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Detection of IgE autoantibodies to BP180 and BP230 and their relationship to clinical features in bullous pemphigoid

Short title: IgE antibodies in BP

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What's already known about this topic?

- Although recent studies indicated that, in addition to IgG autoantibodies, IgE autoantibodies are frequently detected in bullous pemphigoid (BP), diagnostic and pathogenic relevance has not been fully documented.

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- Reliable IgE ELISAs of BP antigens, particularly BP230, have not been established.

What does this study add?

- This study established reliable diagnostic IgE ELISAs for both BP180 and BP230, in which influence of excess amount of IgG autoantibodies were extensively determined.
- This study confirmed frequent detection of IgE autoantibodies to BP180 and BP230. IgE autoantibodies to BP180 correlated with disease activity, and IgE anti-BP230 antibodies correlated with nodular phenotypes but not with inflammatory erythematous phenotype.

What is the translational message?

- We developed reliable IgE ELISAs for both BP180 and BP230, which can be used in clinical practice.
- This study suggested that titers of IgE anti-BP180 autoantibodies correlated with disease activity in BP, and should be clinically useful to select the best treatment, particularly anti-IgE antibody therapy.

Abstract

Background: IgE autoantibodies are considered to be involved in pathogenesis of bullous pemphigoid (BP), particularly inflammatory and erythematous phenotypes.

Objective: To develop reliable ELISAs for detection of IgE autoantibodies to both BP180 and BP230 in BP sera, and to compare the ELISA results with clinical features.

Methods: We used commercially available IgG ELISAs to develop IgE ELISAs for both BP180 and BP230. To determine the influence of excess amount of IgG autoantibodies, all normal and BP sera were tested before and after IgG adsorption. The results of IgE ELISAs were statistically compared among various ELISAs, and with various clinical parameters, including our own severity scores and BP phenotypes.

Results: IgG adsorption generally showed no changes in sensitivity and specificity of IgE ELISAs, although slight cross-reactivity of anti-IgE secondary antibody to IgG and interference of excess amount of IgG autoantibodies to IgE reactivity were suggested. IgE autoantibodies to BP180 and BP230 were found in 21 and 18 of 36 BP sera, respectively. The results of IgG and IgE ELISAs for both BP180 and BP230 well correlated. IgG and IgE anti-BP180 antibodies, but not IgG and IgE anti-BP230 autoantibodies, correlated with disease activity. IgE anti-BP30 autoantibodies correlated with nodular phenotype but not erythematous phenotype.

Conclusion: The results in this study indicated that IgE autoantibodies to both BP180 and BP230 are frequently detected in BP sera. IgE anti-BP180 autoantibodies seemed to be pathogenic, while association between IgE autoantibodies and inflammatory BP phenotype was not indicated.

Introduction

Bullous pemphigoid (BP) is characterized clinically by tense blisters on the entire body, and histopathology by subepidermal blisters with eosinophilic infiltration.^{1,2} In addition, most BP patients show clinically extensive urticarial erythemas with severe itching, serologically increase in eosinophils and IgE, and histopathologically various inflammatory changes.³

BP patients show IgG anti-epidermal basement membrane zone autoantibodies, which react mainly with BP230⁴ and BP180.⁴ While major epitopes for IgG anti-BP180 autoantibodies reside within extracellular BP180 NC16a domain,⁵⁻⁷ IgG antibodies in BP sera react with various domains on entire BP230 molecule.⁸ BP180 is transmembrane protein, and its pathogenic role has been well documented by animal model studies.^{9,10} In contrast, because BP230 is intracytoplasmic protein, pathogenic role of BP230 is questionable. However, a recent study identified a novel disease entity, named BP230-type BP, in which IgG anti-BP230 antibodies are sole autoantibodies and may be pathogenic.¹¹

In addition to IgG autoantibodies, relevance of IgE autoantibodies in pathogenicity in BP is also indicated.¹²⁻¹⁴ Previous studies detected *in vivo* bound/circulating IgE autoantibodies in BP with positive ratios ranging 22%-100%.¹⁵⁻²⁷ The majority of these studies used IgE ELISAs of BP180 recombinant protein (RP) with positive ratios ranging 22%-77%,^{17-21,24,25,27} indicating that there were not yet standardized methods in IgE ELISAs. There were only three studies of IgE BP230 ELISA.^{15,19,20}

Several studies reported possible correlation of IgE anti-BP180 antibodies with BP disease activity.^{15,17,20,21,24} In addition, Omalizumab, a humanized monoclonal antibody to FcεRI, was successfully used in treatments in refractory BP patients.²⁸⁻³⁰ These findings indicated pathogenic role of IgE autoantibodies in BP. In contrast, a study showed no apparent correlation.²⁵ Thus, the pathogenic relevance of IgE autoantibodies in BP is still unclear, although possible mechanisms for development of BP skin lesions by IgE autoantibodies were suggested.³¹⁻³⁴

Serum IgG level (around 14 mg/ml) is approximately 300,000 fold higher than serum IgE level (around 0.00005 mg/ml).³⁵ Therefore, excess amount of IgG autoantibodies may affect the reactivity of IgE autoantibodies in ELISAs. It is highly conceivable that excess amount of IgG antibodies may interfere with reactivity with IgE antibodies, leading to false negative results in IgE ELISAs. Alternatively, anti-IgE antibodies used as secondary antibody may cross-react with IgG antibodies bound to RP coated on ELISA plates, leading to false positive reactivity. Indeed, IgE anti-BP180 autoantibodies were correlated with titers of IgG anti-BP180 autoantibody.²¹ Thus, establishment of reliable IgE ELISAs is needed to overcome the influence of excess amount of concurrent IgG autoantibodies.

In this study, to further examine the presence and pathogenic role of IgE autoantibodies in BP, we detected IgE antibodies to both BP180 and BP230 by our novel ELISAs and compared the results with various clinical parameters. To exclude the possibility of interference of IgG autoantibodies, we

examined all normal and BP sera before and after IgG depletion. The results indicated that IgE antibodies to both BP180 and BP230 are frequently detected in BP, and titers of IgE anti-BP180 autoantibodies correlated with disease activity.

Materials and Methods

Details for information of patients and sera, clinical findings including our own criteria of disease severities and BP phenotypes, IgG adsorption method, IgG and IgE ELISAs of BP180 and BP230, SDS-PAGE and immunoblotting, ELISA for human IgG, receiver operating characteristic curve (ROC) analysis and statistical analyses are described in Supplementary data.

Results

Background of and laboratory findings in BP patients

The 36 BP patients in this study were comprised of 8 (22.2%) males and 28 (77.8%) females with average age of 63.6 years (Table 1). When sera were obtained at initial diagnosis in Kurume University, 16 (44.4%) and one (2.8%) of the 36 patient had systemic PSL and an immunosuppressive drug, respectively. Eventually, systemic PSL was not given in 6 patients during the follow-up period.

White blood cell counts for 34 BP patients ranged 3800-26600/mm³ (mean 9415/mm³), eosinophil counts for 33 patients ranged 0-38.40/mm³ (mean 4.93/mm³), CRP for 29 patients ranged 0-4.66 mg/dl (mean 0.76 mg/dl), and total serum IgE for 10 patients ranged 18.5-3560 IU/ml (mean 1007.65 IU/ml) (Table 1).

Severity scores in BP patients and BP phenotypes

Means of severity scores 1 and 2 were 2.11 and 1.47, respectively (Fig.1, Table 1). BP phenotypes included 12, 10 and 8 of the 36 BP patients were classified as bullous, erythematous, bullous/erythematous phenotypes, respectively (Fig.1, Table 1). In addition, 4 patients were diagnosed as pemphigoid nodularis, and two patients were diagnosed as localized pemphigoid (Brunsting-Perry type and dyshidrosiform type).

