-1 in concentrated culture supernatant of HaCaT cells (IgG) was shown by serum 1 with much reduction with disease course.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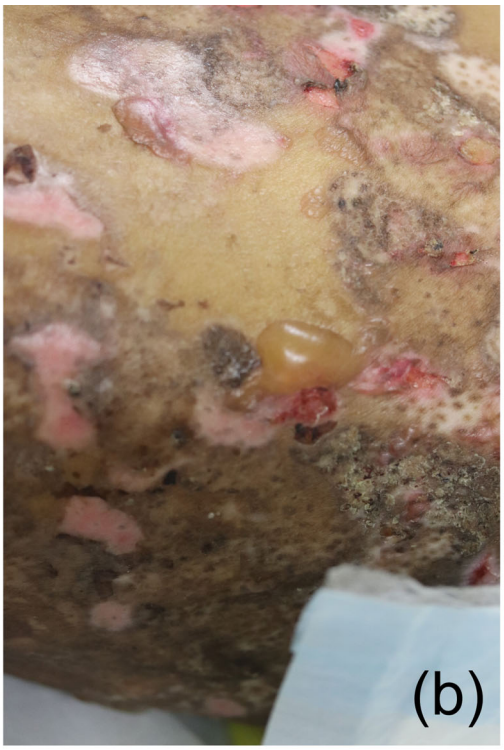
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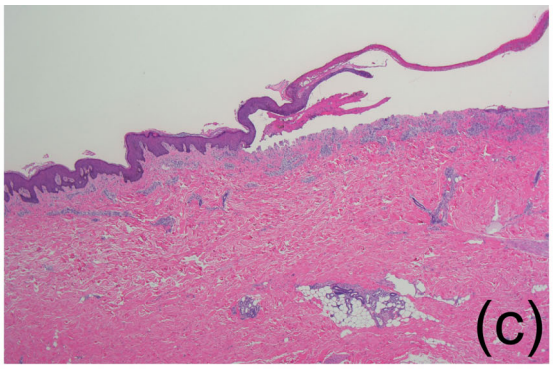
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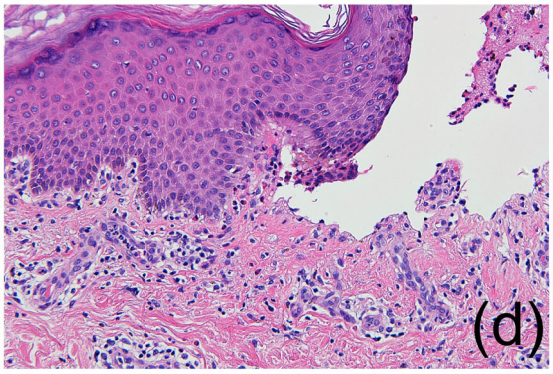
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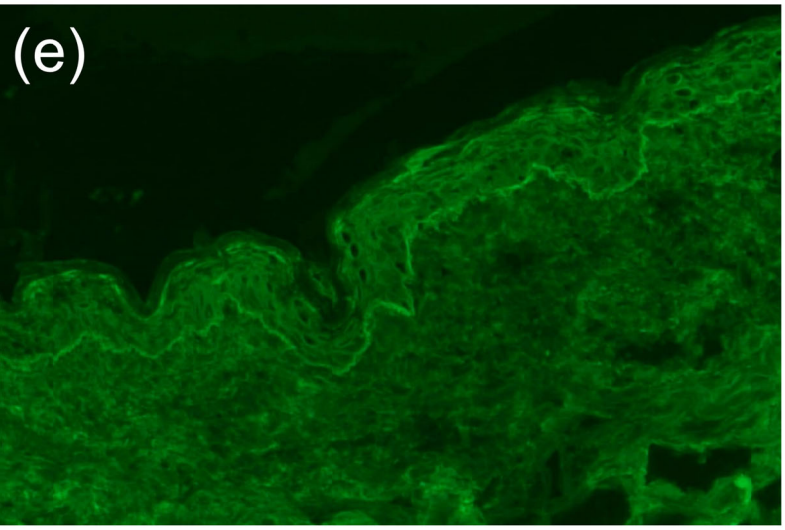
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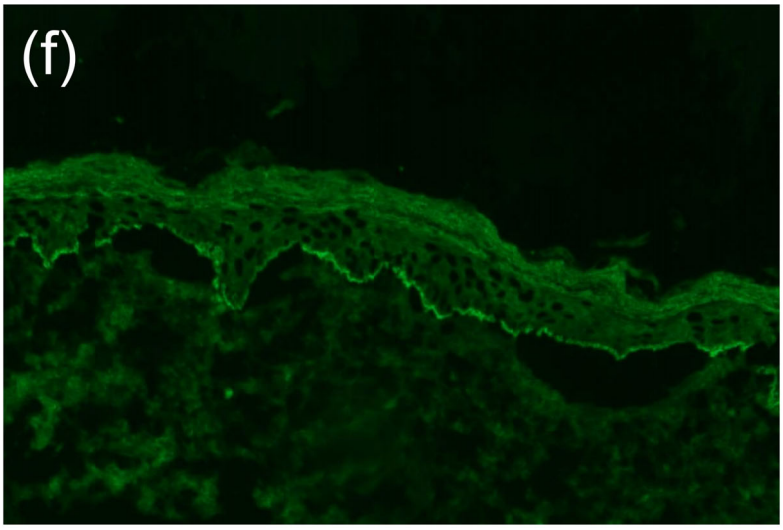
