Optimal Conditions of 1 M NaCl Splitting Technique to Demonstrate Basement Membrane Zone Antigens in Bullous Pemphigoid, Epidermolysis bullosa acquisita and Linear IgA Bullous Dermatoses

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Key Words

Bullous pemphigoid
Linear IgA bullous dermatoses
Epidermolysis bullosa acquisita
Basement membrane zone antibodies

Abstract

We compare a series of conditions for inducing lamina lucida separation through normal skin specimens using 1 M NaCl for the detection of basement membrane zone **auto-antibodies** using indirect immunofluorescence. Skin was split at room temperature and at 4 °C with and without phenylmethylsulphonyl fluoride. Our results demonstrate that for the detection of bullous pemphigoid and **epidermal-binding** linear IgA bullous dermatoses antibodies skin should be split at 4°C with enzyme inhibitors. For epidermolysis bullosa acquisita and **dermal-binding** antibodies skin can be split at room temperature.

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Introduction

Separation of the dermo-epidermal junction through the lamina lucida is an essential technique for diagnosis in sub-epidermal bullous diseases. Results obtained using different methods (induction of suction blisters, incubation in proteo-lytic enzymes and incubation in 1 M NaCl solution) are often conflicting. This may be due to partial degradation of basement membrane zone proteins during these procedures [1]. It has been demonstrated that incubation in 1 M NaCl is a simple cost-effective technique that reliably produces a split through the lamina lucida [1]. Therefore standardization of this technique would allow results from different centres to be compared.

We compare a series of conditions for inducing lamina lucida separation of normal skin specimens using 1 M NaCl for detection of basement membrane zone auto-antibodies using indirect immunofluorescence.

Method

Serum samples were obtained from 40 patients with bullous pemphigoid (BP), 7 patients with epidermolysis bullosa acquisita (EBA) and 20 patients (15 were epidermal-binding and 5 were dermal-binding) with linear IgA bullous dermatoses (LABD). The samples were stored at -20 °C until used. Skin specimens were obtained from surgical patients with no known or previous

history of auto-immune or skin disease. Multiple 4-mm punch biopsies were taken and were incubated in 1 M NaCl solution for: 48 h at room temperature, 48 h at room

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temperature with 1 mM phenylmethanesulphonyl fluoride (PMSF), 72 h at room temperature, 72 h at room temperature with 1 mM PMSF, 72 h at 4 °C with 1 mM PMSF. The solutions were changed every 24 h. The epidermis was then gently separated from the dermis with forceps. The split tissue was embedded in optimum cutting temperature compound, snap-frozen and stored in liquid nitrogen until used.

The level of cleavage was checked in all specimens by staining with type IV collagen and BP antibodies. Four-micrometre-thick cryostat sections were used for indirect immunofluorescence using FITC-labelled antihuman IgG or IgA. Serial dilutions of serum were tested. The principal end-point was the highest titration of fluorescence staining at the separated dermo-epidermal junction. The immunofluorescence staining was read independently by 3 observers.

50
40
30
20
10
W
≡

1/100 1/200
1/400 1/6 Titres
1/1,600 1/3,200

Results

Skin split under the conditions studied showed BP antibodies binding to the roof and type IV collagen binding to the base, hence confirming that the split was always through the lamina lucida.

The end-point titres of all BP sera studied were greatest on skin split at 72 h at 4 °C with PMSF compared with skin split at room temperature at different time intervals with or without PMSF (fig. 1). The end-point titres of all epidermal-binding LABD sera studied were again greater on skin split at 72 h at 4 °C with PMSF than on skin split at room temperature. However, there was no difference in the end-point titres of EBA sera on skin split under the different conditions studied (fig. 2). This was also true for the 5 dermal-binding LABD sera studied.

Discussion

■ 48 h at room temp.
a 48 h at room temp. + PMSF
ü 72 h at room temp.
0 72 h at room temp. + PMSF
◊ 72 h at 4 °C + PMSF

1/8 4-
1/100 1/200
1/1,600 1/3,200
1/400 1/800 Titres

Fig. 1. The difference in end-point titres of BP sera on skin split in 1 M NaCl under different conditions.

Fig. 2. No difference in end-point titres of EBA sera on skin split in 1 M NaCl under different conditions.

Our results show that BP and epidermal-binding LABD antibodies are probably best detected on skin incubated at 4 °C for 72 h in the presence of PMSF, a known proteolytic enzyme inhibitor. This implies that BP and epidermal-binding LABD antigens are susceptible to degradation during incubation in 1 M NaCl. The lack of change in end-point titres of EBA and dermal-binding LABD antibodies suggests that both antibodies are stable and are not susceptible to degradation at room temperature.

Conclusions

For detection of BP and epidermal-binding LABD antibodies we feel that it is advisable to use split skin that has been incubated in 1 M NaCl at 4 °C together with enzyme inhibitors. However, for detection of EBA and dermal-binding LABD antibodies skin can probably be split at room temperature in 1 M NaCl.

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