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Case report

Title: Mucous membrane pemphigoid with generalized blisters: IgA and IgG autoantibodies target both laminin-332 and type XVII collagen

Running head: Mucous membrane pemphigoid with IgA/G reacting with laminin-332 and type XVII collagen

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What's already known about this topic?

- IgA and IgG class autoantibodies directed against type XVII collagen (COL17) or laminin-332 in patients with mucous membrane pemphigoid (MMP) have been well documented.
- MMP with IgA autoantibodies that react with both laminin-332 and COL17 has not been reported.

What does this study add?

- This is the first MMP case in which circulating IgA and IgG class autoantibodies against both laminin-332 and COL17 were detected.

Abstract

Mucous membrane pemphigoid (MMP) is a mucous membrane-dominated, subepidermal autoimmune blistering disease in which autoantibodies usually react with the C-terminal domain of type XVII collagen (COL17) or with laminin-332. Only a few cases of MMP with widespread blisters have been reported. Serologically, IgA and IgG class autoantibodies directed against COL17 or IgG autoantibodies directed against laminin-332 in patients with MMP have been well documented. MMP cases in which IgA reacts with laminin-332, however, are extremely rare. We report a case of MMP in a 67-year-old man. Clinical examination revealed extensive mucosal lesions as well as generalized blisters and erosions that healed with scar formation. He was intractable with systemic steroid treatment. Interestingly, in addition to IgG directed against laminin-332 and the noncollagenous 16A (NC16A) and C-terminal domains of COL17, circulating IgA reacted with laminin-332 and with the NC16A domain of COL17 were also detected. This is the first MMP case with circulating IgA and IgG autoantibodies against both laminin-332 and COL17.

Keywords: cicatrical pemphigoid, autoimmune blistering disease, scar

Introduction

Mucous membrane pemphigoid (MMP) is characterized by blistering and erosive lesions that occur mostly in the oral cavity and conjunctivae, leaving scarring [1]. C-terminal portions of type XVII collagen (COL17) and laminin-332 are known as major autoantigens of MMP [2,3]. IgA and IgG autoantibodies directed against COL17 or IgG autoantibodies directed against laminin-332 in MMP patients have been well described [4,5], and clarified using *in vivo* mouse models [6-9]. In contrast, MMP cases whose IgA autoantibodies react with laminin-332 are extremely rare [5].

We report a case of MMP with extensive mucosal lesions as well as generalized blisters and erosions resulting in scar formation. Interestingly, both IgA and IgG autoantibodies directed against COL17 and laminin-332 were detected.

Case report

A 67-year-old Japanese male had a three-week history of pruritic tense blisters on the hands and feet that gradually spread to entire body. On physical examination, numerous disseminated vesicles, erosions and excoriated papules were observed on the whole body (Fig. 1a, b). In addition, erosions and ulcers were found on the lower lip and the buccal and perianal area (Fig. 1d). The conjunctivae were normal. He also had a sore throat, and endoscopic examination revealed multiple erosions and ulcers on the pharyngeal, laryngeal and esophageal mucosae (Fig. 1c). A biopsy specimen taken from the edge of blister on the back showed subepidermal blister formation with eosinophilic, lymphocytic and neutrophilic infiltrates (Fig. 1h). Enzyme-linked immunosorbent assay (ELISA, MBL, Nagoya, Japan) was positive for IgG antibodies to the NC16A domain of COL17 (index value: 1074; cutoff: 9).

He was initially treated with intravenous prednisolone (1.5 mg/kg per day), followed by oral prednisolone (1 mg/kg per day) for more than a month; however, this failed to sufficiently improve the clinical condition. Since 100 mg of oral azathioprine and 50 mg of diaphenylsulfone daily were added, cutaneous and mucosal lesions started to improve slowly leaving post-inflammatory hyperpigmentation and scar formation (Fig. 1e, f, g).

Material and methods

Immunofluorescence analysis

Direct immunofluorescence (IF) for detecting deposits of IgG, IgA, IgM, C3 and C1q was performed on perilesional skin biopsy specimens from the patient. Indirect IF was performed on normal human skin and 1M NaCl-split normal human skin as described previously [10].

Immunoblot analysis

Epidermal and dermal extracts of normal human skin, supernatants of cultured HaCaT cells and recombinant proteins (NC16A and the C-terminal (BP915) domains of COL17) were prepared as described previously [11-14]. Purified laminin-332 was supplied as a gift from Dr. S. Amano of Shiseido Life Science Research Center, Yokohama, Japan [15,16]. Immunoblotting was performed as described previously [5]. For IgG detection, nitrocellulose membranes were incubated with 1 : 20 diluted serum overnight at 4℃. Bound antibodies were visualized enzymatically using 1 : 100 diluted HRP-conjugated rabbit antihuman IgG for 3 h at room temperature. For IgA detection, membranes were incubated with 1 : 20 diluted serum overnight at 37℃, then incubated in 1 : 50 diluted HRP-conjugated rabbit antihuman IgA for 3 h at room temperature. Colour was

developed with 4-chloro-1-naphthol in the presence of H_2O_2 [5].

Results

Immunofluorescence analysis

Direct IF microscopy showed linear deposition of IgG (Fig. 2a), IgA (Fig. 2b) and C3 (data not shown) at the basement membrane zone (BMZ). Indirect IF of normal human skin demonstrated circulating IgG (titre 1 : 320) and IgA (titre 1 : 32) reacting with the BMZ (data not shown). Indirect IF using 1M NaCl-split normal human skin revealed linear deposition of IgG (Fig. 2c) (titres of 1 : 320 and 1 : 80, epidermal and dermal sides, respectively) and IgA (Fig. 2d) (titres of 1 : 32 and 1 : 16, epidermal and dermal sides, respectively).