Confirmation of adsorption of IgG from the sera

In both Oriole fluorescent protein staining and immunoblotting for human IgG for 5 representative BP sera, the protein bands corresponding to non-reduced IgG, which were seen in comparable intensities in both pre-adsorbed sera and captured IgG samples, was extensively reduced in post-adsorbed sera (Fig.S1a,b). Human IgG ELISA also showed the reduction of IgG in the 36 BP and 8 normal sera to undetectable levels after IgG adsorption (Fig.S1c). These results indicated that IgG adsorption could deplete IgG in sera almost completely.

Determination of cut-off values for various ELISAs in this study

In this study, cut-off index values for IgG ELISAs of BP180 and BP230 were calculated to be 10.120 and 17.960, respectively, from the results of 36 pre-adsorbed normal sera (Table 1, Table S1). These cut-off values were used for both pre-adsorbed and post-adsorbed BP sera, because post-adsorbed normal sera showed extremely low indices. Cut-off OD450 values for IgE ELISAs of BP180 for post-adsorbed and pre-adsorbed sera were 0.030 and 0.026, respectively, and those for IgE ELISAs of

BP230 for post-adsorbed and pre-adsorbed sera were 0.028 and 0.034, respectively (Table 1, Table S1). We also examined 10 atopic dermatitis patient sera with high total IgE levels, which showed no positive results in both IgE ELISAs.

The results in IgG ELISAs of BP180 and BP230

In IgG BP180 ELISA, all of 36 pre-adsorbed BP sera showed positive results with relatively high indices, while, after IgG adsorption, only 8 sera showed positive reactivity with much reduced indices (Fig.2a, Table 1, Table S2). In IgG BP230 ELISA, 22 of pre-adsorbed BP sera showed positive results with relatively high indices, which became negative after IgG adsorption, except for one serum (Fig.2c, Table 1, Table S2). These results further confirmed extensive removal of IgG antibodies after IgG adsorption procedure.

The results in novel IgE ELISAs of BP180 and BP230

In IgE BP180 ELISA, 21 of 36 pre-adsorbed BP sera showed positive results with relatively high OD450 values, while, after IgG adsorption, 19 sera showed positive reactivity (Fig.2b, Table 1, Table S2). In IgE BP230 ELISA, 18 of 36 pre-adsorbed BP sera showed positive results with relatively lower OD450 values, while, after IgG adsorption, 15 sera showed positive reactivity (Fig.2d, Table 1, Table S2). We repeated the IgE ELISA of BP180 for the 15 representative pre-adsorbed BP sera and could reproduce the results, confirming the reproducibility of all the IgE ELISAs.

Validation of the results of IgG and IgE ELISAs by ROC and statistical analyses

The results of ROC analyses showed high sensitivity and specificity of IgG ELISA of BP180 for the diagnosis of BP (AUC: 1.0) (Fig.S2a), while those of IgG ELISA of BP230 were slightly lower (AUC: 0.85) (Fig.S2b). These results confirmed well the previous studies that IgG ELISA of BP180 is highly sensitive diagnostic method for BP and IgG ELISA of BP230 is supportive diagnostic method.^{7,8} The results of ROC analyses showed lower sensitivity and specificity of IgE ELISAs of both BP180 and BP230 for the diagnosis of BP (AUCs: 0.59-0.74) (Fig.S2c-f), compared with those of IgG ELISAs of either BP180 or BP230.

As described above, pre-adsorbed and post-adsorbed sera showed comparable results for either OD450 values or positive ratios in both BP180 and BP230 IgE ELISAs. The similarity of results between pre-adsorbed and post-adsorbed sera was also examined by ROC analyses and statistical analyses. Neither increases nor decreases of AUCs between pre-adsorbed and post-adsorbed sera ROC analyses were found in IgE ELISAs of both BP180 (Fig.S2b,c) and BP230 (Fig.S2e,f), indicating that IgE ELISAs of both BP180 and BP230 had similar sensitivity or specificity between pre-adsorbed and post-adsorbed sera.

Furthermore, statistical analyses for IgE ELISAs of BP180 and BP230 also showed strong positive correlation ($P < 0.0001$) between pre-adsorbed and post-adsorbed sera (Table 2a).

Precise and detailed analyses of the results of IgE ELISAs of both BP180 and BP230 between pre-adsorbed and post-adsorbed sera

Although ROC and statistical analyses indicated similarity between pre-adsorbed and post-adsorbed sera in IgE ELISAs for both BP180 and BP230, detailed inspection of the results identified reduction or increase in OD values for some of the individual BP sera.

We summarized the ratios of increase or decrease of OD450 values after IgG adsorption against values of pre-adsorbed values in both BP180 and BP230 ELISAs for IgE ELISA positive BP sera (Fig.2e). Briefly, OD450 values decreased to less than 60% in 4 and 3 BP sera for BP180 and BP230, respectively, with one each serum with decrease to less than 20%. Oppositely, OD450 values increased to more than 120% in 5 and 2 BP sera for BP180 and BP230, respectively, with one serum with increase to 286% in BP230 ELISA. Positive reactivity with two and three of pre-adsorbed BP sera became negative for BP180 and BP230, respectively, after IgG adsorption. No BP sera became positive after IgG adsorption for either BP180 or BP230.

The results of statistical analysis among ELISA data

Correlation among all ELISAs; i.e., IgG and IgE ELISAs of BP180 or BP230 for pre-adsorbed and post-adsorbed BP sera were statistically analyzed (Table 2a,b).

In addition to the correlations between pre-adsorbed and post-adsorbed sera in IgE ELISAs for both BP180 and BP230 described above (Table 2a), there were a number of pairs of ELISAs with significant positive or negative correlations (Table 2b). Notably, the negative correlations between pre-adsorbed and post-adsorbed sera in IgG ELISAs for both BP180 and BP230 were apparent (Table 2b). We also found a significant positive correlation between IgG and IgE antibodies in both BP180 and BP230 ELISAs. In addition, slight correlation was found between IgG BP230 ELISA and IgE BP180 ELISA.

The results of statistical analysis between the results of ELISAs and clinical features

Regarding IgG ELISAs, because almost all of post-adsorbed BP sera showed negative results in BP230 ELISA, we statistically analyzed other 3 ELISAs for association to clinical parameters (statistically significant results in Table 2c, all results in Table S2).

We found statistically significant association of indices of IgG BP180 ELISAs for both pre-adsorbed and post-adsorbed BP sera with severity scores 1, while indices of IgG BP180 ELISA only for pre-adsorbed sera correlated with genders (higher indices in male patients) and eosinophils. However, no statistical significance was detected in BP230 ELISAs. These results indicated that IgG anti-BP180 antibodies correlated with disease activity in BP.⁷

Regarding IgE ELISAs, we found statistically significant correlation of OD450 values of IgE BP180 ELISAs for both pre-adsorbed and post-adsorbed BP sera with severity scores 1 and 2, whereas no statistical significance was detected in BP230 ELISA for both pre-adsorbed. These results indicated that IgE anti-BP180 antibodies also correlated with disease activity in BP.

Because IgE antibodies have been suggested to play a role in inflammation, i.e., erythematous phenotype, we rigorously inspected the relationship between IgE ELISAs and BP phenotypes. However, we found no significant association of OD450 values with erythematous phenotype (Table S2). Interestingly, IgE anti-BP230 autoantibodies in both pre-adsorbed and post-adsorbed sera positively correlated to nodular phenotype, while IgE anti-BP180 autoantibodies in both pre-adsorbed and post-adsorbed sera negatively correlated to nodular phenotype.

The statistical evaluation using paired t-test revealed significant positive correlation of total serum IgE with ELISAs of both BP180 ($p=0.0096$) and BP230 ($p=0.0095$), indicating that BP sera with high total serum IgE tended to show positive reactivity in IgE ELISAs.

Discussion

In this study, to precisely determine the presence and the significance of IgE autoantibodies in BP, we established reliable IgE ELISAs for both BP180 and BP230.

In addition to extreme excess of IgG to IgE, previous IgG ELISAs for BP antigens and desmogleins showed plateaus in reactivity lines in BP and pemphigus, respectively,^{7,8,36,37} indicating that most patients sera have equal or excess amount of autoantibodies against antigenic epitopes on RPs coated on ELISA plates. Therefore, we were concerned that IgG autoantibodies mask all epitopes for IgE autoantibodies, leading to false negative results in IgE ELISA.