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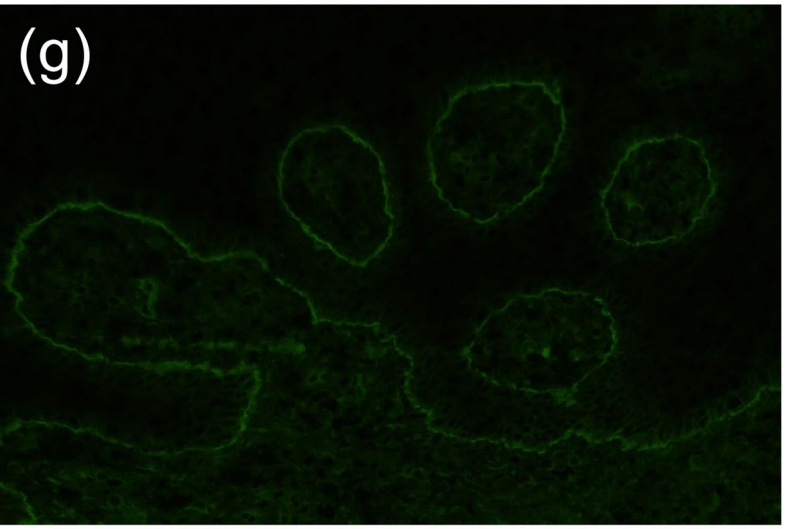
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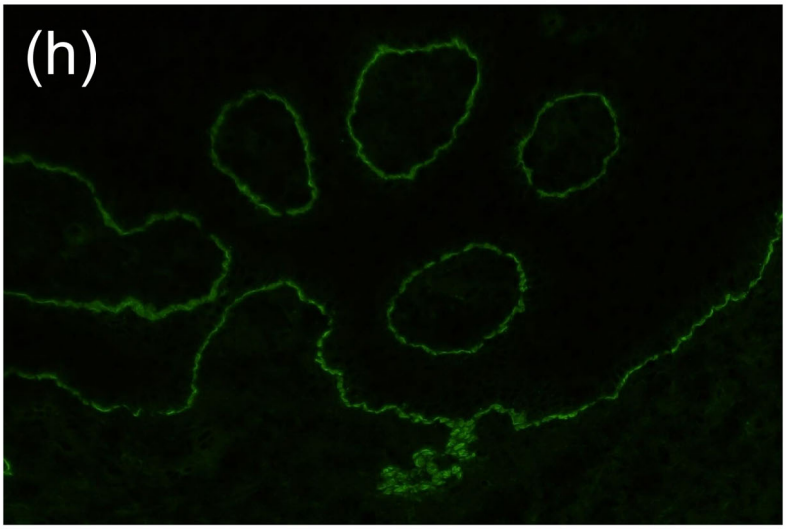
(h)

(a) epidermal extract

serum 3
serum 2
serum 1
BP180.BP230 control
Normal control(IgG)

230 kDa BP230
180 kDa BP180

(b) RP of BP180 NC16a domain

serum 3
serum 2
serum 1
BP180-NC16a control
Normal control(IgG)

RP

(c) RP of BP180 C-terminal domain

serum 3
serum 2
serum 1
C-terminal control
Normal control(IgG)

RP

(d) Concentrated culture supernatant of HaCaT cells

serum 3
serum 2
serum 1
control(IgG)
Normal control(IgG)

120 kDa