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Basement membrane zone IgE deposition is associated with bullous pemphigoid disease severity and treatment results

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Conflicts of interest
The Department of Dermatology at Keio University holds a patent for an anti-BP180 enzyme-linked immunosorbent assay produced by Medical & Biological Laboratories (MBL). MBL has approved submission of the manuscript but has exerted no editorial control of the content.

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Summary

Background A subset of patients with bullous pemphigoid (BP) show deposition of IgE in the basement membrane zone (BMZ), yet the relationship between BMZ IgE and the clinical presentation of BP remains unclear.
Objectives To investigate the relationship between IgE deposition, IgE levels in serum, and disease severity in patients with BP.
Methods We investigated IgE autoantibodies in 53 patients with BP by direct immunofluorescence (DIF), indirect immunofluorescence and enzyme-linked immunosorbent assay.
Results Of 53 patients with BP, 23 (43%) had IgE deposition, 10 (19%) of whom were IgE+ and 13 (25%) IgE± according to DIF analyses. Erosion/blister (E/B) Bullous Pemphigoid Disease Area Index (BPDAI) scores were significantly higher in IgE+ patients than in IgE− patients (n = 15), while no significant differences were found for urticaria/erythema BPDAI scores. IgE+ and IgE± patients took longer to reduce their E/B BPDAI score by 75% after systemic corticosteroid treatment. BP180-IgE levels were significantly higher among IgE+ patients than IgE± or IgE− patients (n = 10). Total IgE levels in the serum and blood eosinophil counts did not differ between IgE+, IgE± and IgE− patients. A significant correlation was detected between BP180-IgG and BP180-IgE, but not between BPDAI scores and any of BP180-IgG, BP180-IgE or blood eosinophil count.
Conclusions IgE deposition in the BMZ is associated with higher E/B BPDAI scores and longer treatment periods. We conclude that IgE binding in the BMZ may contribute to BP pathogenesis by promoting blister formation.

What's already known about this topic?

- BP180-IgE autoantibodies have an important role in the pathogenesis of bullous pemphigoid (BP).
- A subset of patients with BP display deposition of IgE within the basement membrane zone (BMZ) of skin tissue.

What does this study add?

- Patients with *in vivo* IgE deposition in the BMZ displayed higher erosion/blister Bullous Pemphigoid Disease Area Index (BPDAI) scores, while urticaria/erythema BPDAI scores were not significantly different.
- Patients with *in vivo* IgE deposition in the BMZ took longer to reduce their erosion/blister BPDAI score by 75% after systemic corticosteroid treatment.
- BP180-specific IgE levels in serum were higher among patients with linear IgE deposition in the BMZ than in those with granular or no IgE deposition.

Bullous pemphigoid (BP) is an acquired autoimmune skin disease characterized by erythema and fluid-filled blisters, which are located predominantly on the flexible regions of the body, such as the limbs and lower abdomen.[1] The two basement membrane zone (BMZ) autoantigens targeted by BP autoantibodies are BP180 (type XVII collagen) and BP230 (dystonin-e), both of which are hemidesmosomal components of the BMZ.[2] Clinically, patients with BP often experience a prodromal phase of weeks or months with pruritus, excoriations and eczematous lesions before developing fluid-filled blisters.[1,3] Histopathological analyses have revealed that lesion formation is initiated by degranulation of dermal mast cells and is associated with eosinophilic infiltration in the upper dermis.[4,5]

Elevated levels of IgE autoantibodies have been described in as many as 70–85% of BP sera and blister fluid samples.[1] *In vivo* deposition of IgE in the BMZ has been reported in 18–41% of patients with BP.[2,4,6] IgE autoantibodies primarily target the NC16a domain of BP180, although other epitopes have been described.[7] IgE autoantibodies against the NC16a domain of BP180 (BP180-IgE) are detected in 22–100% of patients with BP.[1] There is a report that patients with BP with IgE deposition in the BMZ have significant urticarial papules and plaques.[6] On the other hand, those with IgE but no IgG deposition in the BMZ have atypical disease presentation resembling prurigo nodularis or exfoliative erythroderma.[2] Such patients may require a higher dose of corticosteroids, and more intensive therapies for remission.[8]

Interestingly, there is a report that the disappearance of BP180-IgE is associated with clinical remission,[9] and omalizumab, a humanized monoclonal antibody binding to free human IgE, is an effective treatment for BP.[10–12] Together, these observations suggest a relationship between IgE deposition in the BMZ and BP disease progression. In this study, we focused on the relationship between IgE deposition in the BMZ and clinical or serological assessments in patients with BP.

