

Detection of anti-type VII collagen IgE antibodies in epidermolysis bullosa acquisita

H. Koga ¹, K. Teye,² K. Yamashita,¹ N. Ishii,¹ D. Tsuruta³ and T. Nakama¹

¹Department of Dermatology, Kurume University School of Medicine, Fukuoka, Japan

²Kurume University Institute of Cutaneous Cell Biology, Fukuoka, Japan

³Department of Dermatology, Osaka City University Graduate School of Medicine, Osaka, Japan

Linked Comment: Ludwig. *Br J Dermatol* 2019; **180**:981–983.

Summary

Correspondence

Hiroshi Koga.

E-mail: hiroshi_koga@med.kurume-u.ac.jp

Accepted for publication

6 October 2018

Funding sources

None.

Conflicts of interest

None declared.

DOI 10.1111/bjd.17310

Background Epidermolysis bullosa acquisita (EBA) is a rare pemphigoid disease involving autoantibodies to type VII collagen (COL7), a major structural component of anchoring fibrils. IgE autoantibodies to type XVII collagen (BP180) have been identified in bullous pemphigoid (BP), the prototype of pemphigoid diseases. Although the pathogenic relevance of IgG anti-COL7 has been investigated, that of IgE in EBA remains unclear.

Objectives To reveal the presence and pathogenic relevance of IgE anti-COL7 in EBA.

Methods We examined IgE antibodies in 109 patients with EBA by indirect immunofluorescence (IIF) and enzyme-linked immunosorbent assay (ELISA).

Results IIF with normal human skin revealed IgE reactivity in the basement membrane zone in 29 (26.6%) cases. To verify whether the IgE antibodies were specific to COL7, we performed IIF with 21 clearly positive cases and the skin of a patient with dystrophic EBA, which does not involve COL7. All cases showed negative results, indicating that IgE antibodies were specific to COL7. In a modified IgG COL7 ELISA for IgE, 16 (14.7%) cases were positive (three and 13 cases were negative and positive on IIF, respectively). We compared anti-COL7 IgG and IgE, and found a weak but significant correlation ($r = 0.459$, $P < 0.001$). EBA is clinically divided into a mechanobullous (MB; noninflammatory) type and an inflammatory (INF) type resembling BP. Of the IIF-positive cases, 11 of 30 (37%) had INF and nine of 48 (19%) had MB.

Conclusions This study is the first to demonstrate the presence of circulating anti-COL7 IgE in patients with EBA, which may correlate with the clinical phenotype.

What's already known about this topic?

- Epidermolysis bullosa acquisita (EBA) is a rare pemphigoid disease in which autoantibodies, mainly IgG, target type VII collagen.
- In bullous pemphigoid, the prototype of pemphigoid diseases, IgE autoantibodies to type XVII collagen (BP180) have been detected and are reported to be associated with disease severity.
- However, no study has yet investigated the presence of IgE autoantibodies to type VII collagen in EBA.

What does this study add?

- IgE anti-type VII collagen antibodies were also detected in sera from patients with EBA, with concomitant elevated total IgE.
- IgE anti-basement membrane zone-positive cases appear to occur more frequently in inflammatory-type EBA than in noninflammatory-type EBA.
- IgE anti-type VII collagen may correlate with the clinical phenotype in EBA.

What is the translational message?

- IgE and type VII collagen-specific IgE could be a therapeutic target for EBA.

Pemphigoid diseases are organ-specific autoimmune disorders that are characterized by circulating autoantibodies to structural proteins in the epidermal basement membrane zone (BMZ) and deposition of autoantibodies on the BMZ. Epidermolysis bullosa acquisita (EBA) is a rare pemphigoid disease involving autoantibodies to type VII collagen (COL7), a major structural component of anchoring fibrils.¹ The immunoglobulin isotype of autoantibodies deposited in skin is mainly IgG, but IgA and IgM have also been reported.^{2–4}

In terms of clinical phenotype, EBA is a heterogeneous subepidermal blistering disease that is classified into two major types: (i) the classical/mechanobullous type and (ii) the nonclassical/nonmechanobullous type. The former is characterized by bullous/vesicular lesions or erosions preferentially localized to trauma-prone sites (dorsal hands, elbows, knees and feet) with scarring and milia formation. Nail dystrophy may also be observed. The latter type is characterized by pruritus and tense bullae on erythematous or urticarial skin that develops on the trunk and extremities, resembling bullous pemphigoid (BP), mucous membrane pemphigoid and Brunsting-Perry-type pemphigoid.⁵ Generally, the EBA phenotype can be classified into a mechanobullous (MB) type and an inflammatory (INF) type (BP-like form). The MB type is the most common in Europe, whereas the INF type is more common in Asia.⁵

