

Levels of anti-BP180 NC16A IgE do not correlate with severity of disease in the early stages of bullous pemphigoid

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Abstract Bullous pemphigoid (BP), a common autoimmune skin disease, is associated with IgG autoantibodies against the hemidesmosomal proteins, BP180 and BP230. In addition to IgG, IgE has been shown to play a role in the disease. However, the association between disease activity and IgE specific to the NC16A domain of BP180 in blister fluid remains unclear. Our objective was to evaluate the correlation between BP disease activity and BP180 NC16A-specific IgE sera and blister fluid titers, and to analyze changes during treatment. We evaluated the levels of anti-BP180 IgE autoantibodies in the sera and blister fluids of 37 BP patients using an Enzyme-linked immunosorbent assay. We also observed changes in the levels of these antibodies in 2 BP patients at 4 or 5 different time points (day 0 when the patient first visited our hospital, day 5, day 14, day 39 and day 62 for patient 1; day 0, day 4, day 8 and day 17 for patient 2). We also collected extra serum samples from the 2 patients when the disease was controlled (blister disappeared) (day 85, day 104 and day 146 for patient 1 and day 123, day 158 and day 189 for

the other patient). IgE anti-BP180 antibodies were detected in the serum of 72.97 % of the patients. There was no correlation between disease activity scores and BP180 NC16A IgE titers in serum ($r = -0.077$, $p > 0.05$) or in blister fluid ($r = 0.262$, $p > 0.05$). The levels of the autoantibody in serum were positively correlated with that in blister fluid ($r = 0.6651$, $p < 0.001$); however, the levels continued to rise despite effective control of the disease in the initial two to 6 weeks of diagnosis. The level of this autoantibody reached a peak on day 39 for patient 1 and on day 17 for patient 2 although the systemic and topical medication of steroid had controlled the disease process effectively. We conclude that levels of anti-BP180 NC16A IgE are higher in the sera than blister fluids. These levels could generally reflect disease severity throughout the course of the disease, but not in the early stages of medication.

Keywords Bullous pemphigoid · IgE · Anti-BP180NC16A · Sera · Blister fluid

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Introduction

Bullous pemphigoid (BP), an autoimmune disease of the skin with tense blisters on normal or erythematous skin and even mucous membranes, is predominantly a condition that affects elderly people. The adjusted incidence of BP was 2.4 per 100,000 person-years [1]. BP arises from the development of antibodies that target hemidesmosomal proteins, including BP180 (BPAg 2, 180 kDa) and BP 230 (BPAg 1, 230 kDa), in the basement membrane zone (BMZ). BP180, also known as type XVII collagen, is a transmembrane collagenous protein [7]. The extracellular portion of the 16th non-collagenous (NC16A) domain of

BP180 (BP180 NC16A) has been demonstrated to harbor the immuno-dominant epitopes [8, 13].

Recently, a humanized monoclonal antibody against human IgE autoantibodies has shown to improve the symptoms of BP patients [12]. Many studies have shown that anti-BP180 NC16A IgE antibodies are involved in the pathogenesis of BP [5, 14]. These antibodies can be detected in the majority of patients with BP by enzyme-linked immunosorbent assay (ELISA), and their levels in the circulation have been found to significantly correlate with disease activity [5, 10]. Levels of IgG and IgE autoantibodies decreased after effective medicine [11].

Blister fluid aspiration is a less traumatic procedure than venepuncture, and is a routine procedure for BP patient to relieve the discomfort due to tense bullae. Many studies have shown that anti-BP180NC16A IgG antibodies can be detected in the blister fluid of BP patient and there is no significant difference between the serum and blister fluid using ELISA and indirect immunofluorescence (IIF) techniques. The blister fluid could be used as an alternative to serum for IIF in subepidermal immunobullous diseases, especially in children and those with poor venous access [3].

