

## IgA Antibodies Recognizing LABD97 Are Predominantly IgA1 Subclass

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**Linear IgA bullous dermatosis is a rare acquired subepidermal blistering disease of the skin. A recognized antigen in linear IgA bullous dermatosis is a 97-kDa basement membrane zone protein termed LABD97. Previous studies, using immunofluorescent techniques, have suggested that the IgA response is restricted to the IgA1 subclass. We studied the IgA antibody subclasses in the sera of 6 patients that contained circulating IgA antibodies reactive with LABD97. The methods used included direct and indirect immunofluorescence and Western immunoblot. All patients tested had IgA1 anti-LABD97 antibodies detected by all 3 methods. Two patients had IgA2 antibodies detected by direct immunofluorescence. Three patients had IgA2 antibodies on indirect immunofluorescence. Two of these also had anti-LABD97 IgA2 antibodies and 1 had secretory component containing anti-LABD IgA antibodies on Western immunoblot. We conclude that the predominant IgA antibody subclass reactive with LABD97 in LABD is IgA1, although the IgA2 subclass may be involved in some cases. Key words: skin; basement membrane; blisters.**

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Linear IgA bullous dermatosis (LABD) is a rare acquired subepidermal blistering disease of the skin, which has been defined on the basis of its unique immunopathological finding of linear IgA along the cutaneous basement membrane (1). Immunoelectron microscopy in LABD reveals 3 patterns of IgA deposition: sub-lamina densa deposition; lamina lucida deposition; and deposition in both locations (2–5). We have previously described a 97-kDa antigen (LABD97) extracted from human epidermis, which binds IgA basement membrane zone (BMZ) antibodies from sera of patients with the lamina lucida type of LABD (6). We have shown that this LABD97 antigen is identical to a portion of the extracellular domain of the bullous pemphigoid antigen, BPAg2 (7). The IgA antibodies that bind LABD97 localize antigens in the lamina lucida between the NC16A and carboxyl terminal domains of BPAg2 by immunogold electron microscopy (8, 9). It has been reported by immunofluorescent techniques that the BMZ antibodies in LABD are the IgA1 subtype (10).

Human IgA is present in human serum at about one-fifth the concentration of IgG, but it is the most frequent immunoglobulin in secretions. It occurs in 2 isotypic forms, IgA1 and IgA2, which differ in structure by about 20 amino acids, predominantly located at the hinge region (11). In serum, IgA1 is the predominant subclass, while in secretions

the ratio is more evenly balanced. Secretory IgA differs from serum IgA in that 2 IgA molecules can be complexed with a glycoprotein termed secretory component in the former (12). Secretory component is produced by epithelial cells lining mucosal surfaces and confers stability and increased resistance to proteolysis for the IgA molecule (13).

We tested the sera of 6 patients with LABD known to have IgA circulating antibodies reactive with LABD97 by the specific technique of Western immunoblot in order to determine the antibody subtype reactive with this antigen. We tested the anti-IgA1 and anti-IgA2 antibodies by slot blot to ensure that they demonstrated no significant cross-reactivity. We also searched for the presence of secretory component in order to determine the presence of secretory IgA.

### MATERIAL AND METHODS

#### *Patients and sera*

Six patients (2 females, 4 males) with clinical LABD proven on histology and direct immunofluorescence (DIF) known to have circulating IgA antibodies to LABD97 were studied. The patients' ages were 3, 4, 33, 47, 61 and 71 years at the time that serum was obtained for study. No patient had mucosal disease. No serum had IgG or IgM detected on DIF or indirect immunofluorescence (IIF). Serum from 2 normal volunteers was used as controls.

