Linear IgA bullous dermatosis (LABD) is a distinct disease entity with specific clinical, histopathological, and immunological features. Patients with LABD show circulating IgA antibodies to epidermal basement membrane zone (BMZ), which have been reported to react with heterogeneous antigens, including 290-kDa type VII collagen, 285-kDa protein, BP230 and BP180, 145-kDa protein, LAD-1, and LABD97. Laminin-332, which was also called epiligrin or laminin-5, is an epidermal BMZ-specific laminin trimer of α3, β3, and γ2 subunits and is known to be an autoantigen for a subset of mucous membrane pemphigoid (MMP), anti-laminin-332-type MMP. Although IgA antibodies to laminin-332 are rarely detected in LABD serum samples, serum samples from some patients with LABD show both IgA and IgG autoantibodies. Zone et al first proposed the term linear IgA/IgG bullous dermatosis (LAGBD) for these cases. Linear IgA/IgG bullous dermatosis targets multiple autoantigens, and IgA antibodies in LABD react mainly with various autoantigens for LABD. This heterogeneity in autoantigens may contribute to the variable clinical features. However, because only a few studies have been performed to assess immunoreactivity in LAGBD, the pathogenic mechanisms for induction of mucocutaneous lesions in LAGBD have not yet been elucidated.

In the present study, we describe 3 Japanese cases of LAGBD, which showed prominent IgG and IgA reactivity with laminin-332, in addition to IgG and IgA reactivity with type VII collagen, laminin-γ1, and BP230 and BP180 recombinant proteins.
purified human laminin-332, and concentrated HaCaT cell culture supernatants containing soluble ectodomain of BP180 (LAD-1) were performed as described previously.10-12 Serum samples were diluted at 1:20 and 1:10 to detect IgG and IgA antibodies, respectively.

**Case 1**
An 81-year-old women developed bullous skin lesions 3 weeks before presentation. Physical examination revealed several tense bullae and erosive lesions on the trunk, buttocks, hands, and feet (Figure 1A), as well as erosions on the soft palate, tongue (Figure 1B), and genitalia. The patient had pancreatic cancer for 2 years, which was surgically resected but metastasized to the liver. Radiofrequency ablation and microwave coagulation therapy started 1 month before the onset of skin lesions, but liver metastases still remained. The patient also had a 30-year-history of diabetes mellitus, which was managed with insulin injections. Findings from a laboratory investigation showed slight anemia and increased levels of C-reactive protein (51.1 mg/L [to convert to nanomoles per liter, multiply by 0.0167]).

Findings from histopathological examination of a skin biopsy specimen taken from the right buttock showed a subepidermal blister with mild neutrophilic and eosinophilic infiltration in the blister (Figure 1C). Direct immunofluorescence (IF) of the biopsy revealed linear BMZ deposits of IgG, IgA (Figure 1D), and C3. Indirect IF of normal human skin sections revealed circulating IgG and IgA anti-BMZ antibodies, which reacted with both epidermal and dermal sides of 1M sodium chloride–split normal human skin sections (Figure 1E). Direct IF showed linear BMZ deposits of IgG (Figure 1F), IgA (Figure 1G), and C3. Indirect IF of normal human epidermal extracts revealed that IgG and IgA antibodies did not react with any antigens, including 230-kDa BP230, 210-kDa envoplakin, 190-kDa periplakin, 180-kDa BP180, 160-kDa desmoglein 1 (Dsg1), and 130-kDa Dsg3 (data not shown). Immunoblot analysis of recombinant protein of the BP180 NC16a domain showed no positive reaction for either IgG or IgA antibodies (data not shown), while IgA, but not IgG, antibodies reacted with recombinant protein of the BP180 C-terminal domain (Figure 1I).