Anti-BP180 NC16A IgE antibodies can reproduce the early phase of lesion and microscopic separation at the dermal–epidermal junction associated with neutrophilic and eosinophilic infiltration in human skin grafted onto nude mice [5].

However, the correlation between the level of specific anti-BP180 NC16A IgE in the blister fluid of BP patients and disease severity remains unclear, and details of changes in NC16A-specific IgE antibodies in both sera and blister fluids before and after treatment are rare.

In this study, we quantified the levels of IgE antibodies specific to BP180 NC16A in the serum and blister fluid of untreated BP patients ($n = 2$) and patients treated with corticosteroids ($n = 35$) topically and monitored changes over time.

Materials and methods

Samples

Serum and blister fluid samples were obtained from 37 patients with BP (16 women and 21 men; mean age 69.08 ± 13.94 years; range 29–93), and 18 patients with pemphigus vulgaris and 1 with Stevens–Johnson syndrome (7 women and 12 men; mean age 54.05 ± 12.71 ; range 33–86). All patients gave written consent to participate in this study. All BP patients fulfilled four diagnostic criteria:

(1) characteristic clinical findings: erythema, urticarial plaques and/or tense blisters of the skin associated with pruritus; (2) histopathology: subepidermal blister formation with infiltrating eosinophils and neutrophils; (3) direct immunofluorescence (DIF): linear IgG and/or C3 deposits along dermo-epidermal junction; and (4) IIF on saline split human skin (SSS): IgG serum reactivity with the epidermal side [13]. Pemphigus vulgaris and Stevens–Johnson syndrome patients were also met the clinical, immunologic and histologic features. All samples were collected between July 2011 and October 2012 at the Department of Dermatology in Peking Union Medical College Hospital, Beijing Hospital, Air Force General Hospital, Cangzhou People's Hospital in Hebei Province, and Beijing Hospital of Traditional Chinese Medicine. The anti-BP180 NC16A IgE autoantibody titers were evaluated for all samples by ELISA to investigate the relationship between antibody titer and clinical disease activity. We collected serial both serum and blister fluid samples from the 2 untreated patients at different time points (day 0 when the patient first visited our hospital, day 5, day 14, day 39 and day 62 for patient 1; day 0, day 4, day 8 and day 17 for patient 2). We also collected extra serum samples from the 2 untreated patients when the disease was controlled (blister disappeared) (day 85, day 104 and day 146 for the first patient, day 123, day 158 and day 189 for the other patient). The sera of 28 healthy people (13 males and 15 females; mean age 67.32 ± 11.29 years; range 60–82) served as normal controls.

Clinical assessment

Disease activity scores were rated from 1 to 5 following a protocol described in a previous study [11]: if patients suffered from mucosa involvement, an additional one point was added (Table 1).

BP180 NC16A-specific IgE ELISA

ELISA was performed using recombinant BP180 NC16A protein-coated plates in the anti-BP180 IgG ELISA kit

Table 1 Assessment criteria for disease activity scores

Disease activity scores	Assessment criteria
4	Above 50 % area of eruptions or more than 100 new blisters
3	0–50 % area of eruptions or new blisters forming
2	No blisters but erythema
1	Normal skin or only pigmentation
1	Mucous membrane involvement

(MBL International, Japan). Samples were added into wells (50 μ l; 1:10 dilution) and incubated for 2 h at 30 °C. Horseradish peroxidase (HRP)-conjugated mouse anti-human IgE (Abcam, Britain) was diluted to 1:1000 and added to each well for 1 h at 30 °C. To develop the ELISA, 100 μ l of Tetramethylbenzidine/ H_2O_2 (MBL International, Japan) was added and plates were placed in the dark at room temperature for 30 min. Development was performed using a microplate reader set to 450 nm to reflect the level of anti-BP180. To minimize the effect of plate-to-plate variability, index values were calculated according to the formula: index value = (sample absorbance – low calibrator)/(high calibrator absorbance – low calibrator absorbance). The high calibrator was a sample which showed the highest absorbance in our pre-experiment, while the low calibrator had the lowest absorbance. The cutoff value was established using a receiver operating characteristic curve (ROC) analysis according to a protocol described in a previous study [10]. The optimum threshold values correspond with a maximum Youden's index (J = sensitivity + specificity – 1).