Materials and methods

Patients

In total 53 patients with BP diagnosed at Keio Hospital between 2009 and 2016 were included in this retrospective study. BP diagnosis was based on clinical features, pathological findings and immunological findings. The study protocols were reviewed and approved by the institutional review board of the Keio University School of Medicine, and were conducted following the principles established by the Declaration of Helsinki. Written informed consent was obtained from all patients.

Direct and indirect immunofluorescence

For direct immunofluorescence (DIF) staining, skin biopsy specimens were embedded in optimum cutting temperature compound. Cryosections of these specimens were stained with the following antibodies: polyclonal antihuman IgG-fluorescein isothiocyanate (FITC) (F0202, working dilution 1 : 100; Dako, Glostrup, Denmark), polyclonal goat antihuman complement component (C3)-FITC (F0201, 1 : 20; Dako) and polyclonal goat antihuman IgE-FITC (affinity purified and heavy chain specific, A80-108F, 1 : 100; Bethyl Laboratories, Montgomery, TX, U.S.A.). All conjugated antibodies were diluted in phosphate-buffered saline. All of the slides were incubated for 1 h at room temperature. The intensity of the fluorescence was assessed by at least two trained dermatologists. As a positive control, we stained the tissues of patients with BP with C3 and used healthy human skin tissue as a negative control. For IgE scoring, tissues were classified as 'positive' if the deposition was strong and localized in the BMZ in a linear pattern (IgE+). Tissues were classified as 'weakly positive' if the deposition was relatively weak or appeared more granular than linear (IgE±). Those who had no deposition in the BMZ were classified as 'negative' (IgE−). Representative images of IgE+, IgE± and IgE− tissues are shown in Table S1 (see Supporting Information).

The presence of circulating IgE autoantibodies bound to the epidermal side of 1 mol L^{-1} NaCl-separated normal human skin was demonstrated by indirect immunofluorescence (IIF). Samples were classified as either positive (IgE+) or negative (IgE−). We confirmed that the antihuman IgE-FITC antibody does not nonspecifically bind to IgG by staining skin samples taken from patients with pemphigus vulgaris, and found no IgE deposition by DIF (data not shown).

Bullous Pemphigoid Disease Area Index scores

To evaluate disease severity, we used the Bullous Pemphigoid Disease Area Index (BPDAI), developed in 2011 by the International Pemphigus Committee.[13] Skin and mucosa are scored separately, for extent of erosion/blister (E/B) and for urticaria/erythema (U/E).[13] Higher scores indicate greater disease severity. The total score is calculated by summing the scores from all anatomical regions surveyed and can range from 0 to 120.

BPDAI scores of 15 patients were collected. These patients started prednisolone treatment (typically > 0·5 mg kg^{-1} per day) on day 1 of the study. Day 0 is considered the day prior to initial treatment. Individuals in this cohort had not previously received oral immunosuppressive medication. We classified BPDAI scores taken from day −2 to day 0 as day 0, days 5–9 as day 7, and days 12–16 as day 14. Two patients from the IgE± group were excluded because their day 14 BPDAI scores were not collected.

Enzyme-linked immunosorbent assay

Enzyme-linked immunosorbent assay (ELISA) was used to detect BP180-IgE and BP180-IgG (MBL, Nagoya, Japan). For IgE detection, samples were added into wells (50 μL, working dilution 1 : 100) and incubated for 1 h at 25–30 °C.