#### *Immunofluorescence*

DIF studies were performed on perilesional skin biopsies. IIF studies were performed on 1M NaCl BMZ split normal human skin incubated with serum at varying dilutions, as described previously (6). Substrates were placed in embedding medium (Tissue-Tek, Miles, Laboratories, Naperville, ILL, USA), and 6- $\mu$ m sections were cut on a cryostat. To identify IgA, individual sections were incubated separately with fluorescein-labelled goat anti-human IgA alpha chain (Cappel Laboratories, Malvern, PA, USA). To identify IgA1, sections were incubated first with mouse anti-human IgA1 at a dilution of 1:200 (Chemicon International, Inc, Temecula, CA, USA) and then with fluorescein-labelled goat anti-mouse gamma chain at a dilution of 1:200 (Cappel Laboratories, Malvern, PA, USA). To identify IgA2 antibodies sections were incubated with mouse anti-human IgA2 at a dilution of 1:200 (Zymed Laboratories, San Francisco, CA, USA) and then with fluorescein-labelled goat anti-mouse gamma chain at a dilution of 1:200 (Cappel Laboratories, Malvern, PA, USA). To identify secretory component sections were incubated with goat anti-human secretory component at a dilution of 1:200 (ICN Biomedicals, Inc, Costa Mesa, CA, USA) and then with fluorescein-labelled swine anti-goat gamma chain at a dilution of 1:200 (E-Y Laboratories, San Mateo, CA, USA). Positive controls using human tonsil substrate and negative controls using no secondary antibody stained appropriately. Sections were examined by epifluorescent microscopy. For IIF the limiting titre of anti-basement membrane antibody was determined by epifluorescent microscopy.

#### Preparation of epidermal extract containing LABD97 for Western blots

Epidermis was separated from dermis by incubation in 20 mM EDTA at 4°C for 16 h (14). The separated epidermis was then washed 3 times with 25 mM Tris pH 6.8. Following the washes the separating solution and the wash solutions were pooled and concentrated. All solutions (separation, wash, and extraction) contained a mix of protease inhibitors: 0.2 mM phenylmethyl-sulphonyl fluoride, 1 mM EDTA, 0.1 mg/l leupeptin, and 0.7 mg/l pepstatin. This preparation contained the LABD97 antigen when analysed on Western blots, using LABD serum (diluted 1:10) and anti-human alpha-chain antibody.

#### Slot blot

A total of 400 ng of each immunoglobulin (IgG and secretory IgA, Cappel Laboratories, Malvern, PA, USA; IgA1 and IgA2, The Binding Site, Birmingham, UK) was applied to 0.2 µm nitrocellulose paper under a low vacuum using a Hoefer PR 600 slot blot system (Hoefer Scientific Instruments, San Francisco, CA, USA). The strips containing each of the above immunoglobulins and phosphate buffered saline were then incubated with mouse anti-human IgA1 at a dilution of 1:200 (Chemicon International, Inc, Temecula, CA, USA) or mouse anti-human IgA2 at a dilution of 1:200 (Zymed Laboratories, San Francisco, CA, USA) and developed using biotinylated anti-mouse gamma-chain antibody at a dilution of 1:200 (Vector Laboratories, Burlingame, CA, USA) and an avidin-biotin-peroxidase complex.

#### Western immunoblot

Eighty micrograms of the epidermal proteins in sample buffer were applied to each well of a 6.5% polyacrylamide gel and sodium dodecyl sulphate-polyacrylamide gel electrophoresis was performed (15). Proteins were transferred electrophoretically onto nitrocellulose, and the nitrocellulose strips were incubated with patient sera (16). All sera were diluted 1:10 in phosphate buffered saline with 0.5% bovine serum albumin and 0.05% Tween-20 (ATPBS). Individual strips were then incubated with mouse anti-human IgA1 at a dilution of 1:200 (Chemicon International, Inc, Temecula, CA, USA) or mouse anti-human IgA2 at a dilution of 1:200 (Zymed Laboratories, San Francisco, CA, USA) or goat anti-human secretory component at a dilution of 1:200 (ICN Biomedicals, Inc, Costa Mesa, CA, USA). Immunoblots were then developed using a biotinylated anti-mouse or anti-goat gamma chain antibody at a dilution of 1:200 (Vector Laboratories, Burlingame, CA, USA) and an avidin-biotin-peroxidase complex. Reaction times for all lanes of IgA1, IgA2 and secretory component were identical.

## RESULTS

#### Slot blot

The mouse anti-human IgA1 monoclonal antibody reacted with human IgA1 and secretory IgA, but demonstrated only faint reactivity with human IgA2. It demonstrated no reactivity with human IgG or phosphate buffered saline. The mouse anti-human IgA2 monoclonal antibody reacted with human IgA2 and secretory IgA, but demonstrated no reactivity with IgG, IgA1 or PBS. This demonstrated that the 2 mouse monoclonal antibodies did not cross-react with common antigens to a significant degree.

