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# The presence of anti-basement membrane zone antibodies in the sera of patients with non-bullous lupus erythematosus

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**Summary** We performed indirect immunofluorescence (IF) studies using 1 mol/l sodium chloride split skin to determine whether or not a positive IF is specific to patients with bullous lupus erythematosus (LE). We examined the sera from 21 patients with systemic LE (SLE), three of which were obtained from two SLE patients and one subacute cutaneous LE (SCLE) patient with bullous eruptions. As a comparison, we also studied the sera from patients with discoid LE (DLE, $n = 7$), SCLE ($n = 1$), systemic sclerosis (SSc, $n = 20$), bullous pemphigoid ($n = 2$) and normal individuals ($n = 10$). Sera from 16 SLE, four DLE and two SSc revealed a linear deposition of IgG isotype antibody at the epidermal side and/or the dermal side on indirect IF of split skin. The sera from three patients with bullous eruption and from 12 patients of SLE, SCLE, DLE without bullous eruption or SSc were further analysed by immunoblotting using five defined antigens, i.e. dermal extract, epidermal extract, three fusion proteins of 230 kDa bullous pemphigoid antigen (BPAG), 180 kDa BPAG, and human epidermolysis bullosa acquisita (EBA) antigen. Two SLE sera as well as one of the SCLE and the DLE serum reacted with 230 kDa BPAG in epidermal extract, and one of the SCLE and the DLE serum also reacted with the fusion protein of 180 kDa BPAG. No serum reacted with the dermal extract or the fusion protein of 230 kDa BPAG or EBA antigen. There was no consistent correlation between split-skin IF results and immunoblotting results. These results may suggest that even non-bullous LE patients often have autoantibodies to the basement membrane zone antigens, most of which are less pathogenic. Although we rarely examine the sera from non-bullous LE patients, we should keep this phenomenon in mind to avoid overestimating the results of split-skin test and immunoblotting.

Bullous eruptions in patients with systemic lupus erythematosus (SLE) are caused by several different mechanisms. The bullous eruption characterized by neutrophil infiltration and linear IgG deposition at the dermoepidermal junction is termed bullous SLE (BSLE).[1] BSLE is classified into two types depending on the presence (type I) and the absence (type II) of antibody to type VII collagen.[2] The indirect immunofluorescence (IF) test by 1 mol/l sodium chloride split skin reveals IgG deposition at the dermal side in type I. In contrast, Yell *et al.*[3] have recently reported variable results of IF staining on split skin and immunoblotting for the detection of antibody to type VII collagen in patients with BSLE, suggesting heterogeneity of BSLE. They defined BSLE as an acquired subepidermal blistering disease in a patient with SLE, in which immune reactants are present at the basement membrane zone (BMZ) on either the direct or indirect IF test.

These observations prompted us to examine autoantibodies in the sera from LE patients, with or without a bullous eruption, as revealed by split-skin test. Furthermore, we performed immunoblotting using epidermal and dermal extracts and the fusion protein of the 230 kDa bullous pemphigoid antigen (BPAG), 180 kDa BPAG or human epidermolysis bullosa acquisita (EBA) antigen, in order to confirm whether the autoantibodies react with these defined antigens.

## Materials and methods

### *Patients and sera*

The present study included the sera from 21 patients with SLE, seven patients with discoid lupus erythematosus (DLE), one patient with subacute cutaneous lupus erythematosus (SCLE) and 20 patients with systemic

sclerosis (SSc), who satisfied the proposed criterion[4,5] or were diagnosed by the clinical and histological findings. We also included 10 sera from normal individuals. Three patients (patients 1, 7 and 9 in Table 1) had a history of bullous eruptions. In patient 1, skin biopsy revealed the accumulation of neutrophils in the papillary dermis along with linear deposition of IgG and C3 at the BMZ. Extensive liquefaction degeneration was observed in patients 7 and 9, which appeared to lead to blister formation. No other patients had histories of bullous eruptions. Sera obtained from these patients were kept at −20°C until use.

*Indirect IF studies on 1 mol/l sodium chloride split skin*

Normal human skin was split by 1 mol/l sodium chloride solution for 48 h at 4°C and then used as substrate for indirect IF studies.[6] Split skin was incubated with a sample serum diluted at 1:40 by phosphate-buffered saline.

*Western immunoblot analysis*

Immunoblot analysis was performed using both ethylenediaminetetra-acetate (EDTA)-separated normal human epidermal extract for detection of the 230 kDa BPAG (BPAG1) and the 180 kDa BPAG (BPAG 2),[7,8] and dermal extract for detection of the 290 kDa EBA antigen (type VII collagen).[9] We also used the fusion proteins of BP 230 kDa and 180 kDa BPAGs and EBA antigen as antigen sources.[10] These antigen sources were subjected to sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS–PAGE) and separated proteins were transferred electrophoretically to nitrocellulose sheets (Scheicher & Schuell, Dassel, Germany). Blots were first blocked with 3% skimmed milk in Tris–HCl buffered saline (TBS, pH 8·0), and incubated with sera. After a wash with TBS containing 0·05% Tween 20, blots were incubated with peroxidase conjugated anti-human IgG (Dako, Copenhagen, Denmark) at a dilution of 1 : 100. Finally, positive bands were visualized with 4-chloro-1-naphthol in the presence of hydrogen peroxide. We included reference sera of BP, pemphigus foliaceus and pemphigus vulgaris as a positive control.