Total IgE ELISA

Detection of total IgE using ELISA was performed according to the manufacturer's instructions (Abcam, UK).

Statistical analysis

Statistical analysis was performed using Statistics Analysis System (SAS) 9.2 (SAS Institute Inc., Cary, NC, USA). The Spearman's rank correlation and Wilcoxon signed ranks test were used, and a p value of <0.05 was considered to be significant.

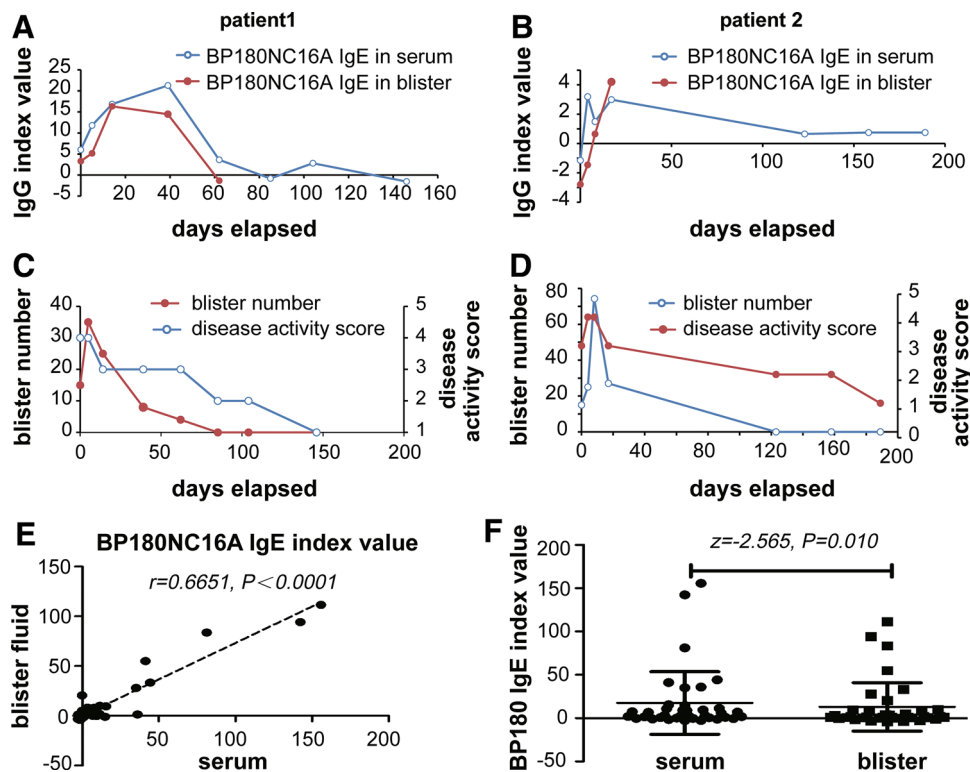
Results

Anti-BP180 NC16A IgE in serum and blister fluid of BP patients

The optimum threshold value for a positive result was determined as 0.519 by the ROC analysis. IgE anti-BP180 NC16A were detected in the serum or blister fluid of 72.97 % (27 of 37) of BP patients. All index values of 18 pemphigus vulgaris patients and 1 Stevens–Johnson syndrome patient were below the threshold value, indicating a high specificity of our method.