Elevation of total serum IgE levels in BP has been reported.^{6,7} Furthermore, IgE antibodies to type XVII collagen (BP180) have also been identified in BP, the prototype of pemphigoid diseases.^{8,9} Serum levels of anti-BP180 IgE were found to correlate with disease severity, scored by the Bullous Pemphigoid Disease Area Index,¹⁰ although conflicting results also have been reported.^{11,12} In one *in vivo* study, anti-BP180 IgE purified from the serum of patients with BP induced erythema on grafted human skin in severe combined immunodeficient (SCID) mice, suggesting an association between anti-BP180 IgE and erythema.¹³ In contrast, the scores for urticarial/erythematous lesions did not correlate with serum levels of anti-BP180 IgE in patients with BP.¹⁰ Regarding IgE-specific treatment, the use of omalizumab, a humanized monoclonal antibody that inhibits IgE binding to its high-affinity receptor (FcεRI), has been reported for the treatment of patients with BP.¹⁴

However, serum IgE levels and the presence of antigen-specific IgE in EBA have not been adequately studied thus far. There is only one case report showing a correlation of IgE levels with disease activity during treatment in a patient with EBA,¹⁵ and other reports have shown deposition of IgE on direct immunofluorescence (DIF).^{16–18} In addition, anti-BMZ IgE was reported in canine EBA.¹⁹ No study has yet evaluated

the presence of anti-COL7 IgE in patients with EBA. Therefore, in this study, we examined IgE antibodies, including their specificity to COL7 and their correlation to the clinical phenotype, in patients with EBA.

Materials and methods

Patients

A total of 109 patients with EBA were enrolled based on (i) compatible clinical features, (ii) IgG reaction on the dermal side by indirect immunofluorescence (IIF) of salt-split skin; and (iii) detection of IgG antibodies to COL7 by Western blotting using a dermal extract of human skin and/or enzyme-linked immunosorbent assay (ELISA). The study was approved by the ethics committee of Kurume University and was performed in adherence with the Declaration of Helsinki guidelines.

Indirect immunofluorescence

Frozen sections of normal human skin were used to detect anti-BMZ IgE antibodies in the patients' sera using the following protocol. After rinsing with phosphate-buffered saline (PBS), frozen sections were blocked with 5% normal goat serum in Tris-buffered saline (TBS; 10 mmol L⁻¹ Tris, 150 mmol L⁻¹ NaCl, pH 7.2) for 30 min at room temperature, followed by incubation with a 1 : 10 dilution of serum samples in TBS containing 0.1% casein and 0.01% Tween 20 for 90 min at room temperature. After washing with PBS, the sections were incubated with DyLight 488-conjugated antihuman IgE (NBP1-74936; Novus Biologicals, Littleton, CO, U.S.A.) diluted 1 : 100 with TBS containing 0.1% casein for 60 min at room temperature. No cross-reactivity of DyLight 488-conjugated antihuman IgE to human IgG was confirmed (Fig. S1; see Supporting Information). The intensity of positivity on BMZ was judged as strongly positive (++), positive (+), weakly positive (+/–) and negative (–) by an investigator unaware of the patient's status. Clearly positive cases (++ and +), obtained by immunofluorescence using normal human skin, were tested along with the skin of a patient with dystrophic epidermolysis bullosa, which does not involve COL7, as confirmed by negative findings on immunofluorescence using anti-COL7 antibody (LH7-2; Sigma, St Louis, MO, U.S.A.).

Enzyme-linked immunosorbent assay

The anti-type VII collagen ELISA Kit (MBL, Nagoya, Japan) was used for the detection of anti-COL7 IgG in the patients'