## Results

No serum revealed positive staining when untreated human skin was used as a substrate for the indirect IF test. By contrast, 16 out of 21 SLE sera showed three types of IF staining patterns along split sites, i.e. dermal type (two of 21), epidermal type (10 of 21) and combined type (four of 21), revealed by split skin test (Fig. 1). Of seven DLE sera, two sera exhibited dermal type and two other sera showed either epidermal type or

**Table 1.** Summary of split-skin test and immunoblotting

| | | | Split skin test | | Immunoblotting | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Patient | Age/sex | Diagnosis | E§ | D‖ | Epidermal extract | FP of BP 230* | FP of BP 180† | Dermal extract | ‡FP of EBA–NC1 |
| 1 | 34/M¶ | SLE | + | + | − | − | − | − | − |
| 2 | 45/M | SLE | + | + | BP230+ | − | − | − | − |
| 3 | 41/F | SLE | + | + | − | − | − | − | − |
| 4 | 56/F | SLE | − | + | − | − | − | − | − |
| 5 | 30/F | SLE | − | + | − | − | − | − | − |
| 6 | 22/F | SLE | − | − | BP230+ | − | − | − | − |
| 7 | 36/F¶ | SLE | − | − | − | − | − | − | − |
| 8 | 41/F | SLE | − | − | − | − | − | − | − |
| 9 | 44/M¶ | SCLE | − | − | BP230+ | − | ± | − | − |
| 10 | 63/M | DLE | − | − | − | − | − | − | − |
| 11 | 54/F | DLE | − | − | − | − | − | − | − |
| 12 | 52/M | DLE | + | − | BP230+ | − | − | − | − |
| 13 | 39/F | DLE | − | − | − | − | + | − | − |
| 14 | 69/F | SSc | − | − | − | − | − | − | − |
| 15 | 31/F | SSc | − | − | − | − | − | − | − |

Fusion protein (FP) of *the 230 kDa BPAG, †the 180 kDa BPAG, or ‡human EBA (non-collagenous domain 1 of type VII collagen). §Epidermal side and ‖dermal side. ¶Patients with bullous eruption.

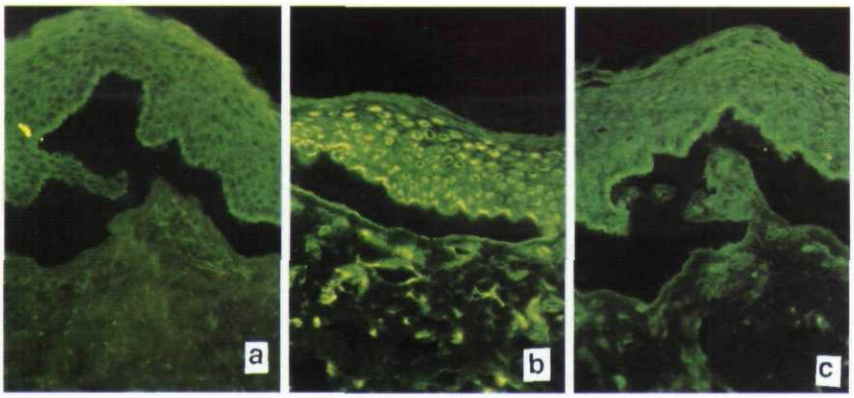


**Figure 1.** Indirect immunofluorescence patterns on 1 mol/l NaCl split skin (×20). (a) epidermal type (patient 12); (b) dermal type (patient 5); (c) combined type (patient 1). The interference with nuclear or cytoplasmic immunofluorescence usually coexists.

combined type. Neither SSc sera nor normal sera revealed positive staining. It is of note that the fluorescence of lupus sera was not so strong and sharp as that of BP sera. The interference with the nuclear or cytoplasmic staining of keratinocytes was frequently observed, which made it difficult to determine staining of the epidermal side.

Immunoblotting analyses revealed that two of the SLE sera and one each of the SCLE and the DLE serum reacted with the 230 kDa BPAG in epidermal extract (Fig. 2) and the SCLE and another DLE serum recognized the fusion protein of the 180 kDa BPAG (Fig. 3). No serum showed any specific reactivity in the dermal extract or the fusion proteins of 230 kDa BPAG or human EBA antigen (non-collagenous domain 1 of type VII collagen). Thus, there was no consistent correlation between the results of split skin IF and immunoblotting (Table 1).

