We report a 68-year-old Japanese female patient with subepidermal blistering disease with autoantibodies to multiple laminins, who subsequently developed membranous glomerulonephropathy. At skin disease stage, immunofluorescence demonstrated IgG anti-basement membrane zone antibodies reactive with dermal side of NaCl-split skin. Immunoblotting of human dermal extract, purified laminin-332, hemidesmosome-rich fraction and laminin-521 trimer recombinant protein (RP) detected laminin γ-1 and α-3 and γ-2 subunits of laminin-332. Three years after skin lesions disappeared, nephrotic symptoms developed. Antibodies to α-3 chain of type IV collagen (COL4A3) were negative, thus excluding the diagnosis of Goodpasture syndrome. All anti-laminin antibodies disappeared. Additional IB and ELISA studies of RPs of various COL4 chains revealed reactivity with COL4A5, but not with COL4A6 or COL4A3. Although diagnosis of anti-laminin γ-1 (p200) pemphigoid or anti-laminin-332-type mucous membrane pemphigoid could not be made, this case was similar to previous cases with autoantibodies to COL4A5 and/or COL4A6.

CASE REPORT

A 68-year-old Japanese woman visited us in 2008, complaining of a 2-month history of bullous skin lesions with severe pruritus. The patient took aspirin 100 mg/day and candesartan cilexetil for hypertension for several years. Physical examination revealed scattered tense vesicles 5–8 mm in size with or without erythematous bases on the limbs, trunk and face (Fig. 1a). Neither psoriatic lesions nor mucosal lesions were found. Histopathology revealed subepidermal bulla with inflammatory infiltrates of neutrophils, eosinophils and lymphocytes (Fig. 1b). Direct immunofluorescence (IF) showed linear deposits of IgG and C3 along epidermal BMZ (Fig. 1c). Indirect IF of normal human skin demonstrated IgG anti-BMZ antibodies, which reacted exclusively with dermal side of 1M NaCl-split skin (Fig. 1d). The results of laboratory tests were within normal ranges, and ELISAs showed negative results for both BP180 and BP230.

Immunoblotting (IB) of normal human dermal extract and recombinant proteins (RPs) of BP180 NC16a and C-terminal domains showed negative results. In contrast, IB detected laminin α-3 (p200) in normal human dermal extract (Fig. 2a), and weakly laminin-α3 and laminin-γ2, but not laminin-β3, in purified human laminin-332 (Fig. 2b). Novel IB of hemidesmosome-rich fraction detected laminin-α3, but not laminin-β3, laminin-γ2 or laminin-γ1 (Fig. 2c). Novel IB of laminin-521 trimer RP detected only laminin-γ1 (Fig. 2d). Details for various IB tests and other methods are described in Appendix S1.

1http://www.medicaljournals.se/acta/content/?doi=10.2340/00015555-2059
Subepidermal blistering disease associated with membranous glomerulonephropathy

Fig. 1. Findings for skin lesions at bullous disease stage. (a) Clinical features on the face. (b) Histopathological features showing subepidermal bulla with inflammatory infiltrates of neutrophils, eosinophils and lymphocytes (H&E staining, ×400). (c) Result of direct immunofluorescence (IF) for C3 showing linear deposit to epidermal basement membrane zone (×400). (d) Results of indirect IF of 1M NaCl-split skin at bullous disease stage showing reactivity exclusively with dermal side (×200). (e) Results of indirect IF of 1M NaCl-split skin at glomerulonephropathy stage showing faint reactivity with dermal side (×200).

Fig. 2. Immunoblotting (IB) results. (a) IB of dermal extract. Anti-LM-γ1 monoclonal antibody (mAb) (lane 1) and patient serum taken at bullous disease stage (Patient 1, lane 2) reacted with the 200 kDa LM-γ1, while patient serum taken at kidney disease stage (Patient 2, lane 3) and normal human serum (lane 4) did not show positive results. (b) IB of purified human LM-332 mucous membrane pemphigoid serum (MMP) (lane 1), but not normal human serum (lane 2), reacted with LM-α3, LM-β3 and LM-γ2 subunits. Patient serum at bullous disease stage (Patient 1, lane 3), but not at kidney disease stage (Patient 2, lane 4), reacted relatively weakly with LM-α3 and LM-γ2. (c) IB of hemidesmosome (HD)-rich fraction. Anti-BP230 mAb (lane 1), anti-BP180 mAb (lane 2), anti-LM-α3 mAb (lane 3), anti-LM-γ2 mAb (lane 4), anti-LM-β3 mAb (lane 5) and anti-LM-γ1 mAb (lane 9) reacted with corresponding antigens. Patient serum at bullous disease stage (Patient 1, lane 6), but not at kidney disease stage (Patient 2, lane 7), and normal human serum (lane 8), reacted relatively weakly with LM-α3. (d) IB of LM-521 trimer RP. Anti-LM-α5 mAb (lane 1), anti-LM-β2 mAb (lane 2), anti-LM-γ1 mAb (lane 3) reacted with corresponding antigens. Patient serum at bullous disease stage (Patient 1, lane 4), but not at kidney disease stage (Patient 2, lane 5) and 4 normal human sera (lanes 6–9), reacted relatively weakly with LM-γ1.
Systemic prednisolone 45 mg/day did not improve the skin lesions. Addition of either 50 mg of diaphenylsulphone or colchicine 0.5 mg/day was also ineffective. Subsequently, the patient underwent 2 cycles of 4 consecutive plasma exchanges, which suppressed significantly the skin lesions. Remission was maintained on prednisolone 15 mg/day. No scar formation was recognised.