Horseradish peroxidase (HRP)-conjugated mouse antihuman IgE (affinity purified and heavy chain specific, A80-108P, working dilution 1 : 1000; Bethyl Laboratories) was added to each well for 1 h at 25–30 °C. We included all of the samples available regardless of their past history of treatment to secure a certain number of patients in each group. We established cut off values based on the average ± 3 SD (0·22 ± 0·092) levels detected in 20 serum samples from healthy people. We also investigated serum IgE BP180 levels of some samples after IgG absorption using protein G beads (working dilution 1 : 16·7, incubated for 1 h at 25–30 °C; GE Healthcare, Waukesha, WI, U.S.A.). Although slight cross-activity of HRP antihuman IgE to IgG was suggested, there was no change in the sensitivity and specificity for IgE ELISA, which was consistent with a previous report.[14]

Statistics

All statistical analyses were performed using GraphPad Prism (GraphPad Software, La Jolla, CA, U.S.A.). Statistical significance was defined as $P < 0·05$. The Mann–Whitney test was used to detect differences among the three groups: IgE+, IgE± and IgE−. To test for correlations, a Spearman rank correlation test was conducted.

Results

In total 53 patients with BP were included in this study: 26 male and 27 female. The mean patient age was 69·4 years (range 34–91). DIF revealed linear depositions of C3 in the BMZ, and 83% of patients (n = 44) tested positive for IgG deposition (IgG+). Out of 53 patients with BP, 19% (n = 10) were IgE+ and 25% (n = 13) were IgE±. In total, 43% (n = 23) of patients with BP had deposition of IgE (Table 1). All IgE+ patients were also positive for IgG and C3 deposition. All IgE± patients had linear deposition of C3, but four of them were negative for IgG deposition. The mean patient ages of the IgE+, IgE± and IgE groups were 64·3 ± 14·5, 76·5 ± 10·8 and 68·1 ± 13·4 years, respectively, with no significant differences.

Erosion/blister Bullous Pemphigoid Disease Area Index scores are significantly higher among IgE+ patients

We examined the relationship between IgE deposition and the BPDAI scores of the 15 patients mentioned above (Fig. 1). The BPDAI E/B scores were 38·0 ± 20·8, 36·3 ± 14·7 and 18·0 ± 11·2 in IgE+, IgE± and IgE− patients, respectively. IgE+ patients had significantly higher E/B scores than IgE− patients. The U/E scores were 24·6 ± 17·9, 28·0 ± 14·9 and 25·3 ± 8·26 in IgE+, IgE± and IgE− patients, respectively. Unlike the E/B scores, there were no significant differences in U/E scores between IgE+ and IgE− patients. It was difficult to compare mucosal BPDAI scores because only four patients had mucosal lesions. Of the four patients, one was IgE+, one was IgE± and the other two were IgE− by DIF analyses. When comparing BPDAI scores assigned to each of the 12 anatomical areas, none of the U/E scores were significantly different, whereas significant differences in E/B scores in several anatomical areas were found (see next section).

Table 1 Summary of staining intensities of IgE, IgG and C3 at the basement membrane zone (BMZ)

	IgG+	IgG−	Total
IgE+	10 (19)	0	10 (19)
IgE±	9 (17)	4 (8)	13 (25)
IgE−	24 (45)	6 (11)	30 (57)
Total	43 (81)	10 (19)	53

The data are presented as n (%). + indicates linear, ± indicates granular or partial, and − indicates no deposition at the BMZ. All of the patients were positive for C3 deposition. In total 23 of 53 samples (43%) had *in vivo* IgE deposition at the BMZ.

Improvements in erosion/blister Bullous Pemphigoid Disease Area Index scores after systemic corticosteroid treatment

Patients were followed after treatment with systemic prednisolone to track changes in BPDAI scores. The average E/B scores on day 7 are shown in Table 2. The scores were still significantly higher in IgE+ than in IgE− patients (Fig. 2a). At day 14 there were no significant differences between IgE+ and IgE− patients (Fig. 2b and Table 2). The average U/E scores on day 7 and day 14 are shown in Table 2; no significant differences were detected on either day (Fig. 2e, f). The E/B scores of the chest, right arm and left arm on day 0, and those of right arm and left arm on day 7 were significantly higher among IgE+ patients than IgE− patients ($P < 0·05$) (Fig. S1; see Supporting Information).

To examine the rate of symptom improvement after systemic prednisolone treatment, we calculated BPDAI score ratios for days 7 and 14 (day 7 BPDAI score ÷ day 0 BPDAI score, or day 14 BPDAI score ÷ day 0 BPDAI score) (Fig. 2). We excluded the patients whose scores were zero on day 0. The average day 7/day 0 and day 14/day 0 ratios for E/B and U/E are shown in Figure 2(c) and Table 2. None of the values were significantly different.