## Discussion

Bullous eruptions in SLE are divided into three categories.[11] The first category is caused by the cleavage of the epidermis from the dermis due to extensive liquefaction degeneration. The second category includes primary blistering diseases such as dermatitis herpetiformis, BP, pemphigus vulgaris and pemphigus foliaceus, EBA, and linear IgA disease. The third category, proposed by Camisa and Sharma,[1] is characterized by a subepidermal blister with neutrophil-predominant inflammation in the papillary dermis without liquefaction degeneration and the linear deposition of immunoglobulin or complement at the BMZ. The presence of antibodies to type VII collagen was considered to be pathogenetic in BSLE.[9,12–14]


**Figure 2.** Immunoblotting using epidermal extract as an antigen. *Indicates the sera of patients with bullous eruption: #bullous pemphigoid; ##pemphigus foliaceus; ### pemphigus vulgaris. Arrowheads indicate the molecular size of 230 kDa (BPAG 1), 180 kDa (BPAG 2), 160 kDa (pemphigus foliaceus antigen; desmoglein 1) and 130 kDa (pemphigus vulgaris antigen; desmoglein 3) from the top to the bottom.

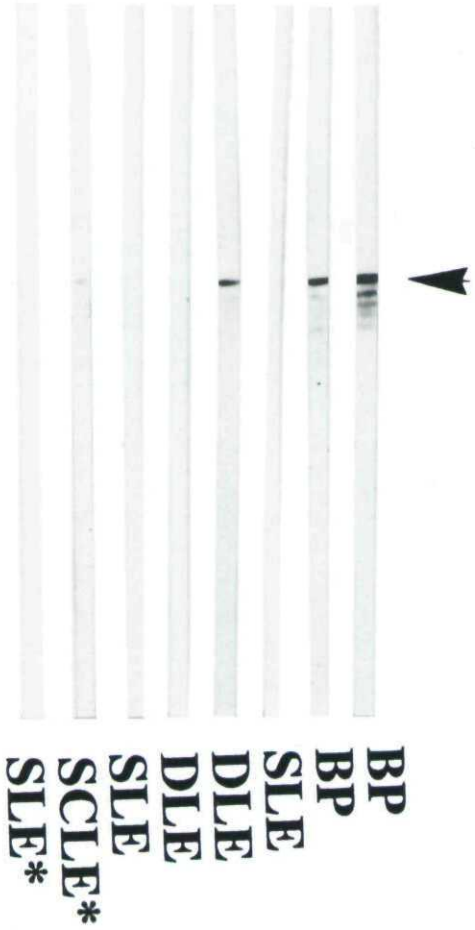


**Figure 3.** Immunoblotting using fusion protein of 180 kDa bullous pemphigoid antigen as an antigen. The arrowhead indicates the molecular size of 180 kDa (BPAG 2).

In the present study, three patients (patients 1, 7 and 9) had a history of bullous eruptions. To date, the only target antigen identified in a subset of BSLE has been type VII collagen. Patient 1, who showed typical histological and direct IF findings to BSLE, revealed a combined type of indirect IF staining and no positive band to any defined antigens on immunoblotting. This patient seems to resemble the case reported by Yell *et al.*[3] We believe that this peculiar type of BSLE may have the antibodies to the BMZ antigens which are prone to be denatured in the process of SDS–PAGE. The serum from patient 9 (SCLE) reacted both with the 230 kDa BPAG in epidermal extract and with the fusion protein of the 180 kDa BPAG. However, negative results of direct IF study and split skin test may exclude the diagnosis of BP. Although the extensive liquefaction degeneration appeared to be a primary event, the clinical significance of the antibodies remains to be elucidated.

Immunoblottings revealed that three sera (patients 2, 6 and 12) reacted with the 230 kDa BPAG of epidermal extract and one serum (patient 13) recognized the fusion protein of the 180 kDa BPAG. Since all these patients had no history of bullous eruption, these antibodies may not be aetiologically important. One possible explanation is that these antibodies reacted with 230 kDa proteins other than the 230 kDa BPAG. Another is that the antibodies are directed against the epitopes on the 230 kDa BPAG different from those recognized by BP sera.

It is of note that sera from lupus patients often showed positive IF stainings at the epidermal side (10 of 21) with less intensity than BP sera. However, two sera (patients 1 and 3) failed to react with any defined antigens on immunoblotting. Thus, some antibodies in the sera of lupus patients may be directed against native antigens that are usually masked in the normal skin and easily destroyed in the process of SDS–PAGE. Furthermore, the findings that no sera with positive IF staining at the dermal side reacted with dermal extract or the fusion protein of EBA antigen may also support this hypothesis. The production of these autoantibodies to the BMZ antigens may be the consequence of both polyclonal B-cell activation and liquefaction degeneration which can expose the masked BMZ antigens.

In conclusion we should not ignore the fact that even patients with non-bullous lupus often have autoantibodies to the BMZ which are considered to be of less clinical significance or not pathogenic.

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