We diagnosed this case as LAGBD with IgG and IgA antibodies to all subunits of laminin-332, as well as IgG antibodies to BP230 and IgA antibodies to laminin-γ1 and BP180 C-terminus. Treatment with oral prednisolone, 30 mg/d (0.6 mg/kg), quickly suppressed the formation of new bullae, although some erosive lesions were refractory. The prednisolone dose was subsequently tapered to 10 mg/d.

**Case 2**
An 88-year-old man with a history of diabetes mellitus developed bullous skin lesions 2 weeks before presentation. Physical examination revealed bullous and erosive lesions on the axillae (Figure 2A), buttocks (Figure 2B), hands, and feet, as well as erosions on the lip. Histopathological examination of a skin biopsy from the buttock revealed subepidermal blister with inflammatory infiltrate of neutrophils and lymphocytes in the blisters and the dermis (Figure 2C).

Direct IF showed linear BMZ deposits of IgG (Figure 2D), IgA (Figure 2E), and C3. Although indirect IF of normal human skin sections revealed neither IgG nor IgA anti-BMZ antibodies, indirect IF of 1M sodium chloride–split skin sections showed the reactivity of IgA, but not IgG, with both epider-
mal and dermal sides. The results of IgG ELISAs for Dsg1, Dsg3, BP180, and BP230 and IgA ELISAs for BP180 and BP230 were all negative.

Immunoblot analysis of purified human laminin-332 revealed IgG and IgA reactivity with the 165-kDa and 145-kDa forms of laminin-α3, 140-kDa laminin-β3, and 105-kDa laminin-γ2 (lane 1), while IgG antibodies in the normal control serum sample showed no reactivity (lane 2). G, In normal human dermal extracts, IgG antibodies in the control epidermolysis bullosa acquisita (EBA) serum sample reacted with 290-kDa type VII collagen (lane 1), and IgG antibodies in the control antilaminin-γ1 pemphigoid (p200) serum sample reacted with 200-kDa laminin-γ1 (lane 2). H, Immunoblot analysis using SuperSignal West Dura Chemiluminescent Substrate showed similar results. I, IgG antibodies in the control anti-BP180-type MMP serum sample (lane 1), but not in the normal control serum sample (lane 2), reacted with recombinant protein (RP) of the C-terminal domain.

Case 3
A 64-year-old man with a history of diabetes mellitus showed itchy skin lesions with a few blisters on the trunk for 2 years, which rapidly spread on the whole body. Physical examination revealed small bullae and erosions with edematous erythemas scattered or in a herpetiform arrangement on the trunk (Figure 3A) and extremities (Figure 3B), as well as erosion on the tongue.

Findings from histopathological examination of a skin biopsy specimen from the thigh showed a subepidermal blister...
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with eosinophilic and neutrophilic infiltration in the blister and the upper dermis (Figure 3C). Direct IF showed linear BMZ deposits of IgG, IgA, and C3. Indirect IF of 1M sodium chloride–split skin sections revealed IgG and IgA reactivity with both epidermal and dermal sides of the split (Figure 3D and E), whereas indirect IF of normal human skin sections revealed no reactivity. The results of IgG ELISAs for Dsg1, Dsg3, BP180, and BP230 and IgA ELISAs for BP230 and BP180 were all negative.

Immunoblot analysis of purified human laminin-332 showed IgG reactivity with the 105-kDa laminin-γ2 and IgA reactivity with the 165-kDa and 145-kDa laminin-α3 subunits (Figure 2F). IgG, but not IgA, antibodies reacted weakly with recombinant protein of the BP180 NC16a domain (Figure 3G). We diagnosed this case as LAGBD with IgG antibodies to laminin-γ2 and BP180 NC16a domain and IgA antibodies to laminin-α3. Combination therapy using dapsone, 75 mg/d, and minocycline hydrochloride, 200 mg/d, suppressed only partially the blister formation. Development of blisters continued, even after oral prednisolone, 10 mg/d, was added. However, an increase of prednisolone dose to 30 mg could suppress completely the blister formation. Then, the doses of dapsone and prednisolone were tapered to 50 mg/d and 5 mg/d, respectively, without blister formation. Because blisters reappeared when the minocycline hydrochloride dose was reduced to 100 mg/d, the dose was increased to 200 mg/d, which led to complete disappearance of the skin lesions.

