Case Report

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

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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.

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 trunc, 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).

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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.)
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 α6β4 integrin expressed on 293T cells and analyzed by flow cytometry.

The patient’s serum IgG preferentially bound to the extracellular domain of α6β4 integrin (Fig 3, A-1; red line), but not to α6β1 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). α6β4 and α6β1 integrin transfectants were highly expressed respective β and α integrins (Fig 3, A-2-4; red and blue lines). 293T cells endogenously expressed both α6 and β1 integrins, but not β4 integrin (Fig 3, A-2-4; gray lines). These data indicate that the patient’s serum specifically recognized α6β4 integrin, possibly the extracellular domain of β4 integrin.

On the basis of these clinical and laboratory findings, we diagnosed this patient’s condition as DPP-4 inhibitor–induced BP with anti-α6β4 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-α6β4 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 α6β4 integrin. In addition, we examined the serum anti-α6β4 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-α6β4 integrin extracellular domain antibodies, except for the patient in our study; this finding suggested that anti-α6β4 integrin extracellular domain antibodies are not common in patients with BP. It is possible that the autoantibodies are directed against the extracellular region of β4 integrin, or alternatively, that they recognize only the α6β4 conformation, and not the α6β1 or other integrin dimer structures (data not shown).

To our knowledge, there have been no reports of physiologic α6β4 integrin extracellular domain autoantibodies in mammalian cells.2-4 Autoantibodies that bind to the extracellular region of the α6β4 integrin may inhibit binding of the α6β4 integrin to laminin 532. Furthermore, in this case, the anti-α6β4 integrin extracellular domain antibody titer was maintained for 3 years, despite improvement in the
anti-BP180 NC16A antibody titer (Fig 3, B). Persistent anti-α6β4 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-α6β4 integrin antibodies by intermolecular epitope spreading under certain circumstances. Furthermore, anti-α6β4 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-α6β4 integrin antibodies. To our knowledge, this is the first study to clearly demonstrate the existence of serum autoantibodies against the extracellular region of α6β4 integrins in a patient with BP and also the first to identify α6β4 integrin autoantibodies in a patient with DPP-4 inhibitor–induced BP.

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Conflicts of interest
None disclosed.

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