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Enzyme-linked immunosorbent assay using bacterial recombinant proteins of human BP230 as a diagnostic tool for bullous pemphigoid

Mariko Yoshida [a], Takahiro Hamada [a], Masayuki Amagai [b], Koji Hashimoto [c], Rie Uehara [d], Kentaro Yamaguchi [d], Koji Imamura [d], Eiji Okamoto [d], Shinichiro Yasumoto [a], Takashi Hashimoto [a,*]

[a] *Departments of Dermatology, Kurume University School of Medicine, 67 Asahi-machi, Kurume, Fukuoka 830-0011, Japan*
[b] *Keio University School of Medicine, Tokyo, Japan*
[c] *Ehime University School of Medicine, Ehime, Japan*
[d] *Medical and Biological Laboratories Co. Ltd, Nagoya, Japan*

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Summary

Background: By immunoblot analyses of normal human epidermal extracts, the 230 kDa bullous pemphigoid antigen (BP230) is recognized by most bullous pemphigoid (BP) sera. We produced different recombinant glutathione-*S*-transferase-fusion proteins, which roughly presented N-terminal domain, central rod domain and C-terminal domain of human BP230.
Objective: In the present study, we developed an enzyme-linked immunosorbent assay (ELISA) using the recombinant proteins for detection of anti-BP230 IgG antibodies and assessed the usefulness of this assay in conjunction with an anti-BP180 ELISA to establish the diagnosis of BP.
Methods: Using the bacterial recombinant proteins of N-terminal and C-terminal domains, we developed an ELISA. A receiver-operating-characteristic (ROC) analysis was performed to determine a cut-off value for the BP230 ELISA.
Results: By this BP230 ELISA, 173 (72.4%) of 239 BP sera were positive, while only one (1.1%) of 94 sera from pemphigus vulgaris and pemphigus foliaceus patients was positive and all the 109 normal control sera were negative. Thus, the sensitivity and specificity of the BP230 ELISA were 72.4 and 99.5%, respectively. Interestingly, while 54 (84.4%) of 64 BP sera in active stage and 113 (64.6%) of 175 BP sera in remission

* Corresponding author. Tel.: +81 942 31 7571; fax: +81 942 34 2620.
E-mail address: hashimot@med.kurume-u.ac.jp (T. Hashimoto).

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were positive in BP180 ELISA, 37 (57.8%) of 64 BP sera in active stage and 136 (77.7%) of 175 BP sera in remission were positive in BP230 ELISA. These results indicate that the titer of anti-BP230 antibodies is not related with disease activity in some BP cases. Most significantly, by combining the results of BP230 ELISA and BP180 ELISA, 232 (97.1%) of 239 BP sera were positive.

Conclusion: The combination of BP230 ELISA and BP180 ELISA is the highly sensitive method for the diagnosis of BP.

1. Introduction

Bullous pemphigoid (BP) is an autoimmune subepidermal blistering disease characterized by the presence of IgG autoantibodies to the epidermal basement membrane zone in the sera. The circulating IgG antibodies in BP sera react with two major BP antigens present in hemidesmosomes [1—3], a 230 kDa intracellular protein termed BP230 (or BPAG1) that is localized to the inner plaque of hemidesmosomes [4—9], and a 180 kDa transmembrane protein termed BP180 (or BPAG2) that shows type II orientation with a long C-terminal extracellular component composed of 15 discontinuous collagenous domains separated by 16 non-collagenous domains [10—15].

BP230 is a member of plakin family protein [16,17], and is considered to play an important role in the stable structure of hemidesmosomes. N-terminal and C-terminal domains of BP230 are considered to be important in interactions with desmosomal transmembrane proteins and with keratin intermediate filaments, respectively, whereas the central alpha-helical coiled-coil domain apparently plays a role in its structural stability and the self-assembly [16].

It is now well known that circulating antibodies in BP sera react with epitopes that are tightly clustered in the 16th non-collagenous (NC16a) domain of BP180 extracellular domain, close to the cell membrane of the basal keratinocytes [12,13,18—21]. These antibodies were later shown to be pathogenic by a newborn mouse animal model [22,23].

In the previous studies, we developed an enzyme-linked immunosorbent assay (ELISA) using bacterial recombinant protein of BP180 NC16a domain, and evaluated its clinical benefit for diagnosis and monitoring disease activity [24,25]. The sensitivity of the ELISA was determined to be 84.4% and the specificity was 98.8%. The index values of the BP180 ELISA tended to fluctuate in parallel with the disease activity along the time course of BP patients, and reflected the disease activity much better than indirect immunofluorescence.

However, although BP230 was found as a BP antigen earlier, the role of anti-BP230 antibodies in the pathogenesis of BP is still unclear. This is mainly because BP230 is an intracytoplasmic protein and the autoantibodies are not considered to access to BP230 in the intact keratinocytes.

The earlier studies using various recombinant proteins and synthetic peptides suggested that BP sera react preferentially with C-terminal domains of BP230 [26—30]. A recent study using eukaryotic expression system showed that multiple epitopes in various domains of BP230 are recognized by BP sera [31]. However, the results of these previous studies are not convincing enough, because none of these studies examined the reactivity of BP sera with the entire molecule of BP230. In addition, the eukaryotic expression system can produce larger recombinant proteins, but can yield only a little amount of the proteins. Therefore, the background reactivity in immunoblotting using the lysates of transfected eukaryotic cells was usually very high, which made the results a little obscure.

In the previous study, as the first step to further characterize the role of anti-BP230 antibodies in the pathogenesis of BP, we prepared three bacterial recombinant fusion proteins that cover the entire molecule of BP230 [32]. These recombinant proteins roughly corresponded to N-terminal domain (BP230-N), middle rod domain (BP230-M) and C-terminal domain (BP230-C). We examined the reactivity of BP sera with the three recombinant proteins by immunoblotting, and found that BP sera reacted specifically with the BP230 recombinant proteins, particularly the C-terminal domain.

In the present study, using the recombinant proteins, we developed an ELISA for BP230, and showed that the ELISA is a very useful tool in diagnosis of BP.

2. Materials and methods

2.1. Sera

We collected 239 BP sera; 64 sera from 64 BP patients in active stage and 175 sera from 41 BP patients in remission. We also used the sera from 37 patients with pemphigus foliaceus, 57 sera from patients with pemphigus vulgaris and 109 normal