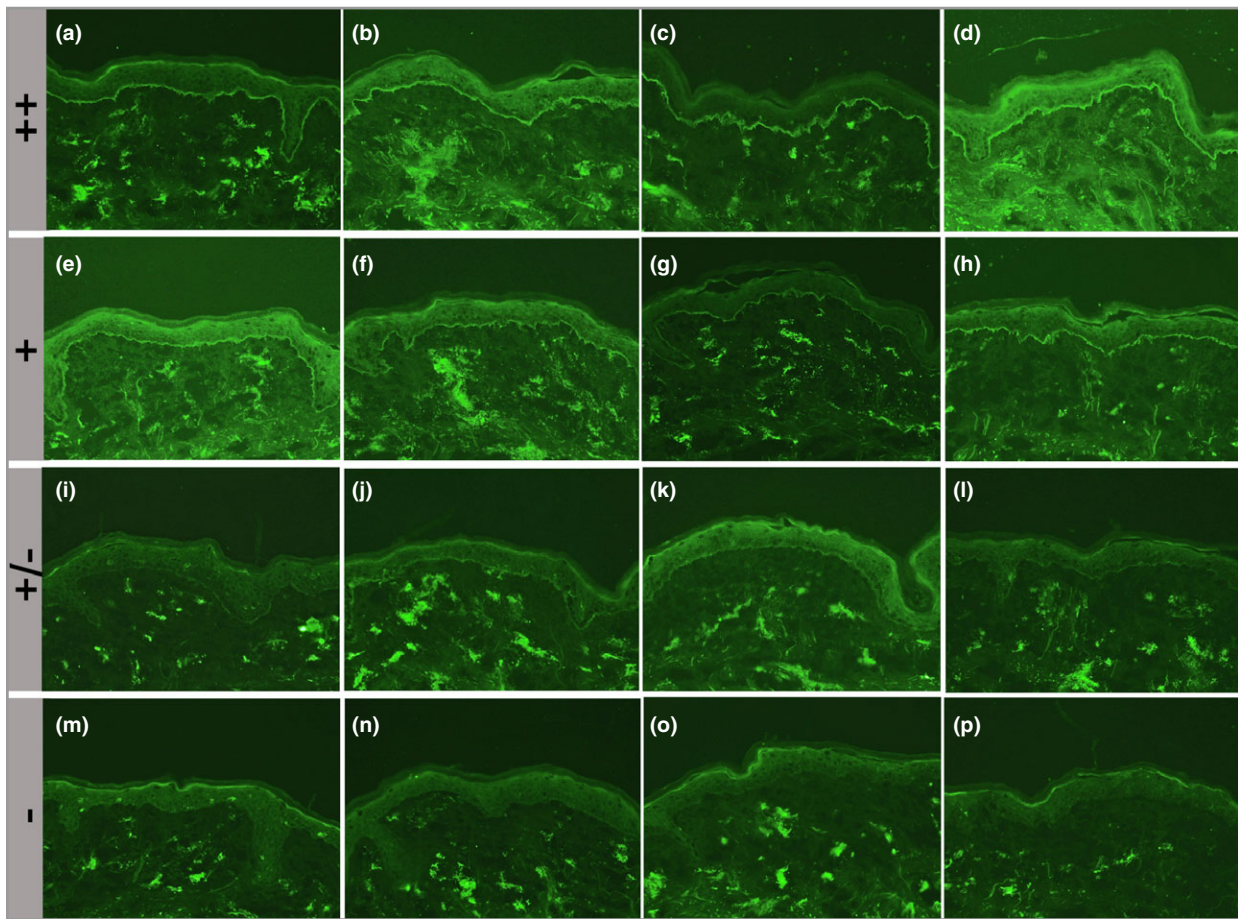


Fig 1. Detection of the anti-epidermal basement membrane zone (BMZ) IgE antibodies in sera from patients with epidermolysis bullosa acquisita (EBA). Indirect immunofluorescence with normal human skin was performed to detect circulating IgE antibodies to BMZ, which were visualized by DyLight 488-conjugated antihuman IgE antibody in sera from patients with EBA. Intensity of positivity on BMZ was judged as (a–d) strongly positive (++); (e–h) positive (+); (i–l) weakly positive (+/-); and (m–p) negative (-). Four representative photos from each group are shown.

sera according to the manufacturer's instructions. To detect specific anti-COL7 IgE in the patients' sera, the same kit was used with a modified protocol as reported previously.⁸ No cross-reactivity of horseradish peroxidase-conjugated antihuman IgE to human IgG was confirmed (Fig. S1; see Supporting Information). The cut-off optical density (OD) value was set as the mean +3 SD (0.0596) of the sera from 41 healthy individuals.

For the measurement of total IgE, the IgE ELISA Quantitation Set (Bethyl Laboratories, Montgomery, TX, U.S.A.) was used according to the manufacturer's instructions.

Statistical analysis

Data are presented as mean $\pm$ SD. Statistical analyses of the results of IIF in the MB and INF types or in cases with and without mucosal involvement were performed using Fisher's exact test. Statistical analysis of the IgE anti-COL7 ELISA of the healthy volunteers (normal) and the patients was performed using the Mann–Whitney U-test (two-tailed). All statistical analyses were performed using GraphPad Prism (Version 6.05;

GraphPad Software, San Diego, CA, U.S.A.). A P-value of 0.05 was considered to be statistically significant.