We also assessed the number of days it took to reduce E/B or U/E BPDAI scores by 75% compared with those before systemic prednisolone treatment started. It took 20 ± 8·0 days for IgE+ and IgE± patients, and 9·4 ± 3·8 days for IgE− patients to reduce their E/B BPDAI scores. IgE+ and IgE± patients required significantly more time than IgE− patients to improve their E/B BPDAI scores: hazard ratio 0·09, 95% confidence interval 0·02–0·55; $P < 0·01$ (Fig. 2d and Table 2). There were no significant differences in the improvement rates of U/E scores. It took 19 ± 5·7 days for IgE+ and IgE± patients, and 13 ± 5·9 days for IgE− patients to reduce their U/E BPDAI scores by 75% (Fig. 2h).

The 15 cases evaluated in this study are summarized in Table S2 (see Supporting Information). No significant

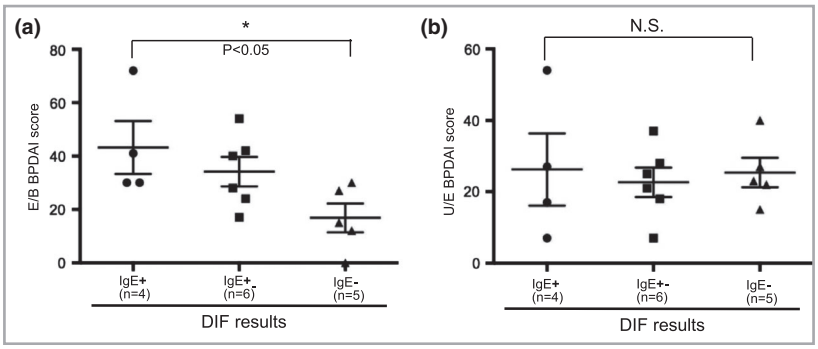

Fig 1. Relationships between IgE intensities and Bullous Pemphigoid Disease Area Index (BPDAI) scores. We divided patients into three groups according to the result of DIF (IgE+, IgE± and IgE−) and compared the erosion/blister (E/B) or urticaria/erythema (U/E) BPDAI scores. (a) The E/B scores of IgE+, IgE± and IgE− patients were 38·0 ± 20·8, 36·3 ± 14·7 and 18·0 ± 11·2, respectively. IgE+ patients had significantly higher E/B scores than IgE− patients ($P < 0·05$). (b) The U/E scores of IgE+, IgE± and IgE− patients were 24·6 ± 17·9, 28·0 ± 14·9 and 25·3 ± 8·26, respectively, with no significant difference (N.S.). The data are presented as the mean ± SEM. DIF, direct immunofluorescence.

Table 2 Changes in erosion/blister (E/B) or urticaria/erythema (U/E) Bullous Pemphigoid Disease Area Index scores after oral corticosteroid treatment

	IgE+	IgE±	IgE−
E/B day 7[a]	23·4 ± 10·2	21·5 ± 21·1	7·0 ± 7·7
E/B day 14[b]	13·8 ± 13·0	18·0 ± 20·9	2·0 ± 2·7
E/B day 7/day 0 ratio[b]	0·64 ± 0·28	0·54 ± 0·40	0·33 ± 0·25
E/B day 14/day 0 ratio[b]	0·38 ± 0·35	0·42 ± 0·34	0·078 ± 0·11
Time to reduce E/B by 75% (days)[c]	20 ± 8·0		9·4 ± 3·8
U/E day 7[b]	14·2 ± 13·7	19·0 ± 13·5	11·5 ± 7·2
U/E day 14[b]	5·3 ± 10·5	12·0 ± 5·0	0·83 ± 1·3
U/E day 7/day 0 ratio[b]	0·66 ± 0·32	0·56 ± 0·34	0·51 ± 0·23
U/E day 14/day 0 ratio[b]	0·097 ± 0·19	0·37 ± 0·22	0·032 ± 0·052
Time to reduce U/E by 75% (days)[b]	19 ± 5·7		13 ± 5·9

[a]$P < 0·05$ for IgE+ vs. IgE−. [b]No significant differences. [c]For IgE+ and IgE± vs. IgE−, hazard ratio 0·09, 95% confidence interval 0·02–0·55; $P < 0·01$.

differences in patients' ages, duration of disease before diagnosis, initial dose of prednisolone, time to remission or recurrence rates were found.