Approximately 3 years after the bullous lesions appeared, symptoms of nephrotic syndrome, including oedema, proteinuria and hypoproteinaemia, developed. Renal biopsy showed sclerotic changes of glomeruli (Fig. 3a). Direct IF showed granular deposition of IgG, IgG1 and IgG4 in glomeruli (Fig. 3b). Electron microscopic study demonstrated electron-dense deposits along subepithelial side of glomerular BMZ (GBM) (Fig. 3c). Circulating anti-GBM antibodies examined by commercial ELISA using RP of α3 subunit of bovine type IV collagen (COL4A3) (NIPRO Co. Ltd., Tokyo, Japan) were negative (<10, cut-off <10 EU). A diagnosis of primary membranous glomerulonephropathy was made.

The serum taken at this stage was also subjected to all of our immunological studies. ELISAs showed negative results for both BP180 and BP230. Indirect IF showed weak reactivity with dermal side of 1M NaCl-split skin (Fig. 1e). All IB studies showed no positive results (Fig. 2a–d).

In addition, we also performed ELISA and IB studies of RPs of various chains of type IV collagen (COL4). These studies suggested that our patient sera reacted with bacterial RP, but not mammalian RP of α5 chain (COL4A5) in IB. In contrast, our patient sera did not react with either α3 chain (COL4A3) or α6 chain (COL4A6) (details are described in Appendix S1).

**DISCUSSION**

When this case first showed skin lesions, clinical, histopathological and immunological features were compatible with anti-LMγ1 (p200) pemphigoid (7). However, while anti-LM-γ1 pemphigoid usually responds well to systemic corticosteroids, this case did not respond to high dose prednisolone. In addition, no psoriatic skin lesions were observed.

Although the presence of autoantibodies to LM-α3 and LM-γ2 suggested the diagnosis of anti-LM-332-type mucous membrane pemphigoid (8, 9), this case showed no mucosal lesions throughout the course. However, antibodies to LM-γ2 may not cause mucosal lesions in some cases (10). Thus, the pathogenic role of anti-LM-332 antibodies in our case remains uncertain. Alternatively, anti-LM-332 antibodies might be produced secondary from anti-LM-γ1 antibodies via an epitope-spreading phenomenon (11). Consequently, we made a tentative diagnosis of subepidermal blistering disease with autoantibodies to multiple laminin subunits. However, our patient might also suffer from a new entity of autoimmune bullous disease. The patient will be followed-up carefully, because autoantibodies to LM-332 may be associated with malignant tumours (12).

Intriguingly, our patient subsequently developed primary membranous glomerulonephropathy and the renal

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**Fig. 3.** Findings from kidney biopsy at glomerulonephropathy stage. (a) Histopathological features, showing sclerotic changes of glomeruli in histopathology (H&E staining, ×200). (b) Result of IgG direct immunofluorescence showing granular deposition (×200). (c) Electron microscopic feature. Arrows indicate electron-dense deposits. Calibration bar = 2 μm.
and skin biopsies showed similar direct IF findings. Since there are several common molecules in the epidermal and glomerular BMZs, including type IV collagen and LM-521 (13), our findings in this case may suggest common causes of bullous disease and membranous glomerulonephropathy.

The time lag from the remission of bullous disease to the onset of glomerulonephropathy was estimated to more than one year. Circulating autoantibodies to various LM subunits were detected by IB of various antigen sources only at skin disease stage, but not at kidney disease stage. Furthermore, no anti-LM-γ1 pemphigoid case associated with membranous glomerulonephropathy has been reported, indicating that the autoantibodies to LM-γ1 did not react with renal BMZ. In addition, a previous study showed that the major LM in kidney BMZ is LM-521, whereas LM-332 is not expressed in kidney tissue at all (13). These issues suggest that antibodies to LMs did not directly cause the kidney diseases.

Anti-GBM nephritis, including Goodpasture syndrome, shows anti-GBM autoantibodies reactive with COL4A3, which is present in glomerular and pulmonary BMZs, but not epidermal BMZ (5, 6). Nonetheless, a case of Goodpasture syndrome showed reactivity with epidermal BMZ (14). In a case of concurrent anti-GBM nephritis reactive with COL4 and bullous pemphigoid reactive with BP180, the kidney and skin lesions were caused by different non-cross reactive antibodies (4). In addition, Ghohestani et al. (5, 6) reported cases with concurrent cutaneous and renal diseases, which showed autoantibodies to COL4A5 and/or COL4A6.

These previous studies prompted us to speculate that our patient had anti-COL4 autoantibodies. Firstly, a diagnosis of anti-GBM nephritis or Goodpasture syndrome was excluded by the pathological findings and absence of anti-GBM (COL4A3) antibodies in both commercial and home-made ELISAs.

Our additional studies using various COL4 RPs showed that this patient reacted with COL4A5, but not COL4A6. The anti-COL4A5 autoantibodies might play a role in development of kidney disease in our patient, as shown in the previous reports (4, 6).

Lastly, a recent study (15) showed that a majority of membranous glomerulonephropathy cases showed antibodies to phospholipase A2 receptor, which should be examined for our patient sera in the future.

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The authors declare no conflicts of interest.

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