Furthermore, ELISA plates used in this study were coated with small RP of BP180 NC16a domain (about 70aa)⁷ and larger RP of BP230 N- and C-terminal domains.⁸ Thus, BP180 RP has larger number of molecules with restricted but concentrated epitopes, while BP230 RP has smaller number of molecules with various epitopes. These differences may also influence sensitivity and specificity in IgE ELISAs.

Upon these reasons, to precisely and extensively determine influence of excess amount of IgG autoantibodies to reactivity of IgE autoantibodies in IgE ELISAs, the first part of this study examined both IgG pre-adsorbed and post-adsorbed samples prepared from all sera.

In general, pre-adsorbed and post-adsorbed BP sera showed comparable results in both BP180 and BP230 ELISAs. Both ROC analyses and statistical analyses indicated consistent results between IgG pre-adsorbed and post-adsorbed samples. Therefore, IgE ELISAs with pre-adsorbed serum samples may be preferably used in future studies, because IgG adsorption procedure is time-consuming and better to be avoided to make IgE ELISA systems simpler.

However, true mechanisms for the sufficient reactivity of IgE with RPs on the ELISA plates are currently unknown. It is plausible that IgE antibodies have extremely higher affinity to antigens than IgG antibodies, or that ratio of IgG autoantibodies against total IgG is very low (possibly only 0.001%). Another possibility may be that ratio of IgE autoantibodies against total amount of IgE is much higher (possibly 10%). Alternatively, IgE autoantibodies may react with considerable different epitopes

from those for IgG autoantibodies. In this case, epitopes for IgE antibodies should be sufficiently apart from those for IgG antibodies, so that binding of IgG autoantibodies to IgG antibody-specific epitopes does not interfere with binding of IgE autoantibodies to IgE antibody-specific epitopes.

Nevertheless, by detailed inspection of our results, multiple sera showed significantly higher or lower OD values in IgE ELISAs after IgG adsorption. Decrease of OD450 values after IgG adsorption may indicate that the anti-human IgE monoclonal antibody used as secondary antibody in our ELISAs cross-reacted with IgG in these sera. Although why such cross-reactivity occurred in particular BP sera is unknown, the anti-IgE secondary antibody had higher affinity with particular IgG subclasses, which were the major IgG subclasses for these BP sera.

Oppositely, increase of OD450 values after IgG adsorption may indicate that excess amount of IgG autoantibodies interfered with IgE reactivity. It is unknown why such interference was evident only in particular BP sera. One possibility is that those sera had very large amount of IgG autoantibodies, although such tendency was not detected in BP sera.

Therefore, to avoid these undesirable reactions, which may cause positive and negative false results, future studies may need improvements of methods of IgE ELISAs, including use of secondary antibody more specific to IgE or inclusion IgG adsorption procedure.

In our IgE ELISAs, 21 (58.3%) and 18 (50%) BP sera showed IgE autoantibodies to BP180 and BP230, respectively. These results are considered to be comparable to ratios (22-77%) in previous studies.^{17-21,24,25,27}

In previous studies, details for relationships either between BP180 and BP230 or between IgG and IgE antibodies were not described.¹²⁻²⁷ Our statistical analyses of various pairs of ELISAs showed significant positive correlations between IgG and IgE autoantibodies to both BP180 and BP230.

Indeed, all 36 BP sera showed positive results in IgG BP180 ELISA, and all 18 IgE BP230 ELISA positive sera were also positive in IgG BP230 ELISA. These results may suggest that the positive IgE reactivity in IgE ELISAs was caused by cross-reactivity of anti-IgE secondary antibody to IgG antibodies. However, this possibility could be excluded by the fact that some BP sera with very high index values in IgG ELISAs of BP180 and BP230 showed completely negative results in corresponding IgE ELISAs.

In the second part, we examined clinical features of BP patients, particularly in terms of relationship to ELISA results. Clinical assessments showed known BP characteristics, i.e., female predominance and slight increases in white blood cell counts, eosinophils counts and CRP. However, our patients were relatively young (mean: 63.56 years), although the reason was unclear.

Indices in IgG BP180 ELISAs antibodies statistically correlated with severity scores 1 and 2 and eosinophil counts. The reason why male patients showed higher indices in IgG BP180 ELISA is unclear. Furthermore, OD450 values in IgE BP180 ELISAs also correlated with severity scores 1

and 2. Therefore, both IgG and IgE BP180 ELISAs were considered to be helpful to determine disease activity in BP patients. In contrast, no statistically significant relationships to clinical parameters were found in BP230 ELISA for either IgG or IgE antibodies.

Although we expected close relationship between IgE autoantibodies and inflammatory BP phenotype, neither BP180 nor BP230 IgE ELISAs showed significant association with clinical phenotypes, particularly erythematous or bullous/erythematous phenotypes. In contrast, interestingly, IgE ELISA for BP230 showed statistically significant association with nodular phenotype, although mechanism for this phenomenon needs to be studied in future.

Our statistical study indicated that BP sera with higher total serum IgE tended to be positive in IgE ELISAs, suggesting that total serum IgE may result in positivity in the IgE ELISAs. However, this possibility could be excluded by two findings in our study; i.e., no correlation between IgE ELISAs of BP180 and BP230, and no positive reactivity in IgE ELISAs by atopic dermatitis sera with high total serum IgE.

In conclusion, we developed reliable novel IgE ELISAs of both BP180 and BP230, and showed significant number of BP sera had IgE autoantibodies. Our results indicated that IgE anti-BP180 antibodies correlated with disease activity in BP patients, but not with BP phenotypes. Previous reports suggested that BP patients with IgE autoantibodies are responsive to Omalizumab.²⁸⁻³⁰ Therefore, our ELISAs should be helpful to select BP patients suitable for Omalizumab therapy. However, relationship between IgE autoantibodies and inflammatory phenotype should be determined in future studies using larger cohort of BP patients.

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Conflict of interests

The authors have no conflict of interest to declare.

Author contributions

THashimoto, SH, DT, MH, NI designed this study. THashimoto, AO, MH, NI contributed in collection of information and serum samples. KT, SN, AO, THachiya performed experiments. TK, KK, MH, THashimoto performed and analyzed ROC and statistical analyses. THashimoto, SH, DT, KT, MH contributed in critical discussions. THashimoto, KT, TK wrote the manuscript.

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Figure Legends

Fig 1. Clinical features of 8 representative BP patients with severity scores 1 and clinical phenotypes.

Fig 2. (a-d) Graphic presentations of the results of IgG and IgE ELISAs of BP180 and BP230 for pre-adsorbed and post-adsorbed samples prepared from 36 normal and 36 BP sera. Horizontal dot lines indicate cut-off values. (e) Table for summary of ratios of increase and decrease of OD450 values in BP180 and BP230 IgE ELISAs after IgG adsorption.