BP180-IgE enzyme-linked immunosorbent assay indices

Of the 53 patients for whom we examined IgE deposition by DIF, we could examine 10 patients for the levels of BP180-IgE by ELISA. The BP180-IgE ELISA indices of those 10 patients in the IgE+, IgE± and IgE− groups were 23·8 ± 14·3, 1·45 ± 1·45 and 7·4 ± 6·96, respectively. Serum levels of BP180-IgE were significantly higher in IgE+ patients than in IgE± and IgE− patients (Fig. 3a and Table 3). We also examined IgE binding to the BMZ in those 10 patients by IIF staining, but there were no significant differences between IgE+ and IgE− patients in the IgE IIF analyses (Fig. 3b).

Next, we referred to the medical charts of the 53 patients, and the BP180-IgG ELISA indices of 33 patients were available. The BP180-IgG ELISA indices were not significantly different among the three groups (Fig. 3c and Table 3). Total IgE levels in the sera of 22 patients, and eosinophil counts in the blood of 33 patients were also available from the charts, and no significant differences were found in serum levels of IgE and blood eosinophil counts (Fig. 3d, e and Table 3).

Lastly, we focused on the relationship between serological findings and clinical presentation. We examined the ELISA indices for BP180-IgE and BP180-IgG of 21 patients with BP whose sera were available, including those for whom we could not perform IgE DIF because there were not enough skin samples. We investigated the correlation among BPDAI scores, BP180-IgG ELISA indices and BP180-IgE ELISA indices. We could obtain BPDAI scores for 12 of the 21 patients, and neither E/B nor U/E BPDAI scores correlated with BP180-IgG or BP180-IgE ELISA indices (Fig. 4a). The ELISA indices for BP180-IgG and BP180-IgE correlated with each other, with BP180-IgE ranging from 0 to 123·1 and BP-IgG ranging from 0 to 435·2 (Fig. 4b). We did not identify any other significant relationships among these three parameters.

Discussion

We found that patients with BP with linear IgE deposition in the BMZ had significantly higher E/B BPDAI scores than those without IgE deposition, while U/E BPDAI scores were not

