Three Cases of Linear IgA/IgG Bullous Dermatosis Showing IgA and IgG Reactivity With Multiple Antigens, Particularly Laminin-332

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IMPORTANCE Linear IgA/IgG bullous dermatosis (LAGBD) is a relatively rare autoimmune bullous disease characterized by both IgA and IgG antibodies to epidermal basement membrane zone. The heterogeneity and pathogenesis of the LAGBD autoantigens have not been fully elucidated.

OBSERVATIONS We report 3 Japanese cases of LAGBD (ages 81, 88, and 64 years; 1 woman and 2 men). The patients showed bullous and erosive lesions on the trunk and extremities with minimal mucosal lesions. Histopathological analysis revealed a subepidermal blister with neutrophilic infiltrates with eosinophils in 2 cases. Direct and indirect immunofluorescence studies disclosed IgG and IgA autoantibodies to various subunits of laminin-332, in addition to IgG and IgA reactivity with type VII collagen, laminin-γ1, and BP230 and BP180 recombinant proteins.

CONCLUSIONS AND RELEVANCE Our studies revealed that the 3 LAGBD cases showed prominent IgG and IgA reactivity with laminin-332, which was only rarely reported. In addition, all cases showed IgG and IgA reactivity with other multiple antigens, indicating the role of epitope-spreading mechanisms initiated from laminin-332. The significance of IgA antibodies to laminin-332 should be studied in larger cohorts of both LAGBD and linear IgA bullous dermatosis.

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Report of Cases

All clinical and laboratory data for the 3 cases are summarized in the Table. For the 3 cases, immunoblot analyses using extracts from normal human epidermis and dermis, recombinant proteins of NC16a and the C-terminal domains of BP180,
purified human laminin-332, and concentrated HaCaT cell culture supernatants containing soluble ectodomain of BP180 (LAD-1) were performed as described previously.\textsuperscript{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 woman developed bullous skin lesions 3 weeks before presentation. Physical examination revealed several tense bullae and erosive lesions on the trunk, buttocks, hands, and feet, 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).

The results of IgG enzyme–linked immunosorbent assays (ELISAs) (Medical and Biological Laboratories)\textsuperscript{33} were weakly positive for BP230 (index, 17.4; normal, <9) but negative for BP180. The results of IgA ELISAs were negative for both BP230 and BP180.

Immunoblot analysis of purified human laminin-332 revealed weak IgG and strong IgA reactivity with all the 165-kDa and 145-kDa forms of laminin-α3 subunit, the 140-kDa β3 subunit, and the 105-kDa γ2 subunit of laminin-332 (Figure 1F). Immunoblot analysis of normal human dermal extracts revealed IgA reactivity with 290-kDa type VII collagen and 200-kDa laminin-γ1, whereas IgG antibodies showed no reaction (Figure 1G). IgA reactivity with laminin-γ1 was confirmed by immunoblot analysis, which used the same antigen sources but with signal detection by SuperSignal West Dura Chemiluminescent Substrate in place of color development (Figure 1H). Immunoblot analysis 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 reactivity 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-
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 (Figure 2F), 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 anti-laminin-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

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. 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 case 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-spread phenomenon. Although the pathomechanism for the production of multiple antibodies is 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