Serum autoantibodies against the extracellular region of α6β4 integrin in a patient with dipeptidyl peptidase-4 inhibitor–induced bullous pemphigoid

Chigusa Yamashita, MD,[a] Noriko Arase, MD, PhD,[a] Shuhei Higuchi, MS,[b] Hisashi Arase, MD, PhD,[b,c] Junichi Takagi, PhD,[d] Satoshi Nojima, MD, PhD,[e] Atsushi Tanemura, MD, PhD,[a] and Manabu Fujimoto, MD, PhD[a,f]
Osaka, Japan

Key words: α6β4 integrin; autoantibody; blister; bullous pemphigoid; dipeptidyl peptidase-4 inhibitors; extracellular domain.

INTRODUCTION

α6β4 Integrin contributes to the stable adhesion of epithelial cells to the basement membrane and the formation of hemidesmosomes.[1] It is one of the important target antigens for autoantibodies in the mucous membrane and skin of patients with bullous pemphigoid (BP).[2-4] Previous studies reported the presence of IgG against the α6 and β4 subunits of α6β4 integrin in both oral[2] and ocular[3] mucous membrane pemphigoid. Furthermore, autoantibodies to β4 integrin have been reportedly observed in the large intracellular region, not the extracellular region.[3]

Recently, cases of BP induced by dipeptidyl peptidase-4 (DPP-4) inhibitors, which are used in the management of type 2 diabetes mellitus, have been reported. Most patients with DPP-4 inhibitor–induced BP have autoantibodies against BP180, and various epitopes have been reported in the autoantibodies to BP180.[5-9] We report a patient with BP with autoantibodies directed against the physiologic extracellular domain of α6β4 integrin. To our knowledge, this finding, as well as anti-α6β4 integrin antibodies in DPP-4 inhibitor–related BP, has not been previously reported.

Abbreviations used:

BP: bullous pemphigoid
DPP-4: dipeptidyl peptidase-4
IgG: immunoglobulin G

CASE REPORT

A woman in her late 60s reported a 1-month history of generalized and perianal skin blistering; a burning sensation preceded blister formation. Her history was significant for type 2 diabetes mellitus that had been treated with linagliptin for the past year. Physical examination revealed noninflammatory painful vesicles, bullae 3 to 10 mm in diameter, and erythema on the trunk, buttocks, and limbs (Fig 1).

Biopsy of a bulla on the upper portion of the left arm showed subepidermal blistering with eosinophils and lymphocytes (Fig 2, *A* and *B*). Direct immunofluorescence demonstrated linear deposits of C3 (Fig 2, *C*) and IgG (Fig 2, *D*) along the basement membrane zone. Indirect immunofluorescence using 1 mol/L NaCl-split human skin as the substrate was positive for IgG on the epidermal side of the artificial blister (Fig 2, *E*).

From the Department of Dermatology, Osaka University Graduate School of Medicine[a]; Department of Immunochemistry, Research Institute for Microbial Diseases, Osaka University[b]; Laboratory of Immunochemistry, World Premier International Immunology Frontier Research Center, Osaka University[c]; Laboratory for Protein Synthesis and Expression, Institute of Protein Research, Osaka University[d]; Department of Pathology, Osaka University Graduate School of Medicine[e]; and the Laboratory of Cutaneous Immunology, World Premier International Immunology Frontier Research Center, Osaka University.[f]

Supported by the Japan Society for the Promotion of Science KAKENHI (grant numbers JP18K08296 and JP21K08344).

IRB approval status: This study has been approved by the Osaka University Research Ethics Review Committee (652), and Ethics Review Committee of Osaka University Hospital (12013).

Correspondence and reprint requests to: Noriko Arase, MD, PhD, Department of Dermatology, Integrated Medicine, Graduate School of Medicine, Osaka University, 2-2, Yamadaoka, Suita, Osaka 565-0871, Japan. E-mail: arase@derma.med.osaka-u.ac.jp.