Results

Detection of anti-basement membrane zone IgE in sera from patients with epidermolysis bullosa acquisita

We examined anti-BMZ IgE antibodies in patients' sera by IIF using normal human skin. IgE reactivity on BMZ was found in 29 (26.6%) of 109 cases (eight strongly positive; 13 positive; eight weakly positive) (Fig. 1).

Anti-basement membrane zone IgE antibodies in sera from epidermolysis bullosa acquisita are specific to type VII collagen

To detect whether anti-BMZ IgE in the sera of patients with EBA are specific to COL7, we further performed IIF, with 21 clearly positive cases using the skin of a patient with dystrophic epidermolysis bullosa, which does not involve COL7.

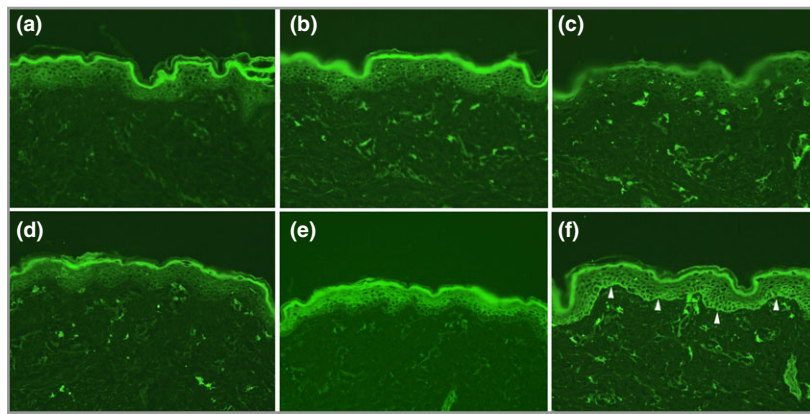


Fig 2. Specificity of IgE antibodies to type VII collagen (COL7) in IgE indirect immunofluorescence (IIF)-positive cases. IIF using skin from a patient with dystrophic epidermolysis bullosa, which does not involve COL7, was performed for clearly positive cases [(++) and (+) in Fig. 1]. Sera from all cases showed negative reactivity to the basement membrane zone (BMZ). (a–d) Representative photos of the results of the patients' sera [they are identical to the cases in (a–d) in Fig. 1], (e) anti-COL7 antibody (LH7·2) as a negative control and (f) serum from a patient with bullous pemphigoid with anti-BP180 IgE antibodies as a positive control are shown. Arrowheads indicate positive findings on BMZ.

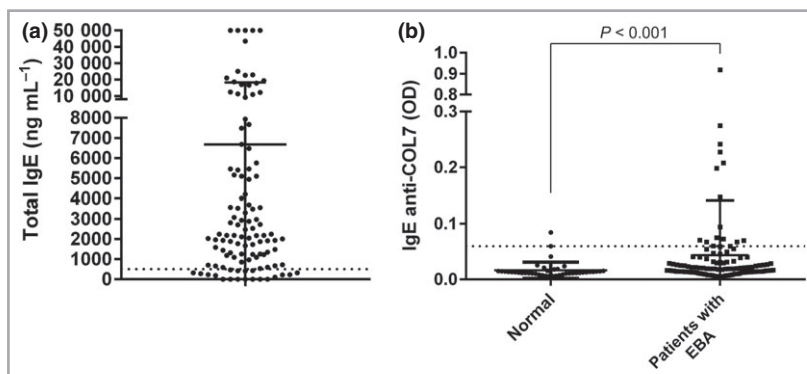


Fig 3. Serum concentrations of total IgE and anti-type VII collagen (COL7) IgE in patients with epidermolysis bullosa acquisita (EBA). (a) Total IgE concentrations in sera from patients with EBA ($n = 109$) were elevated. (b) A commercial COL7 enzyme-linked immunosorbent assay using a modified protocol for IgE detection tested with sera from healthy volunteers ($n = 41$) and sera from patients with EBA ($n = 109$) revealed significantly higher optical density (OD) values in the patients with EBA ($P < 0.001$). The dotted lines indicate the upper normal limit in (a) and the cut-off values in (b). Each dot indicates the results from each sample.

Notably, all 21 cases were negative (Fig. 2a–d). Negative staining with anti-COL7 antibody confirmed the deficiency of COL7 in the skin section (Fig. 2e), whereas positive staining with serum from a patient with BP having anti-BP180 IgE antibodies confirmed the reliability of this assay (Fig. 2f). Together, these results indicated that the anti-BMZ IgE antibodies from the EBA sera were specific to COL7.