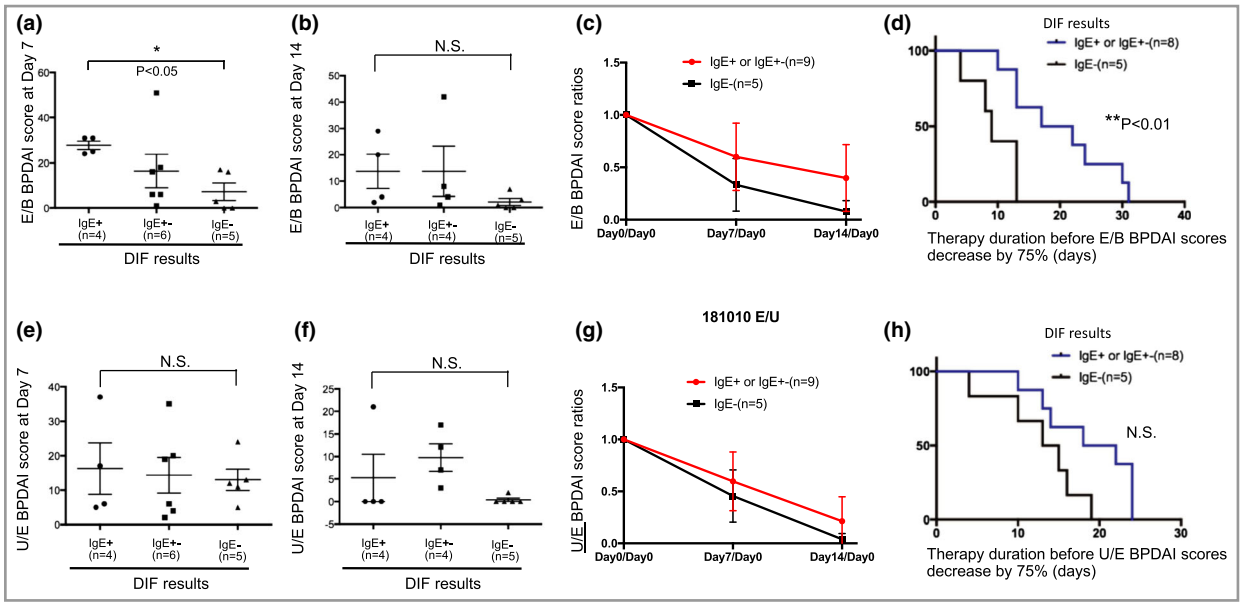

Fig 2. Changes in the erosion/blister (E/B) or urticaria/erythema (U/E) Bullous Pemphigoid Disease Area Index (BPDAI) scores after oral corticosteroid treatment had started. (a) E/B scores on day 7. (b) E/B/ scores on day 14. (c) Day 7/day 0 E/B ratios. (d) Time to reduce E/B scores by 75%. (e) U/E scores on day 7. (f) U/E scores on day 14. (g) Day 7/day 0 U/E ratios. (h) Time to reduce U/E scores by 75%. The data are also tabulated in Table 2. In (d) and (h) the vertical line shows the percentage of patients whose BPDAI score had not decreased by > 75%. N.S., not significant. The data in (a, b, e, f) are presented as the mean ± SEM. DIF, direct immunofluorescence.