JAAD Case Reports 2022;20:65-8.
2352-5126

https://doi.org/10.1016/j.jdcr.2021.12.021

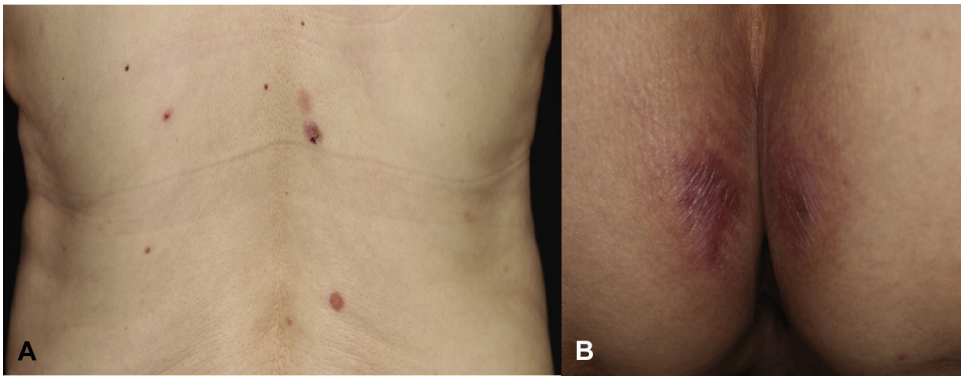

Fig 1. A case of dipeptidyl peptidase-4 inhibitor–induced bullous pemphigoid in a woman in her late 60s. Clinical images of erythema and bullae. **A**, Scattered vesicles with underlying erythema and erythematous macules on the back at the initial examination. **B**, Intact bullae on an erythematous base on the bilateral medial buttock that persisted for 3 years.