Elevation of total serum IgE in patients with epidermolysis bullosa acquisita

In BP, elevated levels of total serum IgE have been reported,^{6,7} whereas only one study showed elevated serum IgE in a patient with EBA.¹⁵ Therefore, we examined the concentration of total IgE in sera from 109 patients with EBA using the commercial ELISA kit. Similar to BP, the serum level of total IgE was also elevated in EBA ($6682 \pm 11658 \text{ ng mL}^{-1}$) (2.4

$\text{ng mL}^{-1} = 1 \text{ IU mL}^{-1}$; normal reference interval in adults $2\text{--}214 \text{ IU mL}^{-1} = 4.8\text{--}513.6 \text{ ng mL}^{-1}$) (Fig. 3a).^{20,21}

Detection of anti-type VII collagen IgE by enzyme-linked immunosorbent assay and correlation to anti-type VII collagen IgG enzyme-linked immunosorbent assay index

We also sought to detect anti-COL7 IgE using a commercially available ELISA kit with a modified protocol as previously reported for BP.⁸ The preadsorption of IgG by protein G may help to increase the sensitivity of the IgE ELISA, although our previous study on BP showed no significant difference in positivity between pre- and postadsorption.⁸ Comparing the OD values of the sera from 16 patients with EBA with and without adsorption by ELISA, the results showed almost identical OD values in the samples with and without adsorption (Fig. S2; see Supporting Information). Based on this observation, we

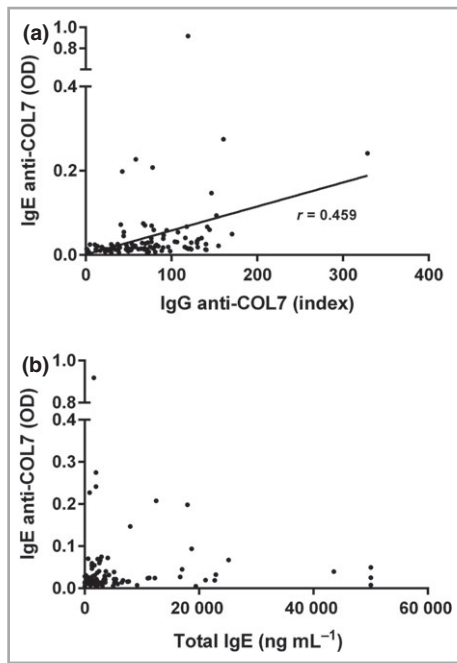


Fig 4. Correlations between anti-type VII (COL7) IgG and IgE and between anti-type VII IgE and total IgE in serum. (a) A scatter diagram shows the correlation between anti-COL7 IgE (optical density; OD) and anti-COL7 IgG (index) concentrations in serum (Pearson's correlation coefficient: $r = 0.459$, $P < 0.001$). (b) A scatter diagram shows the correlation between anti-COL7 IgE (OD) and total IgE (ng mL^{-1}) concentrations in serum. No correlation was significant ($P = 0.84$). Each dot indicates the results from each patient ($n = 109$).

examined the sera of 41 healthy donors and 109 patients with EBA without adsorption of IgG by ELISA. The OD values were significantly higher in the patients with EBA than in the healthy donors (0.043 ± 0.097 vs. 0.016 ± 0.014 ; $P < 0.001$) (Fig. 3b). When the cut-off value was set as the mean +3 SD of the OD of healthy donors, 16 (14.7%) cases of EBA were positive (three and 13 cases were negative and positive on IIF, respectively). When we compared the anti-COL7 IgG (index) and IgE (OD) measured by ELISA in 109 cases of EBA, a moderate correlation was found ($r = 0.459$, $P < 0.001$) (Fig. 4a). We also tested for a correlation between anti-COL7 IgE and total IgE; however, no correlation was found ($P = 0.84$) (Fig. 4b).

Relationship between phenotype and positivity of anti-basement membrane zone IgE in epidermolysis bullosa acquisita

We evaluated the association between anti-BMZ IgE positivity detected by IIF and clinical phenotype (erythema as defined by INF type and oral involvement) (Table 1). Clinical data regarding phenotype and mucosal involvement were available in 78 patients with EBA. Of these, 48 (62%) had the MB type and 30 (38%) had the INF type. Of note, a relatively higher rate of IIF-positive cases was observed in the INF type (37%) than in the MB type (19%), although there was no significant

Table 1 Association between results of indirect immunofluorescence (IIF) and clinical phenotypes and mucosal involvements

	Anti-BMZ IgE in IIF		Total
	Negative	Positive	
Phenotype			
MB type	39 (81)	9 (19)	48 (62)
INF type	19 (63)	11 (37)	30 (38)
Mucosal lesion			
+	44 (76)	14 (24)	58 (74)
–	14 (70)	6 (30)	20 (26)

Data are n (%). BMZ, basement membrane zone; MB, mechanobullous; INF, inflammatory.

difference ($P = 0.11$). Next, a correlation between mucosal involvement and IgE IIF positivity was analysed, and 58 (74%) cases had mucosal involvement. The rates of IIF-positive cases were almost identical in cases with (24%) and without (30%) mucosal lesions.