#### Immunofluorescence

On DIF testing, all biopsies demonstrated BMZ anti-alpha chain reactivity. All of the 5 biopsies tested demonstrated

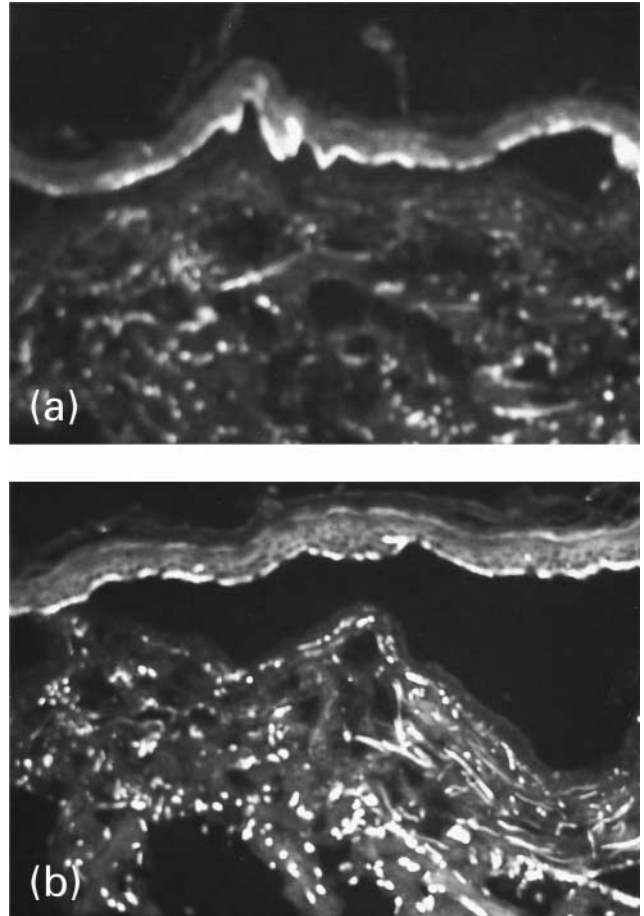


Fig. 1. Indirect immunofluorescence demonstrating IgA1 and IgA2 antibodies binding to the basement membrane zone. (a) IgA1 and (b) IgA2 antibodies binding to the epidermal side of the basement membrane zone split.

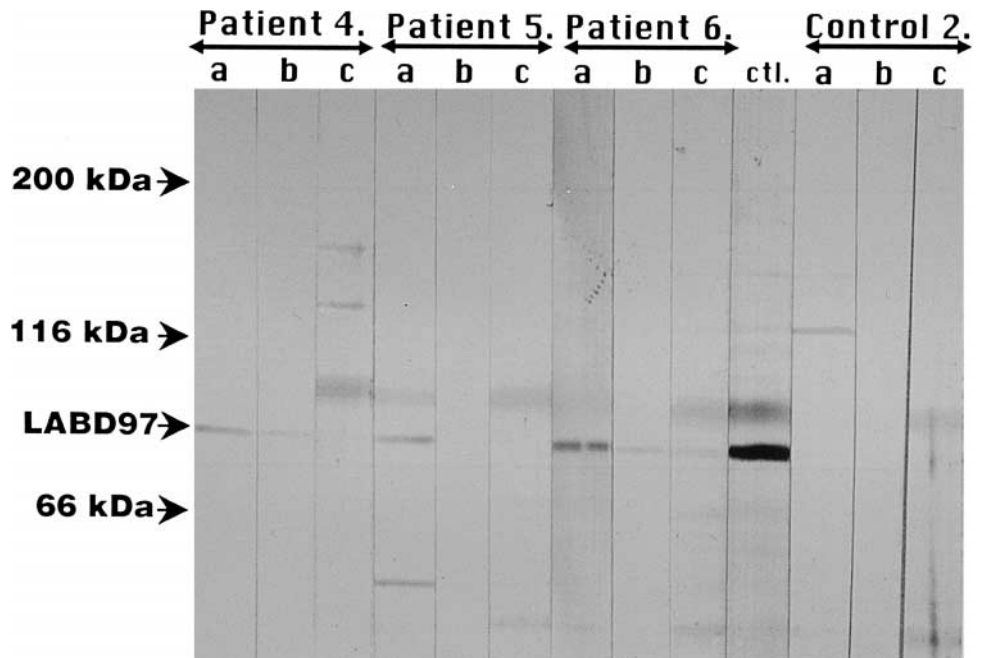
BMZ reactivity with anti-IgA1 antibodies, while 2 biopsies also had BMZ reactivity with anti-IgA2 antibodies. None of the 5 biopsies tested had reactivity with secretory component containing IgA antibodies.