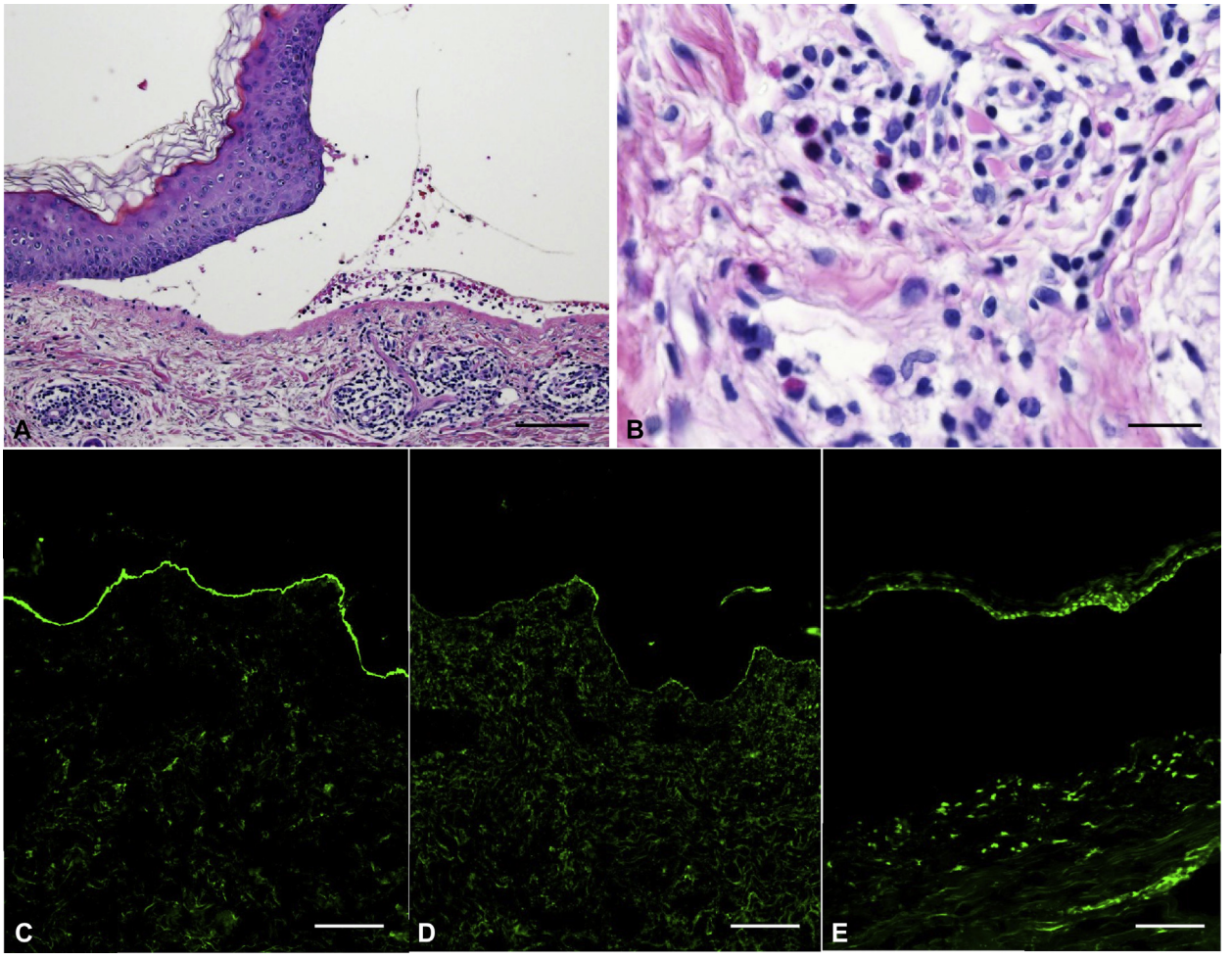

Fig 2. Histopathology of lesion tissue. **A** and **B**, A subepidermal bulla with eosinophilic and neutrophilic infiltration of the upper dermis. **C** and **D**, Linear deposition of C3 (**C**) and IgG (**D**) along the basement membrane zone by direct immunofluorescence. **E**, Linear deposition of IgG autoantibodies along the epidermal side of artificial blister by saline-substrate indirect immunofluorescence. (**A** and **B**, Hematoxylin-eosin stain; original magnifications: **A**, **C**, **D**, and **E**, ×100; **B**, ×400. Scale bars: **A**, **C**, **D**, and **E**, 100 μm; **B**, 20 μm.)

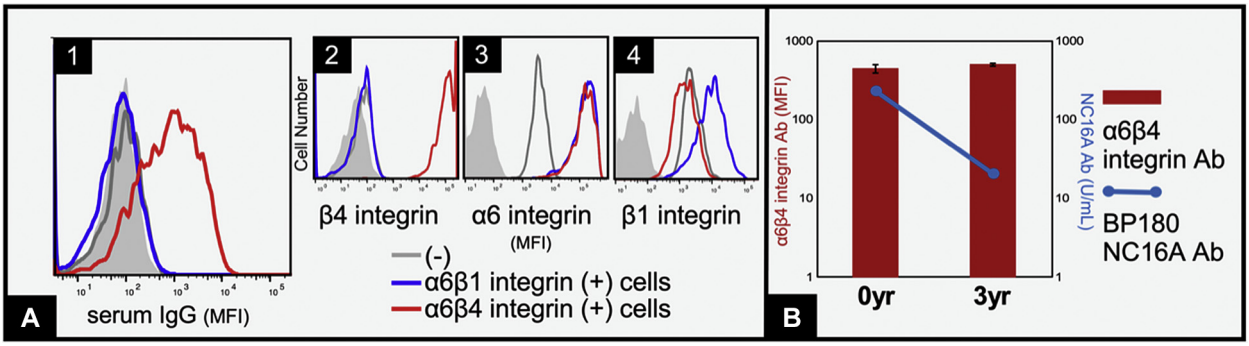

Fig 3. Specific reaction of the patient's serum autoantibodies to the extracellular region of the $\alpha6\beta4$ integrins. **A**, Expression vectors containing the extracellular and transmembrane regions of $\alpha6$ and $\beta4$ integrins or $\alpha6$ and $\beta1$ integrins are transiently transfected into 293T cells in the presence of green fluorescent protein (GFP) vectors. The diluted serum (1) and respective monoclonal antibodies (2-4) were reacted with the transfectants; the fluorescence intensities are analyzed within the GFP^+ transfected cells. Transfectants of $\alpha6\beta4$ integrins (*red lines*), $\alpha6\beta1$ integrins (*blue lines*), and GFP (*gray lines*) are shown. Filled histograms show secondary antibodies only. **B**, The *red bars* show no significant change in serum $\alpha6\beta4$ integrin extracellular domain antibody titers in the patient at onset (0 yr) and 3 years later (3 yr). The *blue line* shows a decrease in the BP180 NC16A titer. IgG: immunoglobulin G; MFI: mean fluorescence intensity.