Discussion

The most important finding in this study was that our antigen detection system using various immunoblot and ELISA analyses revealed the prominent IgA and IgG reactivity with laminin-332 in all 3 cases. Therefore, the present study was considered to be the first report for multiple cases with IgA antibodies to laminin-332. Interestingly, in addition to the reactivity with laminin-332, all 3 cases showed IgA and IgG antibodies to multiple cutaneous antigens in various patterns.

Case 1 showed several mucous membrane lesions in addition to relatively intractable skin lesions. Case 1 also showed
IgG antibodies to BP230 and IgA antibodies to type VII collagen, laminin-γ1, and BP180 C-terminal domain, in addition to IgG and IgA antibodies to all laminin-332 subunits. This complex antibody profile might contribute to the refractory lesions. Clinical data revealed that all 3 cases involved relatively elderly individuals, and no sex specificity was present. Clinical features resembled those of bullous pemphigoid; thus, blisters and erosions with or without erythemas developed mainly on the trunk and extremities. Some cutaneous lesions in case 3 showed annular and herpetiform arrangements similar to LABD. Mild oral mucosal lesions were found in all cases. Findings from histopathological examination showed a subepidermal blister with neutrophilic infiltrate in all cases, with slight eosinophilic infiltration in 2 cases. These clinical and histopathological findings indicate that LAGBD resides in the spectrum between bullous pemphigoid and LABD.

All the patients showed multiple autoantibodies. We speculate that autoantibodies to various BMZ antigens were produced through an epitope-spreading mechanism, defined as a specific T- or B-lymphocyte response to self-antigen proteins that differ from and do not cross-react with original epitopes. Although the original epitopes in our cases are unknown, the first immune response may target laminin-332 because of the strong and constant reactivity with laminin-332. Cases 1 and 2 had pancreatic cancer and colorectal cancer, respectively, whereas case 3 showed no malignancy. Anti-laminin-332-type MMP is well known to be associated frequently with malignant tumors. Because all 3 cases had oral mucosal lesions and IgA and IgG autoantibodies to laminin-332, the diagnosis of anti-laminin-332 MMP had to be differentiated. However, we could not make a diagnosis of MMP because all cases did not show extensive gingival lesions or any...
ocular lesions, which are a hallmark of MMP. Therefore, the relationship between malignant tumors and antibodies to laminin-332 is currently unknown.

Case 1 had liver metastasis of pancreatic cancer, and LAGBD lesions developed after radiofrequency ablation and microwave coagulation therapy. Recently, a case of hepatocellular carcinoma, which also developed anti-laminin-332-type MMP after radiofrequency ablation and microwave coagulation therapy, was reported.19 From the similarity between these 2 cases, it is tempting to speculate that tissue damage due to the physiological therapy may expose laminin-332 or other antigens to the immune system. Finally, all 3 patients had diabetes mellitus, although it is still unknown whether this association is specific to LAGBD.

In conclusion, we reported 3 cases of LAGBD with IgA and IgG autoantibodies to multiple antigens, predominantly various subunits of laminin-332. The production of the multiple antibodies may be explained by an epitope-spreading phenomenon. Although the pathomechanisms for the production of multiple antigens are not clear, it may be speculated that the complicated immune responses were triggered by prominent IgG and IgA reactivity with laminin-332. In addition, to know the difference between LAGBD and LABD and to explore the pathogenesis in LABD in more detail, we need to study LABD cases with exclusive IgA antibodies to laminin-332. Finally, these LAGBD case studies should provide insight on the mechanisms of immunoglobulin class switching in human diseases, which have not been well investigated.

REFERENCES