Discussion

We found circulating IgE antibodies to BMZ in 29 (26.6%) of 109 cases with EBA. IIF using the skin of a patient with dystrophic epidermolysis bullosa revealed that the IgE antibodies were specific to COL7 in all 21 tested cases whose sera clearly showed IgE reactivity to BMZ in normal human skin. Like BP, the autoantigen in EBA, COL7 was recognized by IgE subclass antibodies in patients with EBA. We also found an elevation in total IgE in the sera from patients with EBA. These results suggest that IgE and IgE switch cytokines, such as interleukin (IL)-4 and IL-13, may be implicated in the pathogenesis of EBA.²²

In addition, we showed that a commercially available COL7 ELISA kit was useful for the detection of anti-COL7 IgE in EBA using a modified protocol; 16 (14.7%) cases were positive in this study. We tested sera with and without adsorption by

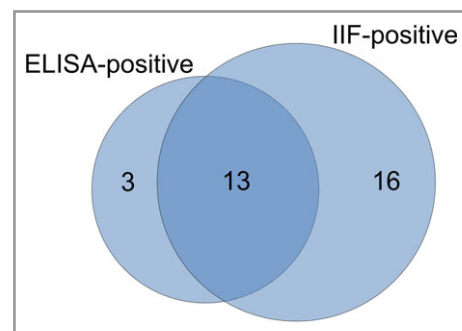


Fig 5. Correlation between indirect immunofluorescence (IIF) and enzyme-linked immunosorbent assay (ELISA) positivity in patients with epidermolysis bullosa acquisita. A Venn diagram indicates the pattern of results on IIF and ELISA. Each number indicates a positive case.

protein G. Similar to the sera of patients with BP, antigen-specific IgE antibodies in the sera of patients with EBA could be effectively measured without adsorption. The commercially available COL7 ELISA kit from MBL is made with recombinant proteins of the noncollagenous domains (NC-1 and NC-2) of COL7, which are recognized by the IgG of patients with EBA. The IIF and ELISA results are summarized in Figure 5. The finding that 16 of 29 IIF-positive cases showed negative IgE ELISA results suggested that some anti-COL7 IgE recognized epitopes outside of the NC-1 and NC-2 domains. Meanwhile, variations in reactivity and sensitivity between IIF and ELISA should be considered. To validate this possibility, further epitope mapping to anti-COL7 IgE is required.

We previously demonstrated a correlation between anti-BP180 IgG and IgE in patients with BP.⁸ Similarly, in the present study, anti-COL7 IgG (index) and IgE (OD) measured by ELISA had a moderate relevance ($r = 0.459$, $P < 0.001$). Although this possibly resulted in the cross-reactivity of the anti-IgE secondary antibody to anti-COL7 IgG antibodies, we demonstrated its specificity (Fig. S1; see Supporting Information). The finding of no correlation between anti-COL7 IgE and total IgE in this study ($P = 0.84$) suggested that an elevation of total IgE in serum does not simply result in anti-COL7 IgE. These results may suggest that an immunoglobulin class switch in B cells recognizing COL7 is involved in the pathogenesis of EBA. To further elucidate this, a fine epitope mapping or B-cell receptor sequence would have to be performed.

The higher prevalence of IgE IIF-positive cases in the INF type than in the MB type suggested that anti-BMZ IgE, namely anti-COL7 IgE in this study, was associated with the inflammatory phenotype (erythema) in EBA, although there was no statistical significance. One limitation of this study was that we were unable to obtain information regarding the clinical manifestation from all the patients, although the frequency of mucosal involvement (74%) in our study was similar to previous reports.²³ In addition, we tested only serologically positive cases; therefore, this study also had a limitation in terms of case enrolment, as the serration pattern in DIF is a more sensitive test for EBA than ELISA and/or IIF.²⁴ To reveal the association between anti-COL7 IgE and the inflammatory phenotype in EBA, a large number of patients with EBA should be included in further studies.