Anti-BP180 NC16A IgG by chemiluminescent enzyme immunoassay was elevated at 228 U/mL (range, 0-9 U/mL). In addition, the serum was analyzed for autoantibodies against the extracellular region of $\alpha6\beta4$ integrin expressed on 293T cells and analyzed by flow cytometry.

The patient's serum IgG preferentially bound to the extracellular domain of $\alpha6\beta4$ integrin (Fig 3, *A*-1; *red line*), but not to $\alpha6\beta1$ integrin (Fig 3, *A*-1; *blue line*) nor to 293T cells themselves (Fig 3, *A*-1; *gray line*). To confirm the expression of integrins on 293T cells and the transfectants, they were stained with several anti-integrin monoclonal antibodies (Fig 3, *A*-2-4 and data not shown). $\alpha6\beta4$ and $\alpha6\beta1$ integrin transfectants were highly expressed respective β and α integrins (Fig 3, *A*-2-4; *red and blue lines*). 293T cells endogenously expressed both $\alpha6$ and $\beta1$ integrins, but not $\beta4$ integrin (Fig 3, *A*-2-4; *gray lines*). These data indicate that the patient's serum specifically recognized $\alpha6\beta4$ integrin, possibly the extracellular domain of $\beta4$ integrin.

On the basis of these clinical and laboratory findings, we diagnosed this patient's condition as DPP-4 inhibitor–induced BP with anti-$\alpha6\beta4$ integrin extracellular domain antibodies. DPP-4 inhibitor was discontinued, and oral prednisolone (0.25 mg/kg/day) was started. Her skin symptoms started to gradually improve after 10 days and significantly improved after 1 year to a state in which she could be treated with only topical steroids. Three years after the start of treatment, her BP180 antibody titer decreased (index: 20; Fig 3, *B*; *blue line*); however, she had occasional recurrent intractable painful bullae on the bilateral medial buttocks (Fig 1, *B*), with persistent high titers of anti-$\alpha6\beta4$ integrin antibody (Fig 3, *B*; *red bars*).

DISCUSSION

In this study, we demonstrated that the serum of this patient with BP specifically bound to the extracellular region of $\alpha6\beta4$ integrin. In addition, we examined the serum anti-$\alpha6\beta4$ integrin extracellular domain antibodies in 42 other patients with autoimmune bullous diseases and 20 healthy individuals. The 42 patients with autoimmune bullous disease included 32 patients with BP (5 patients receiving DPP-4 inhibitors), 6 with mucous membrane pemphigoid, 3 with linear IgA bullous dermatosis, and 1 with acquired epidermolysis bullosa. All patients and controls were negative for anti-$\alpha6\beta4$ integrin extracellular domain antibodies, except for the patient in our study; this finding suggested that anti-$\alpha6\beta4$ integrin extracellular domain antibodies are not common in patients with BP. It is possible that the autoantibodies are directed against the extracellular region of $\beta4$ integrin, or alternatively, that they recognize only the $\alpha6\beta4$ conformation, and not the $\alpha6\beta1$ or other integrin dimer structures (data not shown).

To our knowledge, there have been no reports of physiologic $\alpha6\beta4$ integrin extracellular domain autoantibodies in mammalian cells.[2-4] Autoantibodies that bind to the extracellular region of the $\alpha6\beta4$ integrin may inhibit binding of the $\alpha6\beta4$ integrin to laminin 332. Furthermore, in this case, the anti-$\alpha6\beta4$ integrin extracellular domain antibody titer was maintained for 3 years, despite improvement in the

anti-BP180 NC16A antibody titer (Fig 3, *B*). Persistent anti-$\alpha 6\beta 4$ integrin extracellular domain antibodies may be responsible for the residual refractory bullae on the perianal region in this patient (Fig 1, *B*).