Table 1 Summary of background, severity scores, BP phenotype, laboratory data and the results of IgG and IgE ELISAs for both BP180 and BP230

Patient numbers	Ages	Genders	Severity score 1	Severity score 2	BP phenotypes	WBC (mm ³)	Eosinophils (%)	CRP (mg/dl)	Total IgE (IU/ml) Normal <170	IgG BP180 (pre) c/o 10.120	IgG BP180 (post) c/o 10.120	IgE BP180 (pre) c/o 0.030	IgE BP180 (post) c/o 0.026	IgG BP230 (pre) c/o 17.960	IgG BP230 (post) c/o 17.960	IgE BP230 (pre) c/o 0.028	IgE BP230 (post) c/o 0.034
1	40	F	3	2	E	13700	19.50%	<0.04	n.d.	217.041	4.830	0.172	0.190	15.972	-1.228	0.004	0.005
2	63	M	2	2	E	10700	38.40%	1.16	n.d.	219.694	22.919	1.341	0.415	134.690	2.559	0.754	0.124
3	65	F	3	2	E	16900	23.50%	0.04	n.d.	212.245	20.555	1.010	0.773	119.532	3.275	0.185	0.108
4	39	M	3	3	BE	7000	17.00%	4.17	n.d.	200.306	8.839	1.123	1.096	5.900	-0.716	0.015	0.005
5	46	F	1	1	B	7100	2.30%	0.58	n.d.	171.735	6.783	0.323	0.141	8.647	0.409	0.006	0.010
6	53	F	2	1	N	7200	6.20%	<0.04	561	122.449	2.569	0.004	0.007	139.166	4.708	0.490	0.337
7	76	F	1	0	O	5310	10.2	0.04	18.5	91.837	1.233	0.002	0.008	91.251	0.512	0.010	0.004
8	82	F	2	0	E	7500	1.50%	1.3	n.d.	164.490	5.447	0.032	0.005	126.144	0.921	0.199	0.036
9	89	F	2	0	E	5000	8.5	1.14	234	106.020	1.747	-0.006	0.015	5.595	-1.433	-0.004	0.004
10	47	F	1	0	O	4800	5.2	<0.04	n.d.	50.918	0.822	-0.004	0.014	26.246	-1.126	0.005	-0.001
11	37	F	3	3	BE	11800	0	<0.04	1914	93.980	1.336	0.245	0.305	27.365	0.000	0.046	0.040
12	66	F	1	1	E	10000	5%	1.08	n.d.	31.939	0.103	0.151	0.143	66.022	-0.614	0.079	0.049
13	1	F	2	3	BE	26600	11.50%	n.d.	n.d.	187.245	3.597	0.005	0.002	9.766	-1.126	0.005	0.007
14	76	F	1	1	B	6900	8.50%	0.16	n.d.	185.510	10.277	0.010	0.002	151.679	28.762	0.455	1.303
15	69	F	2	1	E	6100	2.50%	0.13	n.d.	115.612	1.439	0.042	0.031	36.928	0.512	0.028	0.028
16	59	F	2	1	B	6600	19.5	0.07	n.d.	94.796	0.514	0.003	0.013	120.956	0.307	2.395	2.252
17	64	M	2	2	B	13200	3.1	0.06	n.d.	151.633	5.242	0.017	0.013	11.902	-1.228	0.021	0.014
18	62	M	2	1	E	10200	21.9	0.18	3560	126.224	0.719	0.317	0.269	76.297	-0.614	0.585	0.467
19	57	M	3	3	BE	13200	1.7	0.5	n.d.	44.898	0.103	0.058	0.052	7.833	-0.614	0.011	0.008
20	71	F	2	1	BE	8800	0.5	n.d.	n.d.	46.939	0.411	0.019	0.003	15.259	-0.614	0.004	0.004
21	65	F	3	2	B	8400	4.4	0.06	n.d.	148.934	1.464	1.463	1.411	12.878	0.633	0.009	0.002
22	67	M	3	3	B	13000	13.5	0.39	n.d.	225.279	39.958	1.471	1.807	6.244	0.271	0.004	0.009
23	74	F	3	3	BE	6900	1.6	0	97	207.919	25.105	1.669	1.991	11.902	1.357	0.009	0.007
24	55	F	1	1	N	n.d.	n.d.	n.d.	n.d.	55.736	0.732	0.002	-0.001	102.244	2.896	0.012	0.017
25	70	F	3	3	B	8300	0.7	0.74	n.d.	193.909	16.946	0.720	0.997	6.341	0.543	0.008	0.004
26	79	F	2	1	N	6600	6.2	n.d.	n.d.	64.569	1.987	0.008	0.009	144.000	11.674	0.939	0.917
27	67	F	2	1	E	4700	n.d.	<0.04	668	176.447	3.138	0.567	0.344	7.220	1.357	0.010	0.004
28	90	F	2	1	B	6600	5	0.04	141	108.223	1.674	0.006	0.001	14.732	0.362	0.007	0.018
29	73	M	3	2	B	10000	5.5	0.92	1623	210.660	21.653	0.935	1.153	32.293	2.081	0.041	0.056
30	87	F	2	1	B	n.d.	n.d.	n.d.	n.d.	119.391	4.603	0.059	0.052	83.024	2.805	0.399	0.315
31	56	F	2	2	E	11300	8.9	n.d.	n.d.	174.924	3.138	0.087	0.073	26.927	0.181	0.024	0.013
32	74	F	3	2	N	7200	6.2	n.d.	n.d.	63.046	0.837	0.003	0.004	90.341	0.362	0.817	0.702
33	74	M	2	2	BE	10200	15.3	0.59	1260	208.122	28.766	0.546	0.704	71.707	1.357	0.031	0.020
34	77	F	1	0	B	15100	8.5	4.66	n.d.	12.386	1.255	0.000	0.010	57.073	0.362	0.059	0.038
35	74	F	2	1	BE	3800	14.1	0.12	n.d.	12.183	1.360	0.009	0.003	67.317	1.448	0.059	0.026

36	44	F	1	0	B	9400	1.3	0.04	n.d.	50.254	1.151	0.037	0.021	84.976	1.267	0.500	0.427
	mean	M(8),	mean	mean	E(10),	mean	mean	mean	mean	mean	mean	mean	mean	mean	mean	mean	mean
	63.56	F(28)	2.11	1.47	B(12),	9415.00	4.93	0.76	1007.65	129.652	7.035	0.346	0.335	56.010	1.711	0.228	0.205
					N(4),					p/n 36	p/n 8	p.m 21	p/n 19	p/n 22	p/n 1	p/n 18	p/n 15
					O(2)												

M, male.; F, female; pre, pre-adsorbed;. post,post-adsorbed.

B, bullous type; E, erythematous type; BE, bullous/erythematous type.

N, nodular type; O, other types (localized pemphigoid).

c/o, cut-off value.

p/n, positive number.

Table 2 Significant results in statistical analyses of comparisons among the results of various ELISAs and between the results of various ELISAs and clinical parameters

(a) Comparisons of the results in IgE ELISAs for BP180 and BP230 between pre-adsorbed and post-adsorbed BP sera

Parameter A	Parameter B	r_s	P values
IgE BP180 (pre)	IgE BP180 (post)	0.869305	<0.0001*
IgE BP230 (pre)	IgE BP230 (post)	0.902703	<0.0001*

(b) Comparisons among the results various ELISAs, except for two pairs shown in (a)

Parameter A	Parameter B	r_s	P values
IgG BP180 (pre)	IgG BP180 (post)	0.880438	<0.0001 ^{*,n.c.}
IgG BP230(pre)	IgG BP230 (post)	0.621042	0.000239 ^{*,n.c.}
IgG BP180 (pre)	IgE BP180 (pre)	0.672844	<0.0001*
IgG BP180 (pre)	IgE BP180 (post)	0.583526	0.000556*
IgG BP230 (pre)	IgE BP230 (pre)	0.621042	0.000239*
IgG BP230 (pre)	IgE BP230 (post)	0.80296	<0.0001*
IgG BP230 (pre)	IgE BP180 (post)	0.38533	0.02263*

(c) Comparison between the results of various ELISAs and clinical parameters

Parameter A	Parameter B	r_s	P values
IgG BP180 (pre)	Severity score 1	0.5003861	0.003073*
	Severity score 2	0.559943978	0.001095*
	Genders		0.024767662*
	Eosinophils	0.357453209	0.04317*
IgG BP180 (post)	Diseae severity score 1	0.398005148	0.018541147*
	Diseae severity score 2	0.446848739	0.009172595*
IgG BP230 (pre)	Nodular phenotype		0.010253177*