On IIF testing, all of the sera had IgA circulating antibody titres of at least 1:80 on the epidermal side of BMZ split normal human skin. When tested at a dilution of 1:10 all sera demonstrated IgA1 antibody deposition, while 3 sera demonstrated IgA2 antibody deposition on the epidermal side of BMZ split normal human skin (Fig. 1). No serum had detectable secretory component containing IgA antibodies to LABD97. The positive control tonsil substrate demonstrated anti-alpha chain, IgA1 and IgA2 reactivity. The no secondary antibody controls were all negative.

#### Western immunoblot

On Western immunoblot, all 6 sera had IgA1 antibody binding to LABD97, while 2 of the sera had IgA2 antibody binding to LABD97. These sera also demonstrated IgA2 binding on IIF. One serum demonstrated weak secretory component containing IgA antibodies binding to LABD97 (Fig. 2).

Fig. 2. Western immunoblot containing LABD97 extract. Lanes a are developed for IgA1. Lanes b are developed for IgA2. Lanes c are developed for secretory component containing IgA antibodies. The ctl lane contains a positive LABD97 IgA control serum developed for IgA. Controls are normal human sera. Patients 4, 5, and 6 demonstrate IgA1 LABD97 specific antibodies. Patients 4 and 6 also contain IgA2 LABD97 specific antibodies. Patient 6 also demonstrates secretory component containing LABD97 specific antibodies.



## DISCUSSION

Our findings suggest that in the majority of patients with LABD, LABD97 antigen specific antibodies are serum IgA1 subclass. However, in some patients, IgA2 antibodies also react with LABD97. The patients with IgA2 antibodies had no unique clinical characteristics, such as age of disease onset, disease duration, gastrointestinal symptoms, or malignancies. Secretory IgA appears to be less important in the humoral immune response to LABD97 than serum IgA. These findings support the previous work of Wojnarowska et al. (10), although they did not find IgA2 deposition in any of the 32 patients they examined by immunofluorescent techniques. IgA2 BMZ deposition has been found in a minority of LABD patients in other studies. Hall & Lawley (17), in a study of IgA subclasses in dermatitis herpetiformis patients found that in 11 patients with "DH-linear" now known to be LABD, IgA1 deposition was found in 10 patients and IgA2 deposition in 1 patient. Adachi et al. (18) found IgA2 deposition in a patient who had sub-lamina densa deposition of antibody by immunoelectron microscopy. Onodera et al. (19), described a patient with IgA1 and IgA2 BMZ antibodies on DIF with only the presence of circulating IgA1 antibodies.

Because IgA1 and IgA2 differ by only a small number of amino acids, it is important to ensure that there is no cross-reactivity in antigen specificity between the anti-IgA1 and anti-IgA2 antibodies. We have tested many different anti-IgA1 and anti-IgA2 antibody preparations before we were satisfied that there was no significant cross reactivity in antigenic specificity between them. In this study we demonstrated this clearly by slot immunoblot. Other investigators have not reported verification of the specificity of their monoclonal antibodies to IgA subclasses. Another problem that presented in the testing of many different IgA1 and IgA2 preparations was the non-specific binding of some antibody preparations to the BMZ of normal human skin. Because of this we felt it important to confirm LABD antigen specificity by doing Western immunoblot. The results of both of these

techniques strongly suggest that our finding of IgA2 antibodies is real.

In conclusion, our results confirm previous immunofluorescence findings that the IgA subclass deposited at the BMZ is IgA1. We further show that circulating IgA antibodies reactive with LABD97 are also predominantly IgA1. However, some patients also have IgA2 BMZ specific antibodies.

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