Patients with DPP-4 inhibitor–associated BP produce autoantibodies against multiple sites of BP180,[5-9] and some of these autoantibodies can also target BP230.[6] Previous studies have shown that BP230 antibodies are produced by intermolecular epitope spreading after the production of autoantibodies against BP180 NC16A.[10] In this patient, it is possible that anti-BP180 antibodies were produced first, followed by anti-$\alpha 6\beta 4$ integrin antibodies by intermolecular epitope spreading under certain circumstances. Furthermore, anti-$\alpha 6\beta 4$ integrin antibodies persisted for 3 years, even after discontinuation of DPP-4 inhibitor. This suggests that the DPP-4 inhibitor may have unmasked a predisposition to BP in this patient rather than directly inducing anti-$\alpha 6\beta 4$ integrin antibodies. To our knowledge, this is the first study to clearly demonstrate the existence of serum autoantibodies against the extracellular region of $\alpha 6\beta 4$ integrins in a patient with BP and also the first to identify $\alpha 6\beta 4$ integrin autoantibodies in a patient with DPP-4 inhibitor–induced BP.

Technical assistance was provided by Yumiko Fujii, BS and Sumiko Matsuoka, MS.

Conflicts of interest

None disclosed.

REFERENCES

1. Miyazaki N, Iwasaki K, Takagi J. A systematic survey of conformational states in $\beta 1$ and $\beta 4$ integrins using negative-stain electron microscopy. *J Cell Sci*. 2018;131: jcs216754. https://doi.org/10.1242/jcs.216754
2. Rashid KA, Gürcan HM, Ahmed AR. Antigen specificity in subsets of mucous membrane pemphigoid. *J Invest Dermatol*. 2006;126:2631-2636. https://doi.org/10.1038/sj.jid.5700465
3. Bhol KC, Dans MJ, Simmons RK, Foster CS, Giancotti FG, Ahmed AR. The autoantibodies to $\alpha 6\beta 4$ integrin of patients affected by ocular cicatricial pemphigoid recognize predominantly epitopes within the large cytoplasmic domain of human $\beta 4$. *J Immunol*. 2000;165:2824-2829. https://doi.org/10.4049/jimmunol.165.5.2824
4. Li X, Tsuchisaka A, Hua Q, et al. Linear IgA/IgG bullous dermatosis reacts with multiple laminins and integrins. *Eur J Dermatol*. 2015;25(5):418-423.
5. Oyama N, Setterfield JF, Powell AM, et al. Bullous pemphigoid antigen II (BP180) and its soluble extracellular domains are major autoantigens in mucous membrane pemphigoid: the pathogenic relevance to HLA class II alleles and disease severity. *Br J Dermatol*. 2006;154:90-98. https://doi.org/10.1111/j.1365-2133.2005.06998.x
6. Izumi K, Nishie W, Mai Y, et al. Autoantibody profile differentiates between inflammatory and noninflammatory bullous pemphigoid. *J Invest Dermatol*. 2016;136:2201-2210. https://doi.org/10.1016/j.jid.2016.06.622
7. Fania L, Salemme A, Provini A, et al. Detection and characterization of IgG, IgE and IgA antoantibodies in patients with bullous pemphigoid associated with dipeptidyl peptidase-4 inhibitors. *J Am Acad Dermatol*. 2018;78:592-595. https://doi.org/10.1016/j.jaad.2017.09.051
8. Diez IG, Lleo MI, Aventin DL, et al. Bullous pemphigoid induced by dipeptidyl peptidase-4 inhibitors. Eight cases with clinical and immunological characterization. *Int J Dermatol*. 2018;57:810-816. https://doi.org/10.1111/ijd.14005
9. Mai Y, Nishie W, Izumi K, Shimizu H. Preferential reactivity of dipeptidyl peptidase-IV inhibitor-associated bullous pemphigoid autoantibodies to the processed extracellular domains of BP180. *Front Immunol*. 2019;10:1224. https://doi.org/10.3389/fimmu.2019.01224
10. Didona D, Zenzo GD. Humoral epitope spreading in autoimmune bullous diseases. *Front Immunol*. 2018;9:779. https://doi.org/10.3389/fimmu.2018.00779