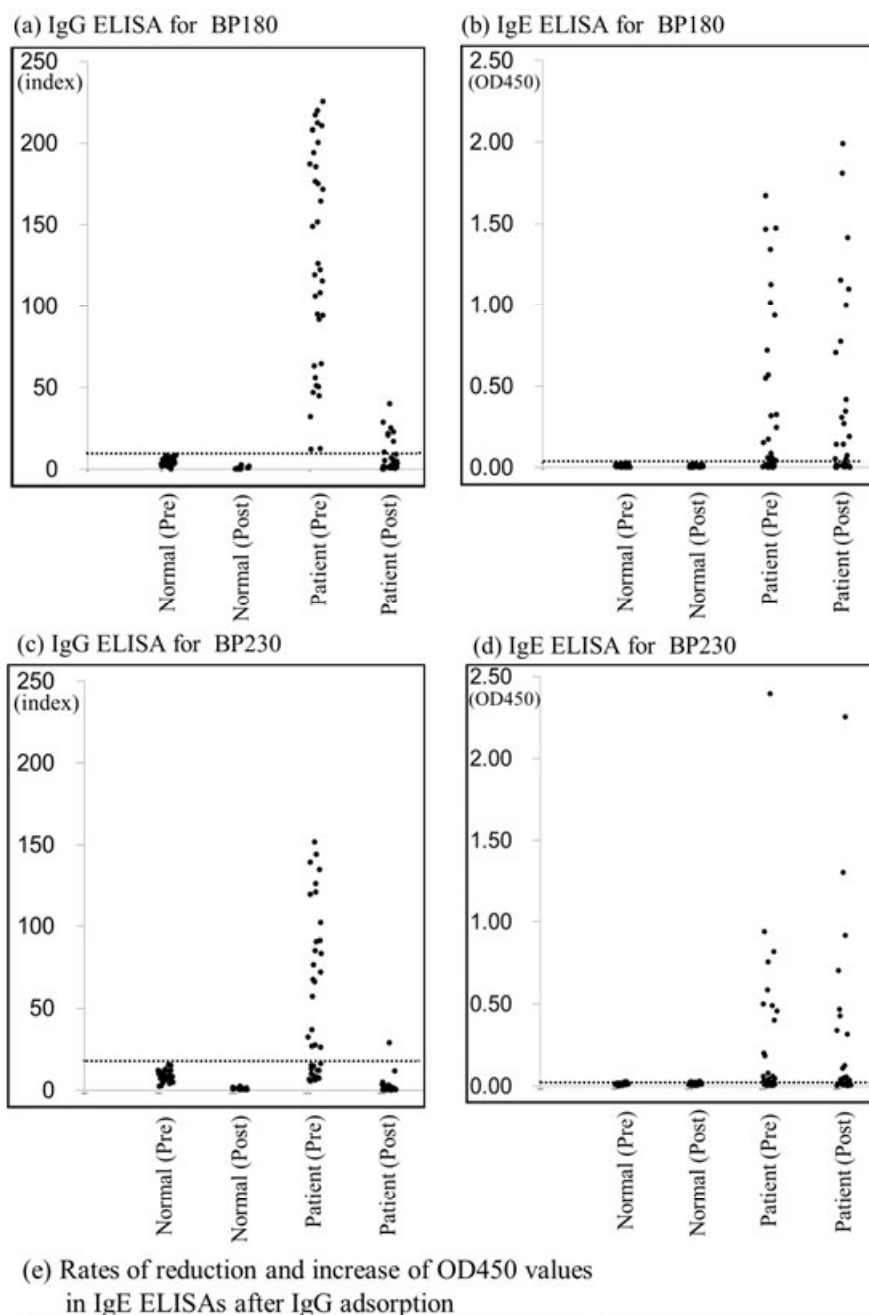
IgE BP180 (pre)	Severity score 1	0.598326898	0.000400507*
	Severity score 2	0.640896359	0.000186202*
	Nodular phenotype		0.026772766*,n.a.
	Genders		0.010790378*
	Total serum IgE		0.0096*
IgE BP180 (post)	Severity score 1	0.59028314	0.000479127*
	Severity score 2	0.537115	0.001736844*
	Nodular phenotype		0.020586219*,n.a.
	Genders		0.010790378*
IgE BP230 (pre)	Nodular phenotype		0.044065401*
	Total serum IgE		0.0095*
IgE BP230 (post)	Nodular phenotype		0.044065401*

pre, pre-adsorbed BP sera; post, post-adsorbed BP sera; *, statistically significant; n.c., negative correlation; n.a., negative association.

Figure 1



Figure 2



Rates (%) of alterations	BP case numbers in IgE BP180 ELISA	BP case numbers in IgE BP230 ELISA
<20%	8	28
20-40%	2	
40-60%	5, 36	3, 35
60-80%	3, 15, 27	6, 12, 18, 30, 33, 34
80-100%	4, 12, 18, 19, 21, 30, 31	11, 15, 16, 26, 32, 36
100-120%	1, 23	15
120-140%	11, 22, 25, 29, 32	19
286%		14
total	21	18

Red numbers indicate BP sera which changed from positive to negative after IgG adsorption

SUPPLEMENTARY DATA

MATERIALS AND METHODS

Patients and sera

This study was approved by Ethical Committee of Kurume University, and informed consents were obtained from all patients and normal individuals. We examined 36 patients with BP who visited Kurume University Hospital between 2006 and 2013. Final diagnoses of BP were made based on typical clinical and histopathological features, IgG and/or C3 deposition to BMZ in direct immunofluorescence, and the positive results of serological studies, including indirect immunofluorescence of normal human skin and 1M NaCl-split-normal human skin and IgG ELISAs of BP180 and/or BP230 (MBL, Nagoya, Japan). Sera from 35 healthy volunteers were used as normal controls. Sera from 10 atopic dermatitis patients with high levels of total serum IgE were also used as controls to verify the reliability of IgE ELISA.

Background of BP patients and laboratory findings, as well as assessments of disease severity and BP phenotypes by our own criteria

Background of the patients (i.e., ages and genders), white blood cell counts, serum eosinophils and CRP were obtained from medical charts at Kurume University Hospital. Clinical pictures were taken at the first visit for all patients.

Because this was a retrospective study including patients seen about 10 years ago, precise determination of BP disease activity index (BPDAI) for extents of either blisters/erosions or urticarias/erythemas was difficult. Therefore, instead of BPDAI, we assessed disease severity by two sets of our own relatively simple criteria using information in medical charts and clinical photographs kept in our university. In severity score 1, we scored the disease severity by disease extent. Thus, score 1 was given to patients with localized skin lesions, score 2 to patients with moderate diffuse skin lesions, and score 3 to patients with extensive severe generalized skin lesions. In severity score 2, we scored the disease severity by maximum dose of systemic steroids calculated as prednisolone (PSL) during the disease course. Thus, score 0 was given to patients treated without systemic steroids, score 1 to patients treated with PSL less than 0.5 mg/kg/day, score 2 to patients treated with PSL 0.5-1.0 mg/kg/day, and score 3 to patients treated with PSL more than 1.0 mg/kg/day.

We also determined BP phenotypes using information in medical charts and clinical

photographs. We classified most BP patients into bullous, erythematous and erythematous/bullous phenotypes, which showed predominant bullous lesions, predominant urticarial/erythematous lesions, and both erythematous and bullous lesions, respectively. A few patients were categorized as either pemphigoid nodularis or localized pemphigoid.

IgG adsorption

After washed 3 times with 10 bed volume of TBS (10mM Tris, pH 7.2, 150mM NaCl), 60µl of settled Protein G beads (EMD Millipore, Billerica, MA, USA) in 0.5ml microtubes were suspended in 180µl of TBS and 6µl sera were added and mixed. After incubated by rotation at low speed for 40 minutes, beads were removed by centrifugation in micro bio-spin columns (BioRad Laboratories, Hercules, CA) and the flow-through was collected in 2ml tubes. After the centrifugation, 120µl of TBS was added to the beads in the spin column and centrifuged again into the same tube containing the initial 180µl flow-through to obtain 300µl of sample. The total 300µl flow-through samples were used as post-adsorbed sera. Tubes without beads were similarly processed and used as pre-adsorbed sera

After post-adsorbed serum samples were obtained, captured IgG on the beads were washed 5 times with TBS buffer and suspended in 300µl of TBS buffer. To verify the IgG removal, 5 pre-adsorbed and post-adsorbed serum samples and captured IgG were examined by SDS-PAGE followed by both total protein staining and immunoblotting, while pre-adsorbed and post-adsorbed serum samples for all the 36 BP and 8 normal sera were subjected to human IgG ELISA kit (RD-Biotech, Besançon, France) as described below.