For the two untreated patients, the levels of autoantibodies in the serum and blister fluid at different time points showed BP180 NC16A IgE levels in blister fluid followed a trend similar to those in serum during the disease course in both patients, although the levels of the autoantibodies in blister fluid were lower (Fig. 1a, b). Anti-BP180 NC16A IgE levels in blister fluid correlated positively with that in

Fig. 1 BP180 NC16A IgE autoantibodies in blister fluid followed a similar trend to those in serum in patient 1 (a) and patient 2 (b). Detection of serum anti-BP180 NC16A IgE, as well as disease activity score throughout treatment. Both patients had active diseases and were untreated at the time of the first blood draw, and were in remission in the last time point. Levels of this autoantibody were correlated with disease severity generally, but in the first few weeks they continued to increase despite effective control of the disease in patient 1 (c) and patient 2 (d). There was a positive correlation in the levels of anti-BP180 IgE in serum and blister fluid ($r = 0.6651$, $p < 0.0001$) (e), but the levels were significantly higher in serum ($Z = -2.565$, $p = 0.010$) (f)



serum ($r = 0.6651$, $p < 0.001$) (Fig. 1e). Levels of anti-BP180 NC16A IgE in serum and blister fluid were significantly different, with higher levels detected in serum than those in blister fluid ($Z = -2.565$, $p < 0.05$) (Fig. 1f).

Correlation between anti-BP180NC16A IgE and total IgE in serum of 37 patients

To evaluate the correlation between anti-BP180NC16A IgE and total IgE, levels of anti-BP180NC16A IgE and total IgE in serum samples of 37 patients were quantified by ELISA. There was no significant difference between anti-BP180NC16A IgE and total IgE in sera from 37 BP patients ($r = 0.545$, $p > 0.05$) according to the Spearman test correlation analysis.

Association of anti-BP180 NC16A IgE with disease activity

We also evaluated the association between anti-BP180 NC16A IgE level and disease activity. In general, titers of the autoantibodies reflected disease severity. After the disease was controlled, the level of IgE anti-BP180 NC16A decreased. However, there was no correlation between disease activity scores and BP180 NC16A IgE titers either in serum ($r = -0.077$, $p > 0.05$) or in blister fluid ($r = 0.262$, $p > 0.05$) according to the Spearman test correlation analysis.

Trends of anti-BP180 NC16A IgE in serum in 2 BP patients throughout treatment

We tested the anti-BP180 NC16A IgE autoantibody titers of 2 BP patients, one for 8 sequential time points (during 146 days) and one for 7 (during 189 days). We assessed disease activity scores at all time points. At the initial blood draw (day 0), patient 1 was in a serious condition with a disease activity score of 4, while patient 2 had a score of 3 which relates to active disease.

On the whole, the levels of autoantibody reflected the clinical conditions. However, the levels of this antibody continued to increase, despite effective control of the disease in the initial 2–6 weeks (Fig. 1c, d). The trend of an increase in autoantibody level declined after effective treatment was given. Overall, autoantibody levels were elevated at the initial time points (day 0), then declined gradually to normal levels with standard immunosuppressive treatment. This paralleled the disease severity score. Following about 62 days, anti-BP180 NC16A IgE decreased to normal levels in patient 1, despite progression of the disease being retarded earlier in the treatment course. However, anti-BP180 NC16A IgE was still found when the patient was symptom free, at the last blood draw.

Autoantibodies reached their peaks on day 39 in patient 1 and on day 17 in patient 2. In the initial 39 days for patient 1 and 17 days for patient 2, autoantibody levels continued to rise, despite successful control of the disease via systemic and topical steroid medication.

Discussion

In this study, we examined the BP180 NC16A IgE levels in the serum and blister fluid in 37 BP patients using ELISA. Anti-BP180 NC16A IgE antibody can be detected in the serum or blister fluid of 72.97 %, consistent with previous reports [3, 9]. To the best of our knowledge, studies related to the level of IgE anti-BP180NC16A in blister fluid are rare. Our study confirmed that the level of IgE anti-BP180NC16A antibodies in serum was consistent with that in blister fluid.