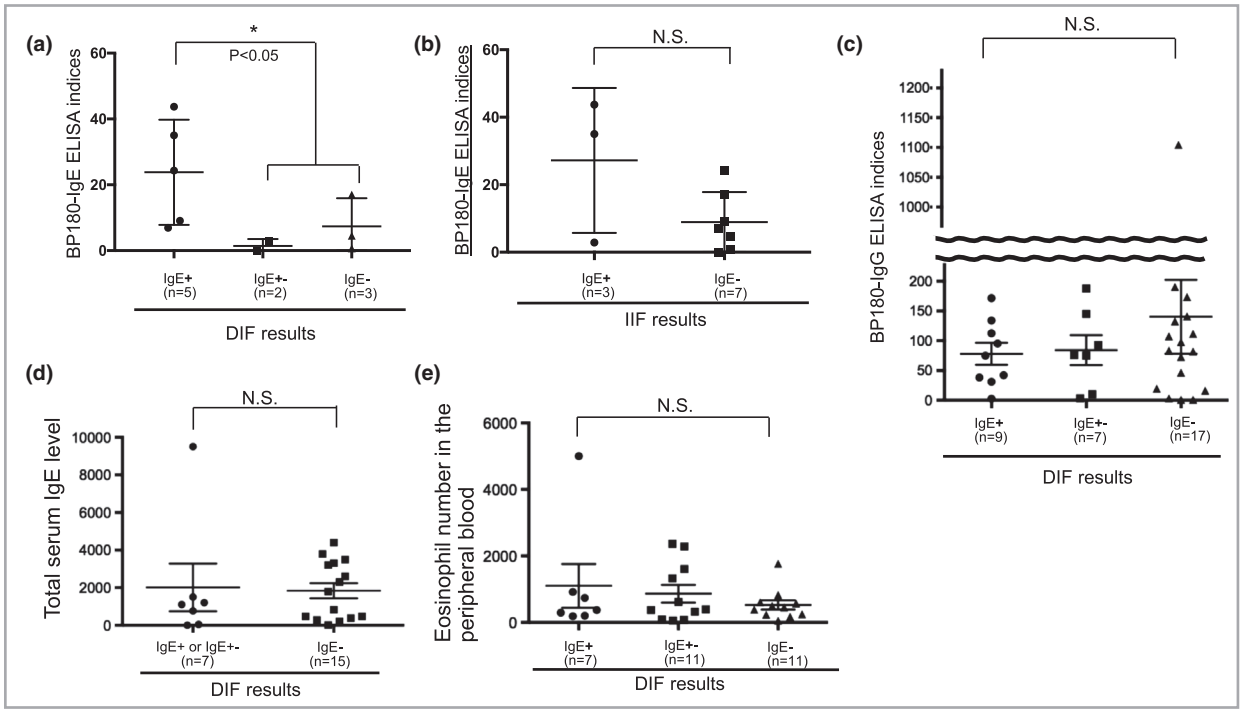

Fig 3. Relationships between serological features and direct and indirect immunofluorescence (DIF and IIF) results. (a) Ten patients whose serum was taken within 2 days of the date of biopsy were examined. (b) IIF using serum samples from the same 10 patients. (c) BP180-specific serum IgG enzyme-linked immunosorbent assay (ELISA) indices. (d) Total serum IgE levels. (e) Average eosinophil counts. The data are also tabulated in Table 3. N.S., not significant. The data are presented as the mean ± SEM.

significantly different. Although no significant difference was found, the average E/B BPDAI scores were highest in the IgE+ group, followed by IgE± and IgE−. Therefore, it appears that the relative quantity of IgE bound to skin may have some influence on E/B BPDAI scores. In addition, it took IgE+ and IgE± patients significantly longer than IgE− patients to improve their

Table 3 Relationships between serological features and direct and indirect immunofluorescence (DIF and IIF) results

	IgE+	IgE±	IgE−
BP180-IgE ELISA DIF[a, b]	23·8 ± 14·3	1·45 ± 1·45	7·4 ± 6·96
BP180-IgE ELISA IIF[c]	27·2 ± 17·5	–	8·9 ± 8·2
BP180-IgG ELISA[c]	78·1 ± 51·8	84·4 ± 61·7	140 ± 248
Serum IgE (IU mL^{-1})[d]	2010 ± 3100		1836 ± 1489
Eosinophil count (cells μL^{-1})[c]	1001 ± 1391	800 ± 831	891 ± 1292

ELISA, enzyme-linked immunosorbent assay. [a]$P < 0·05$ for IgE+ vs. IgE± or IgE−. [b]5·02 ± 6·12 for IgE+ and IgE± combined. [c]No significant differences. [d]IgE+ and IgE± were combined due to limited numbers.

E/B BPDAI scores after treatment with systemic prednisolone. Our results suggest that IgE deposition in the BMZ influences the formation or excoriation of erosions and blisters. We also confirmed that IgE+ patients had significantly higher serum levels of BP180-IgE than IgE− patients, and that there is a positive correlation between serum BP180-IgE and BP180-IgG levels. Collectively our observations imply that BP180-IgE autoantibodies are involved in the pathogenesis of BP. We did not find any relationship between serum BP180-IgE levels and BPDAI scores. This may be partly because the number of patients was limited, but we can also postulate that binding of mast cells in the dermis has some influence on the pathogenesis in BP. It is of interest to investigate the relationships between mast cells in the dermis and serum IgE levels.

The possible disease mechanism of IgE involves IgE receptor (FcεRI)-dependent and FcεRI-independent pathways. A previous report showed that anti-BP180 IgE autoantibodies decreased the number of hemidesmosomes in the BMZ in primary keratinocytes or organ-cultured skin by internalization of BP180, which occurred independently of FcεRI activation.[15] The internalization of BP180 possibly weakened the adhesive strength of the BMZ.[4] Likewise, the release of the cytokines interleukin-6 and interleukin-8 from cultured human keratinocytes after incubation with IgE isolated from BP sera has been reported.[15,16] Together, these reports lend support to our hypothesis that anti-BP180 IgE leads to the development of blisters rather than erythemas in BP. FcεRI-dependent mechanisms have also been described in several reports. Mast cell degranulation represents an early phase of BP disease progression. Studies have shown that BP180 and FcεRI-bound IgE colocalize on mast cells in BP skin samples.[4,17] Furthermore, basophils and eosinophils in the blood may be activated by the binding of autoantibodies to BP180.[1,18] Crosslinking of IgE derived from BP sera with BP180 in FcεRI-expressing rat basophils resulted in robust degranulation of these cells.[17] Together, these findings indicate that IgE antibody–antigen crosslinking to FcεRI initiates a signalling cascade that promotes the release of proinflammatory mediators such as histamines and cytokines.