Modified method for IgG ELISAs of BP180 and BP230

Commercially available ELISAs of BP180 NC16a domain RP (MEASACUP BP180 text, MBL)¹ and RPs of BP230 N- and C-terminal domains (BP230 ELISA kit, MBL)² were performed with several modifications. For two purposes to use similar method between IgG and IgE ELISAs and to obtain large amount of sample, we used the same dilution buffer prepared for IgE ELISAs as below. 100µl of both pre-adsorbed and post-adsorbed serum samples were added to the plates. The plates were washed 4 times with TBS buffer containing 0.05% Tween 20.

Establishment of novel IgE ELISAs for both BP180 and BP230

We applied commercially available IgG ELISAs (MBL) to development of IgE ELISAs

for BP180 and BP230. To achieve final reaction condition of 1:100 serum dilution in TBS containing 2% bovine adult serum and 0.01% Tween 20 (dilution buffer), 300µl of 2X dilution buffer was added to each of 300µl of pre-adsorbed and post-adsorbed serum sample, 100µl of resulting samples were added to ELISA plates and incubated for 1 hour at room temperature (RT). The plates were washed 4 times with TBS containing 0.05% Tween 20, and incubated with 100µl of horse-radish peroxidase (HRP)-conjugated anti-human IgE monoclonal antibody (mAb) (Clone B3102E8, Southern Biotechnology, Birmingham, AL) diluted 1:4000 in dilution buffer for 1 hour at RT. After washing again as above, TMB substrate was added and incubated for 30 minutes at RT. After the reaction was stopped by addition of 100µl 0.5M HCl, optical density (OD) values at 450 nm were measured by Infinite-F50 Microplate reader (Tecan, Grödig, Austria). Cut-off values were determined as mean+3SD of OD₄₅₀ of 35 normal controls for all ELISAs.

SDS-PAGE, protein staining and immunoblotting for detection of IgG

Non-reducing SDS-PAGE was performed for pre-adsorbed sera, captured IgG and post-adsorbed sera for 5 representative BP patient sera. Briefly, 11µl of both pre-adsorbed and post-adsorbed serum samples and bead suspension of captured IgG, all diluted 1:500 in TBS, was added to 4µl of 5X non-reducing sample buffer, heated at 95°C for 5 min, and run on 5-20% SDS-PAGE gels (ATTO, Nagoya, Japan). Total protein on the gel was stained with Oriole fluorescent gel stain (Bio-Rad). Proteins on another gel were transferred onto nitrocellulose membrane using iBlot transfer device (Invitrogen, Carlsbad, CA). The membrane was treated with HRP-conjugated anti-human IgG polyclonal antibody (Dako Denmark, Glostrup, Denmark) and subsequently color was developed with TMB substrate (ATTO).

ELISA for human IgG

IgG amount in the pre-adsorbed and post-adsorbed samples of all patient sera and 8 normal sera were detected by human IgG ELISA kit (RD-Biotech) according to the manufacturer' instruction.

CLEA for human IgE

Total IgE amounts in patients sera were detected by human IgE CLEA kit (Siemens Immulyze total IgE III, Siemens Healthcare Diagnostics Co. Ltd. Tokyo, Japan) according to the manufacturer's instruction. Normal range is less than 170 IU/ml, and median value is 20.4 IU/ml (1 IU=2.4ng).

Receiver operating characteristic curve (ROC) analysis

ROC analysis was performed using StatFlex version 6.0 software (Artech, Osaka, Japan).

Statistical analyses

Differences among qualitative results were compared using the Chi-square test.

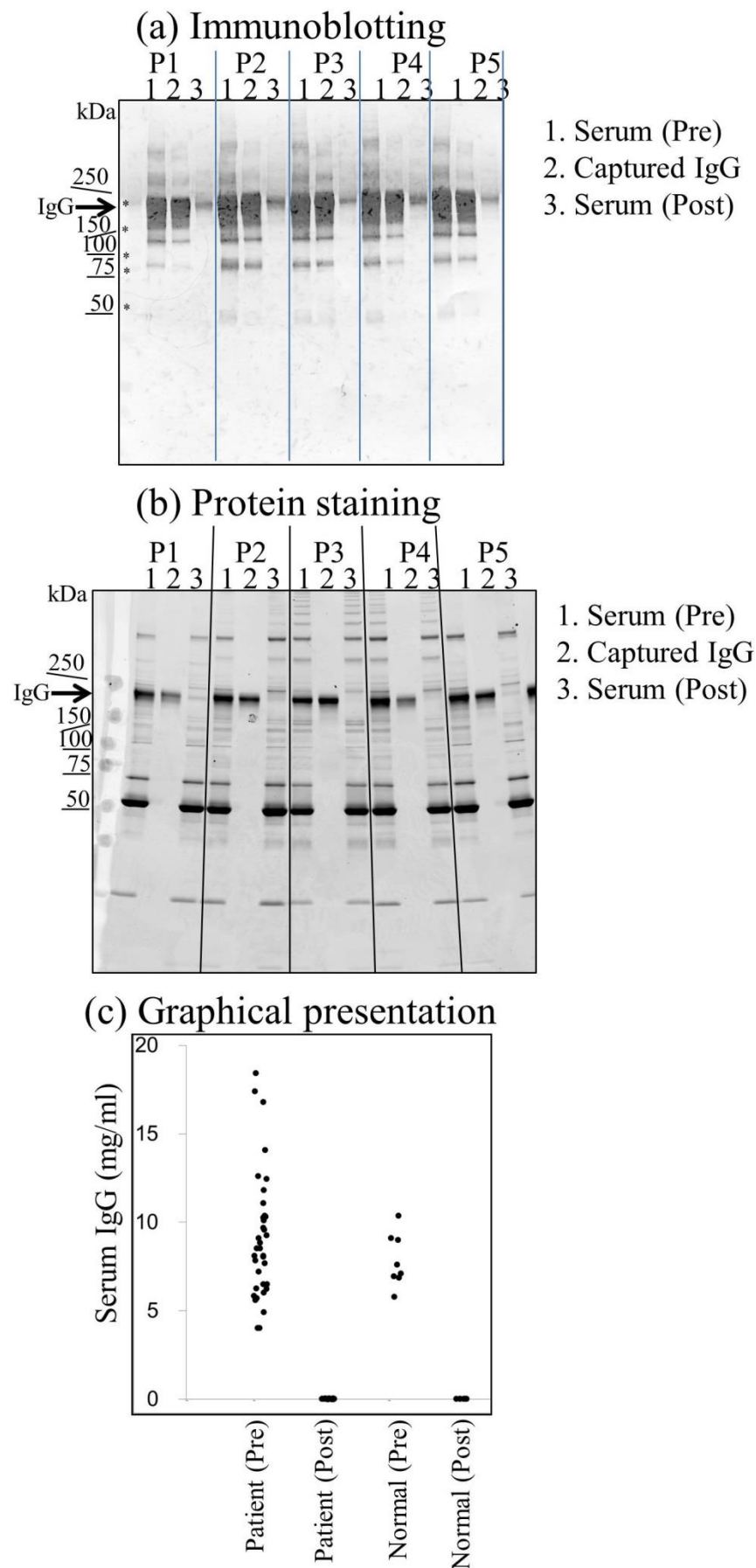
Differences among quantitative parameters between groups were assessed using the Mann-Whitney U test and Spearman's rank correlation test. A P-value of less than 0.05 was considered to be statistically significant.