In general, the titers of the autoantibodies reflected disease severity, and this is consistent with the results of other researchers [11]. When the disease was controlled, the levels of IgE antibodies decreased. However, if we added the data at different time points, especially the data at very early stage, the association is not significant. These results did not match other current indications perfectly. Our study found that BP180 NC16A IgE ELISA scores did not correlate with disease activity in the early stage of the disease, either in serum or in blister fluid, different from that of anti-BP180 NC16A IgG and anti-BP180 NC16A IgE reported by other researchers [2, 11].

To elucidate the exact process of changes in IgE antibody titer in serum or blister fluid, we collected sera and blister fluid samples at sequential time points. Our results showed that the time that the sample is taken is critical. The key issue of our research is the time of sampling. If you take a blood sample or blister fluid at the very early stage of the disease, the level of anti-BP180 NC16A IgE may not be very high. The highest level of anti-BP180NC16A IgE may not be detected in serum or blister fluid in the very early stage of the disease, but may be detected in the later stage, though the disease was treated. The time of taking samples influenced to evaluate the correlation between level of IgE and disease activities.

Our conclusions differ from those of previous studies because of the following reasons. Firstly, BP180 NC16A IgE levels changed greatly in the first few days of the disease course. In our study, during the first two to 6 weeks of oral and topical steroid treatment, anti-BP180 NC16A IgE levels continued to rise, even though the disease was being controlled effectively. The Abs levels reached a peak on days 39 and 17, in patients 1 and 2, respectively. Normalization of these autoantibody levels took both patients more than 2 months. Therefore, we considered that

fluctuation of anti-BP180 NC16A IgE also contributed to this difference. Secondly, utilizing three different immunoserological techniques, anti-BP180 NC16A IgE can be detected in elderly patients with pruritic dermatoses who may represent a prodromal phase of BP (who do not yet fulfill all the BP criteria) [6], though the typical lesions of BP are not present. This result indicates that IgE antibodies may not be related to the disease activity. Based on the above facts, we speculate that the level of IgE antibodies may be still high after steroid treatment even if the disease activity is low.

Conclusion

We demonstrated that the levels of anti-BP180 NC16A IgE are higher in serum than blister fluid. These levels could generally reflect disease severity throughout the course of the disease, but not in the initial one or 2 months after the onset of BP. We do not recommend detecting the levels of anti-BP180 NC16A IgE to evaluate disease activity in the very early stages of BP.

Limitations

This is a monocentric research study, though several hospitals were enrolled to collect samples. The sample size is small. The majority of our patients have been treated with steroid topically before they came to our hospitals. It is very difficult to collect samples from patients without any prior treatment, since the majority of patients in China choose local hospitals for primary treatment when they first become ill. If the disease is not controlled, these patients would then approach a bigger hospital in a metropolis, like Beijing or Shanghai. This phenomenon results in the complexity of the patient's condition.

The approach used in this study is limited. We only reported the results of ELISA tests of the level of IgE specific to anti-BP180 NC16A. Other assays such as IIF should be included for future studies. It is reported that 7.8 % of sera from BP patients react to regions of BP180 outside of NC16A [4]. Other regions of BP180 exclusively outside of NC16A were not identified. Meanwhile, the NC16A domain harbors more than one epitopes recognized by anti-BP180 IgE. It is another limit to use the whole NC16A domain to reach their conclusion.

Another limitation in this study is the time points we collected samples were inconsistent. For patient 1, we collected sera samples on days 0, 5, 14, 39, 62, 85, 104 and day 146. For patient 2, the time points were days 0, 4, 8, 17, 123, 158 and day 189.

In conclusion, here we demonstrated that anti-BP180 NC16A IgE can be detected in the majority of BP patients not only in serum but also in blister fluid. The levels of anti-BP180 NC16A IgE did not reflect disease severity precisely, at least in the initial one or two months after the onset of BP.

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Compliance with ethical standards

Conflict of interest The authors have no conflicts of interest to disclose.

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