Anti-COL7 IgE may play a role in the pathogenesis of EBA. Unfortunately, we did not clearly address this point in this study. However, we suspect the pathogenicity of anti-COL7 IgE via eosinophils and mast cells, expressing FcεRI themselves, given the previous results that BP antigen-specific IgE was associated with inflammatory cells (eosinophils and mast cells) in a previous study on BP.²⁵ In line with this hypothesis, a previous report of an EBA case with IgE deposition on the BMZ showed eosinophil infiltration on histology.¹⁷ Among our cases, we found descriptions of eosinophil infiltration on histology in two cases that were positive for anti-type VII collagen on IgE ELISA and IgE IIF. Continued accumulation of cases is necessary to analyse the association between anti-COL7 IgE and eosinophils.

Alternatively, it is possible to analyse the pathogenesis of anti-COL7 IgE in patients with EBA by injecting purified IgE from a patient with positive reactivity on COL7 IgE ELISA into human skin-grafted SCID mice, according to a previous study on BP;¹³ however, we did not have a sufficient amount of serum that showed positive reactivity on COL7 IgE ELISA. Further investigations are necessary to determine the pathogenesis of anti-COL7 IgE in patients with EBA.

To conclude, this study revealed that IgE antibodies specific to the related antigen exist in EBA, similar to BP. Specific treatment to target IgE, such as omalizumab, may be useful in EBA.

Acknowledgments

We appreciate the technical assistance of Ms Michiru Kubo and Kyoko Hiromatsu.

References

- Schmidt E, Zillikens D. Pemphigoid diseases. *Lancet* 2013; **381**:320–32.
- Delgado L, Aoki V, Santi C *et al.* Clinical and immunopathological evaluation of epidermolysis bullosa acquisita. *Clin Exp Dermatol* 2011; **36**:12–18.
- Hashimoto T, Jin Z, Ishii N. Clinical and immunological studies for 105 Japanese seropositive patients of epidermolysis bullosa acquisita examined at Kurume University. *Expert Rev Clin Immunol* 2016; **12**:895–902.
- Ludwig RJ, Vanhoorelbeke K, Leypoldt F *et al.* Mechanisms of autoantibody-induced pathology. *Front Immunol* 2017; **8**:603.
- Prost-Squarcioni C, Caux F, Schmidt E *et al.* International Bullous Diseases Group: consensus on diagnostic criteria for epidermolysis bullosa acquisita. *Br J Dermatol* 2018; **179**:30–41.
- Soh H, Hosokawa H, Asada Y. IgE and its related phenomena in bullous pemphigoid. *Br J Dermatol* 1993; **128**:371–7.
- Kalowska M, Ciepiela O, Kowalewski C *et al.* Enzyme-linked immunoassay index for anti-NC16a IgG and IgE auto-antibodies correlates with severity and activity of bullous pemphigoid. *Acta Derm Venereol* 2016; **96**:191–6.
- Hashimoto T, Ohzono A, Teye K *et al.* Detection of IgE autoantibodies to BP180 and BP230 and their relationship to clinical features in bullous pemphigoid. *Br J Dermatol* 2017; **177**:141–51.
- Ujiie H. IgE autoantibodies in bullous pemphigoid: supporting role, or leading player? *J Dermatol Sci* 2015; **78**:5–10.
- van Beek N, Luttman N, Huebner F *et al.* Correlation of serum levels of IgE autoantibodies against BP180 With bullous pemphigoid disease activity. *JAMA Dermatol* 2017; **153**:30–8.
- Kamiya K, Aoyama Y, Noda K *et al.* Possible correlation of IgE autoantibody to BP180 with disease activity in bullous pemphigoid. *J Dermatol Sci* 2015; **78**:77–9.
- Bing L, Xiping Z, Li L *et al.* Levels of anti-BP180 NC16A IgE do not correlate with severity of disease in the early stages of bullous pemphigoid. *Arch Dermatol Res* 2015; **307**:849–54.
- Fairley JA, Burnett CT, Fu CL *et al.* A pathogenic role for IgE in autoimmunity: bullous pemphigoid IgE reproduces the early phase of lesion development in human skin grafted to nu/nu mice. *J Invest Dermatol* 2007; **127**:2605–11.
- Fairley JA, Baum CL, Brandt DS *et al.* Pathogenicity of IgE in autoimmunity: successful treatment of bullous pemphigoid with omalizumab. *J Allergy Clin Immunol* 2009; **123**:704–5.