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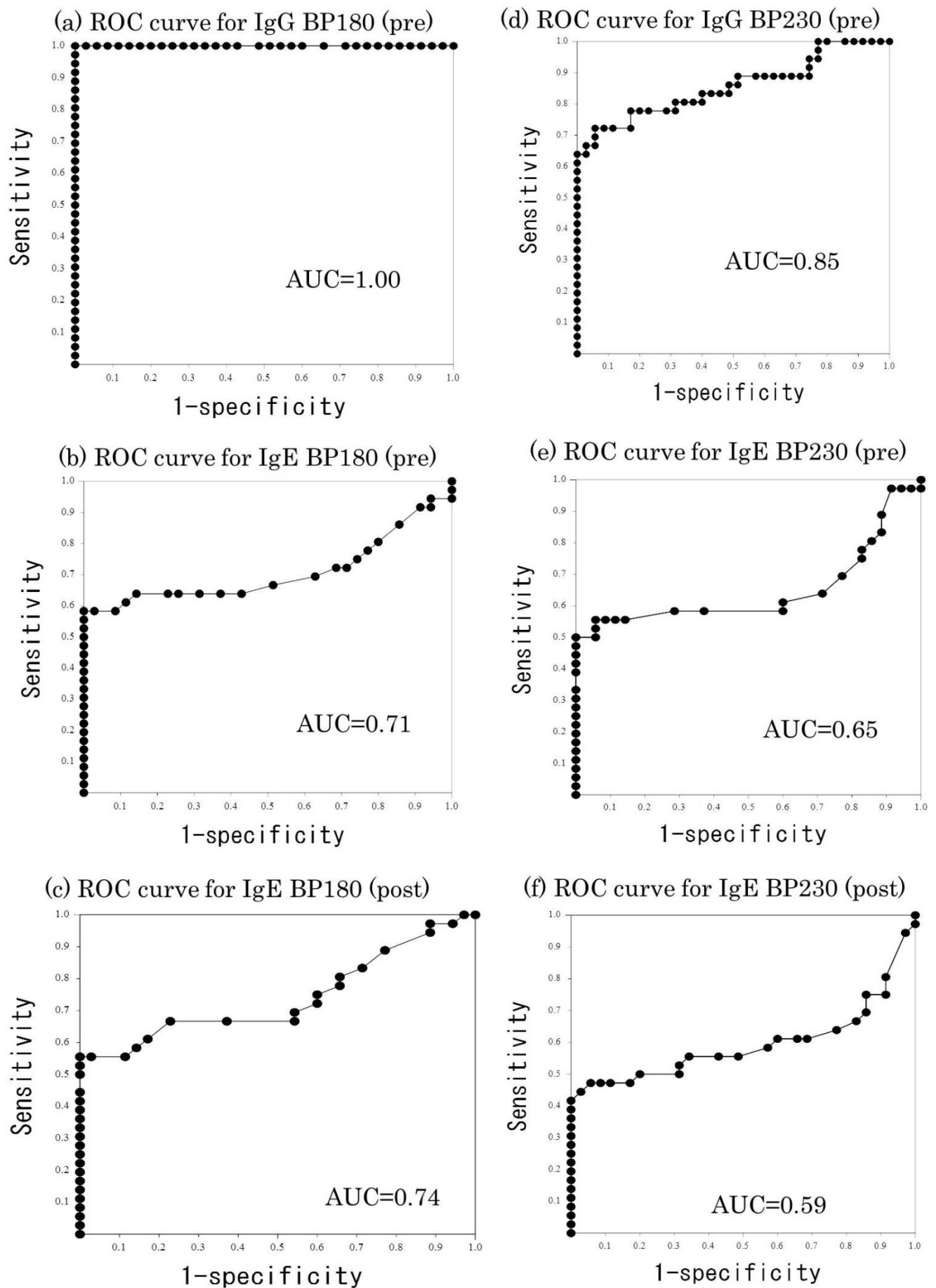
Supplementary Figures

Figure S1



Supplementary Figure 1 The results of confirmation studies on IgG depletion after IgG adsorption. (a) The results of immunoblotting study. (b) Protein staining figure. (c) Graphic presentation of the results of ELISA for human IgG

Figure S2



Supplementary Figure 2 The results of ROC analyses for IgG BP180 and BP230 ELISAs for pre-adsorbed sera and for IgE BP180 and BP280 ELISAs for both pre-adsorbed and post-adsorbed sera.

Supplementary Tables

Table S1 The results of all ELISAs for both pre- and post-adsorbed sera

	BP180(G) (pre) c/o 10.120	BP180(G) (post) c/o 10.120	BP180(E) (pre) c/o 0.030	BP180(E) (post) c/o 0.026	BP230(G) (pre) c/o 17.960	BP230(G) (post) c/o 17.960	BP230(E) (pre) c/o 0.028	BP230(E) (post) c/o 0.034
N01	5.005	-0.797	0.010	0.009	4.632	-0.302	0.013	0.016
N02	3.264	0.000	0.009	0.012	7.158	1.613	0.011	0.019
N03	3.264	0.797	0.014	0.016	5.474	2.016	0.014	0.024
N04	3.700	-0.199	0.015	0.020	2.421	1.109	0.025	0.014
N05	7.726	-0.299	0.011	0.009	6.947	1.109	0.013	0.020
N06	4.570	-0.299	0.009	0.012	6.526	0.605	0.013	0.019
N07	2.394	-0.100	0.011	0.011	7.158	1.714	0.013	0.014
N08	6.964	-0.896	0.010	0.012	10.000	1.310	0.009	0.016
N09	5.876	-0.797	0.007	0.016	11.789	1.210	0.015	0.017
N10	3.047	0.797	0.012	0.014	4.211	0.706	0.019	0.021
N11	5.114	-0.598	0.010	0.012	10.947	0.202	0.014	0.015
N12	8.379	-0.598	0.009	0.011	8.421	0.504	0.015	0.015
N13	4.026	2.590	0.012	0.013	12.105	0.806	0.016	0.021
N14	2.720	-0.299	0.015	0.015	4.632	0.605	0.015	0.012
N15	4.353	-0.896	0.008	0.011	8.316	2.419	0.011	0.013
N16	4.461	0.896	0.013	0.016	6.421	0.504	0.018	0.019
N17	5.223	-0.299	0.015	0.011	15.474	0.202	0.015	0.019
N18	7.173	0.427	0.001	0.003	8.291	-0.514	0.002	0.028
N19	5.274	-0.427	0.028	0.004	15.251	0.309	0.013	0.010
N20	7.384	-0.641	0.003	0.004	12.078	-1.132	0.004	0.010
N21	4.114	0.107	0.006	0.013	6.960	-0.309	0.015	0.023
N22	2.954	-0.427	0.020	0.002	7.267	-0.823	0.013	0.026
N23	4.008	0.214	0.004	-0.001	12.794	0.206	0.013	0.012
N24	2.637	0.321	-0.003	0.011	10.031	0.309	0.001	0.006
N25	0.211	2.564	0.009	0.007	2.866	-0.412	0.003	0.016
N26	2.321	1.923	0.017	0.012	6.755	-0.514	0.011	0.014
N27	4.114	-0.214	0.019	0.003	3.992	-1.029	0.006	0.009
N28	1.793	-0.748	0.020	0.000	4.606	-1.543	0.014	0.004
N29	1.899	-0.641	0.002	0.000	6.141	-1.029	0.011	0.010
N30	3.586	0.214	0.005	0.011	8.700	-0.926	0.013	0.009
N31	3.797	0.214	0.013	-0.002	9.417	-1.646	0.009	0.002
N32	3.586	-0.748	-0.003	0.007	12.078	-1.543	0.025	0.011
N33	2.004	-1.068	0.003	0.002	7.062	-1.337	0.010	0.008
N34	4.747	0.107	0.008	0.002	8.802	-0.926	0.010	0.006
N35	8.228	-0.641	0.002	0.002	10.031	-1.029	0.007	0.004
P01	217.041	4.830	0.172	0.190	15.972	-1.228	0.004	0.005
P02	219.694	22.919	1.341	0.415	134.690	2.559	0.754	0.124
P03	212.245	20.555	1.010	0.773	119.532	3.275	0.185	0.108
P04	200.306	8.839	1.123	1.096	5.900	-0.716	0.015	0.005
P05	171.735	6.783	0.323	0.141	8.647	0.409	0.006	0.010
P06	122.449	2.569	0.004	0.007	139.166	4.708	0.490	0.337
P07	91.837	1.233	0.002	0.008	91.251	0.512	0.010	0.004
P08	164.490	5.447	0.032	0.005	126.144	0.921	0.199	0.036
P09	106.020	1.747	-0.006	0.015	5.595	-1.433	-0.004	0.004
P10	50.918	0.822	-0.004	0.014	26.246	-1.126	0.005	-0.001
P11	93.980	1.336	0.245	0.305	27.365	0.000	0.046	0.040
P12	31.939	0.103	0.151	0.143	66.022	-0.614	0.079	0.049
P13	187.245	3.597	0.005	0.002	9.766	-1.126	0.005	0.007
P14	185.510	10.277	0.010	0.002	151.679	28.762	0.455	1.303
P15	115.612	1.439	0.042	0.031	36.928	0.512	0.028	0.028
P16	94.796	0.514	0.003	0.013	120.956	0.307	2.395	2.252
P17	151.633	5.242	0.017	0.013	11.902	-1.228	0.021	0.014
P18	126.224	0.719	0.317	0.269	76.297	-0.614	0.585	0.467
P19	44.898	0.103	0.058	0.052	7.833	-0.614	0.011	0.008
P20	46.939	0.411	0.019	0.003	15.259	-0.614	0.004	0.004
P21	148.934	1.464	1.463	1.411	12.878	0.633	0.009	0.002
P22	225.279	39.958	1.471	1.807	6.244	0.271	0.004	0.009
P23	207.919	25.105	1.669	1.991	11.902	1.357	0.009	0.007
P24	55.736	0.732	0.002	-0.001	102.244	2.896	0.012	0.017
P25	193.909	16.946	0.720	0.997	6.341	0.543	0.008	0.004
P26	64.569	1.987	0.008	0.009	144.000	11.674	0.939	0.917
P27	176.447	3.138	0.567	0.344	7.220	1.357	0.010	0.004
P28	108.223	1.674	0.006	0.001	14.732	0.362	0.007	0.018
P29	210.660	21.653	0.935	1.153	32.293	2.081	0.041	0.056
P30	119.391	4.603	0.059	0.052	83.024	2.805	0.399	0.315
P31	174.924	3.138	0.087	0.073	26.927	0.181	0.024	0.013
P32	63.046	0.837	0.003	0.004	90.341	0.362	0.817	0.702
P33	208.122	28.766	0.546	0.704	71.707	1.357	0.031	0.020
P34	12.386	1.255	0.000	0.010	57.073	0.362	0.059	0.038
P35	12.183	1.360	0.009	0.003	67.317	1.448	0.059	0.026
P36	50.254	1.151	0.037	0.021	84.976	1.267	0.500	0.427
	mean 129.652	mean 7.035	mean 0.346	mean 0.335	mean 56.010	mean 1.711	mean 0.228	mean 0.205
	p/n 36	p/n 8	p/n 21	p/n 19	p/n 22	p/n 1	p/n 18	p/n 15