A pathogenic role for IgE has also been postulated for chronic spontaneous urticaria (CSU). This skin condition is associated with elevated serum levels of IgE, which displays affinity for several autoantigens including membrane-bound proteins.[19] The effects of omalizumab on CSU have been demonstrated in several double-blinded randomized placebo-controlled studies.[19] In one study, IgG autoantibodies against FcεRI or IgE were detected in almost half of patients with CSU and were found to be associated with a longer duration of disease and poor treatment responses to antihistamines.[19] It is possible that anti-BP180 IgE exacerbates symptoms by acting jointly with IgG in BP.

IgE involvement is also indicated in other bullous diseases and atopic dermatitis (AD). Elevated IgE levels have been described in patients with linear IgA bullous dermatosis, herpes gestationis and endemic pemphigus foliaceus.[1] In a patient with epidermolysis bullosa acquisita, high serum IgE levels

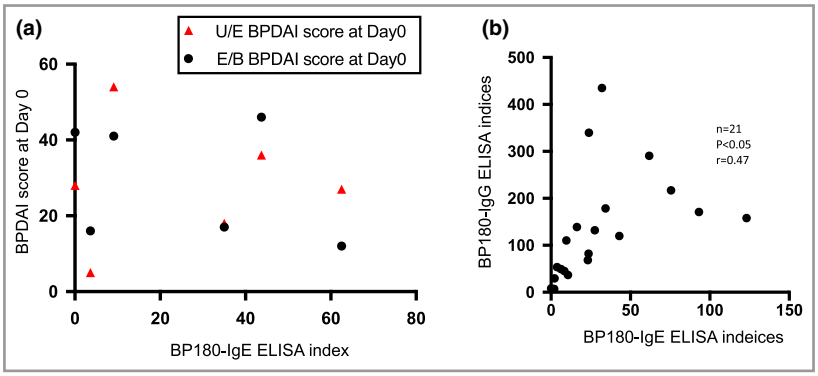

Fig 4. Relationships between BP180-IgE, BP180-IgG and Bullous Pemphigoid Disease Area Index (BPDAI) scores. The BP180-IgE enzyme-linked immunosorbent assay (ELISA) indices ranged from 0 to 123·1, and those of BP180-IgG ranged from 0 to 435·2. Patients whose BPDAI scores were taken within 2 days from the date of biopsy were examined. Neither erosion/blister (E/B) nor urticaria/erythema (U/E) BPDAI scores correlated with BP180-IgE ELISA index. BP180-specific IgG and BP180-specific IgE were found to correlate with each other ($P = 0·03$, $r = 0·47$). We could not find any other correlations among these parameters.

were correlated with disease activity.[20] IgE binding in the BMZ was found in 69% of patients with mucous membrane pemphigoid.[6] In addition, although AD has conventionally been considered an allergic disease, sera of patients with severe AD contain IgE for autoantigens linked to the development of skin lesions.[21]

The IgE immune response occurs via FcεRI or FcεRII.[22] IgE binding to its high-affinity receptor, FcεRI, is well known to facilitate degranulation of mast cells and basophils and promote T helper 2 immunity.[19] Low-affinity engagement of FcεRI reduces degranulation and cytokine production but can promote immune cell recruitment and inflammation in tissues.[23] Therefore, assessing the affinity of antigens to IgE represents an important key to understand the pathomechanism of BP.

In conclusion, our results suggest that IgE autoantibodies in the BMZ contribute to the development or exacerbation of erosions and blisters in BP. In line with previous work, our results indicate that anti-IgE therapy such as omalizumab is effective for curing not only erythema and urticaria but also erosions and blisters. Clarifying the mechanisms by which BMZ-associated IgE has its effects is essential in the effort to find safe and effective therapies for patients with BP.

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's website:

Fig S1. Erosion/blister Bullous Pemphigoid Disease Area Index scores from each of the 12 anatomical areas at (a) day 0 and (b) day 7.

Table S1 Intensity classification in the direct immunofluorescence staining of specimens from patients with bullous pemphigoid.

Table S2 Disease demographics and response to therapy of 15 patients with bullous pemphigoid followed with the Bullous Pemphigoid Disease Area Index.