Immunoblot analysis

Immunoblot analysis using epidermal and dermal extracts from normal human skin, the recombinant NC16A and C-terminal domains of COL17, purified laminin-332 and a cell culture supernatant of HaCaT cells, from which the 120-kDa soluble ectodomain (LAD-1) of COL17 was isolated[17], were performed. We found circulating IgG autoantibodies against the NC16A domain (Fig. 3a), the C-terminal domain (Fig. 3b) and the 120-kDa soluble ectodomain (Fig. 3c) of COL17, and the γ2 subunit of laminin-332 (Fig. 3d).

Further immunoblotting revealed that IgA autoantibodies reacted with the γ2 subunit of laminin-332 (Fig. 3d) and faint reactivity with the NC16A domain and the 120-kDa soluble ectodomain of COL17 (data not shown). IgA autoantibody against the C-terminal domain of COL17 was negative.

Neither IgG nor IgA against BP230, type VII collagen or p200 protein were detected in epidermal and dermal extracts (data not shown).

Discussion

We describe an uncommon case of MMP with multiple mucosal involvement as well as generalized blisters, which predominantly healed with scar formation. Initially, the widespread bullae and circulating IgG against the NC16A domain of COL17 led us to diagnose bullous pemphigoid (BP). Detailed immunohistochemical examination, however, showed that both IgA and IgG reacted with laminin-332 as well as with COL17. From these results, the patient's disease could be diagnosed as MMP with generalized blisters, BP with extensive mucosal involvement or subepidermal autoimmune blistering disease with overlapping features of MMP and BP. In this case, because of multiple mucosal lesions and the unusual scar formation, we finally made the diagnosis of MMP with generalized blisters.

A variety of different autoantigens are recognized by circulating autoantibodies from patients with MMP and it is possible that the unusual autoimmune profile developed as a result of epitope spreading. In this case, in addition to IgG directed against laminin-332 and the noncollagenous 16A (NC16A) and C-terminal domains of COL17, circulating IgA reacted with laminin-332 and with the NC16A domain of COL17 were also detected. Previously, antibodies against laminin-332 are found in about 10% to 20%

of MMP patients [18]. Passive transfer studies in newborn and adult mice have shown that polyclonal antibodies to human laminin-332, generated from rabbits, bind epidermal basement membrane and produce subepidermal blisters of skin and mucous membranes like those seen in patients with MMP [6,19]. In contrast, approximately 40 to 70% of patients with MMP show autoantibodies to multiple sites on COL17 [19]. Rabbit antibodies, generated against the murine homologue of the NC16A domain of human COL17, induce subepidermal blisters when passively transferred into neonatal mice, and that resemble those seen in patients with BP and some forms of MMP [19,20]. The *in vivo* pathogenicity of autoantibodies to the C-terminal domain of COL17 has not yet been demonstrated [21].

In contrast to BP, which is the most common autoimmune blistering disorder induced by autoantibodies against the NC16A domain of COL17, cutaneous involvement of MMP is usually limited to small areas [22-24]. To date, there have been only a few cases of MMP with widespread blisters [23,24]. Interestingly, these cases had autoantibodies against the NC16A domain of COL17 [23,24]. Our patient also presented with widespread cutaneous blisters, and ELISA and immunoblot analysis revealed antibodies against the NC16A domain of COL17. These findings suggest that the presence of autoantibodies against

the NC16A domain of COL17 may be associated with the clinical involvement of wide spread blisters in MMP patients.

IgG is the main immunoglobulin subtype that has been confirmed as an autoantibody against laminin-332 and/or COL17 in sera from patients with MMP [4,5]. In addition, IgA autoantibodies against COL17 are another major immunoglobulin subtype found in sera from MMP patients, and the presence of both IgG and IgA anti-COL17 antibodies has been associated with more severe and persistent clinical features [21,25,26]. However, to the best of our knowledge, there are no reports of IgA autoantibodies against both laminin-332 and COL17 being detected in MMP sera [5]. In the previous study, a patient with IgA autoantibodies against laminin-332 had severe conjunctival involvement with multiple bullae on the extremities and was refractory to systemic steroid therapy [5]. Our patient was also intractable to steroid treatment and needed relatively high doses of oral prednisolone, azathioprine and diaphenylsulfone. The presence of IgA anti-laminin-332 might be associated with poor response to steroid treatment, although the correlation between clinical manifestations and the profile of autoantibody subtypes is difficult to determine due to limited number of patients.

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Figure legends

Fig. 1. Clinical manifestations and histopathological findings

Multiple erythema, bullae and erosions on the back (a) and forearm (b), and esophageal erosions (c) were observed at the initial visit. Refractory ulcers on the perianal area were also observed (d). After 3 months of treatment with systemic prednisolone, azathioprine and diaphenylsulfone, the skin lesions gradually healed with hyperpigmentation and scar formation (e, f). The esophageal erosions also slowly resolved (g).

A skin biopsy specimen obtained from the edge of blister on the back showed a subepidermal blister with inflammatory cell infiltrates, including eosinophils, lymphocytes and neutrophils (h). (Haematoxylin and eosin stain, original magnification x200)

Fig. 2. Immunofluorescence studies

Direct immunofluorescence of the cutaneous lesions revealed *in vivo* linear IgG (a) and IgA (b) deposition at the BMZ (arrowheads). Indirect immunofluorescence microscopy of 1M NaCl-split normal human skin showed that circulating IgG (c) and IgA (d) anti-BMZ antibodies reacted with both epidermal and dermal sides (arrowheads). (original magnification x200; indirect immunofluorescence, serum dilutions were 1 : 40 (c) and 1 :