pre, pre-adsorbed; post, post-adsorbed; c/o, cut-off value.

Table S2 Comparisons between the results of all ELISAs and all clinical parameters

(a) Comparisons between the results of all IgG ELISAs and all clinical parameters

Parameter A	Parameter B	r_s	P values
IgG BP180 (pre)	Severity score 1	0.5003861	0.003073*
	Severity score 2	0.559943978	0.001095*
	Bullous phenotype		0.75437185
	Erythematous phenotype		0.157731385
	Bullous/erythematous phenotype		0.103044581
	Erythematous phenotype+bullous/erythematous		0.326693057
	Nodular phenotype		0.14435483
	Genders		0.024767662*
	Ages	-0.122715573	0.467841763
	White blood cell counts	0.295110772	0.090021878
	Eosinophils	0.357453209	0.04317*
	CRP	0.072167488	0.702554492
IgG BP180 (post)	Severity score 1	0.398005148	0.018541147*
	Severity score 2	0.446848739	0.009172595*
	Bullous phenotype		0.382788201
	Erythematous phenotype		0.791094772
	Bullous/erythematous phenotype		0.262807109
	Erythematous phenotype+bullous/erythematous		0.80018414
	Nodular phenotype		0.246968443
	Genders		0.070696638
	Ages	0.124710425	0.460637925
	White blood cell counts	0.177005348	0.309239871
	Eosinophils	0.218582888	0.21627591
	CRP	0.170812808	0.36607154
IgG BP230 (pre)	Severity score 1	-0.258944659	0.125537917
	Severity score 2	-0.367647059	0.032054341
	Bullous phenotype		0.729481968
	Erythematous phenotype		0.723949243
	Bullous/erythematous phenotype		0.986861536
	Erythematous phenotype+bullous/erythematous		0.241748259
	Nodular phenotype		0.010253177*
	Genders		0.253649788
	Ages	0.181660232	0.282501708
	White blood cell counts	-0.05737204	0.741719395
	Eosinophils	0.262533422	0.137513812
	CRP	-0.0591133	0.754433995

(b) Comparisons between the results of all IgE ELISAs and all clinical parameters

Parameter A	Parameter B	r_s	P values
IgE BP180 (pre)	Severity score 1	0.598326898	0.000400507*
	Severity score 2	0.640896359	0.000186202*
	Bullous phenotype		0.960598853
	Erythematous phenotype		0.243811504
	Bullous/erythematous phenotype		0.255852644
	Erythematous phenotype+bullous/erythematous		0.061946653
	Nodular phenotype		0.026772766*,n.a.
	Genders		0.010790378*
	Ages	-0.184491634	0.275067344
	White blood cell counts	0.31038961	0.074577748
	Eosinophils	0.008856952	0.960040719
	CRP	0.088669951	0.638928632
	Total serum IgE		0.0096*
IgE BP180 (post)	Severity score 1	0.59028314	0.000479127*
	Severity score 2	0.537115	0.001736844*
	Bullous phenotype		0.856256872
	Erythematous phenotype		0.216402856
	Bullous/erythematous phenotype		0.182250809
	Erythematous phenotype+bullous/erythematous		0.486398021
	Nodular phenotype		0.020586219*,n.a.
	Genders		0.010790378*
	Ages	-0.197619048	0.242351238
	White blood cell counts	0.252559206	0.146823812
	Eosinophils	0.073529412	0.677450129
	CRP	0.07955665	0.673773905
IgE BP230 (pre)	Severity score 1	-0.053346203	0.752305838
	Severity score 2	-0.134733894	0.432086128
	Bullous phenotype		0.86920084
	Erythematous phenotype		0.684622447
	Bullous/erythematous phenotype		0.830487967
	Erythematous phenotype+bullous/erythematous		0.486398
	Nodular phenotype		0.044065401*
	Genders		0.760817246
	Ages	0.102574003	0.543959766
	White blood cell counts	0.010847976	0.95031049
	Eosinophils	0.209308155	0.236402879
	CRP	0.15455665	0.413450313
	Total serum IgE		0.0095*
IgE BP230 (post)	Severity score 1	-0.058687259	0.728442194
	Severity score 2	-0.108333333	0.52759178
	Bullous phenotype		0.680570921
	Erythematous phenotype		0.901620125
	Bullous/erythematous phenotype		0.598224316
	Erythematous phenotype+bullous/erythematous		0.268142205
	Nodular phenotype		0.044065401*
	Genders		0.804651825
	Ages	0.123166023	0.466209655
	White blood cell counts	0.129717341	0.456169322
	Eosinophils	0.214488636	0.225003313
	CRP	0.13226601	0.48399854

pre, pre-adsorbed BP sera; post, post-adsorbed BP sera; *, statistically significant; n.a., negative association.