- 15 Miyake H, Morishima Y, Komai R *et al.* Epidermolysis bullosa acquisita: correlation of IgE levels with disease activity under successful betamethasone/dapsone combination therapy. *Acta Derm Venereol* 2001; **81**:429.
- 16 Luke MC, Darling TN, Hsu R *et al.* Mucosal morbidity in patients with epidermolysis bullosa acquisita. *Arch Dermatol* 1999; **135**:954–9.
- 17 Borradori L, Caldwell JB, Briggaman RA *et al.* Passive transfer of autoantibodies from a patient with mutilating epidermolysis bullosa acquisita induces specific alterations in the skin of neonatal mice. *Arch Dermatol* 1995; **131**:590–5.
- 18 Rubenstein R, Esterly NB, Fine JD. Childhood epidermolysis bullosa acquisita. Detection in a 5-year-old girl. *Arch Dermatol* 1987; **123**:772–6.
- 19 Favrot C, Dunston SM, Paradis M *et al.* Isotype determination of circulating autoantibodies in canine autoimmune subepidermal blistering dermatoses. *Vet Dermatol* 2003; **14**:23–30.
- 20 Amarasekera M. Immunoglobulin E in health and disease. *Asia Pac Allergy* 2011; **1**:12–15.
- 21 Martins TB, Bandhauer ME, Bunker AM *et al.* New childhood and adult reference intervals for total IgE. *J Allergy Clin Immunol* 2014; **133**:589–91.
- 22 Geha RS, Jabara HH, Brodeur SR. The regulation of immunoglobulin E class-switch recombination. *Nat Rev Immunol* 2003; **3**:721–32.
- 23 Iranzo P, Herrero-Gonzalez JE, Mascaro-Galy JM *et al.* Epidermolysis bullosa acquisita: a retrospective analysis of 12 patients evaluated in four tertiary hospitals in Spain. *Br J Dermatol* 2014; **171**:1022–30.
- 24 Terra JB, Jonkman MF, Diercks GF *et al.* Low sensitivity of type VII collagen enzyme-linked immunosorbent assay in epidermolysis bullosa acquisita: serration pattern analysis on skin biopsy is required for diagnosis. *Br J Dermatol* 2013; **169**:164–7.
- 25 Freire PC, Munoz CH, Stingl G. IgE autoreactivity in bullous pemphigoid: eosinophils and mast cells as major targets of pathogenic immune reactants. *Br J Dermatol* 2017; **177**:1644–53.

Supporting Information

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

Fig S1. Specificity of antihuman IgE antibody used in this study.

Fig S2. IgE type VII collagen enzyme-linked immunosorbent assay with/without adsorption by protein G.

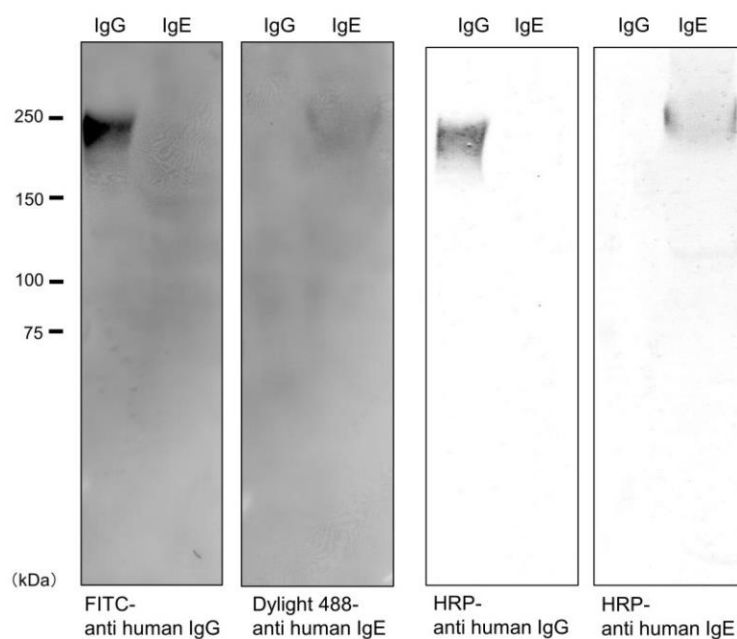


Figure S1. Specificity of anti-human IgE antibody used in this study.

Approximately 10 ng of blotted human IgG (purified from serum by Protein G) or IgE (reference standard) in Enzyme Immunoassay for the Quantitative Determination of Immunoglobulin E (IgE) kit (Immunospec, Canoga Park, CA) were incubated with FITC-conjugated anti-human IgG (Dako, Denmark), Dylight 488-conjugated anti-human IgE (Novus Biologicals, Littleton, CO), HRP-conjugated anti-human IgG (MBL Co. Ltd, Nagoya, Japan), or HRP-conjugated anti-human IgE (Clone B3102E8, Southern Biotechnology, Birmingham, AL), respectively. The bands were visualized using a Typhoon 9500 laser scanner (GE Healthcare, Freiburg, Germany) or with TMB (ATTO, Tokyo, Japan).

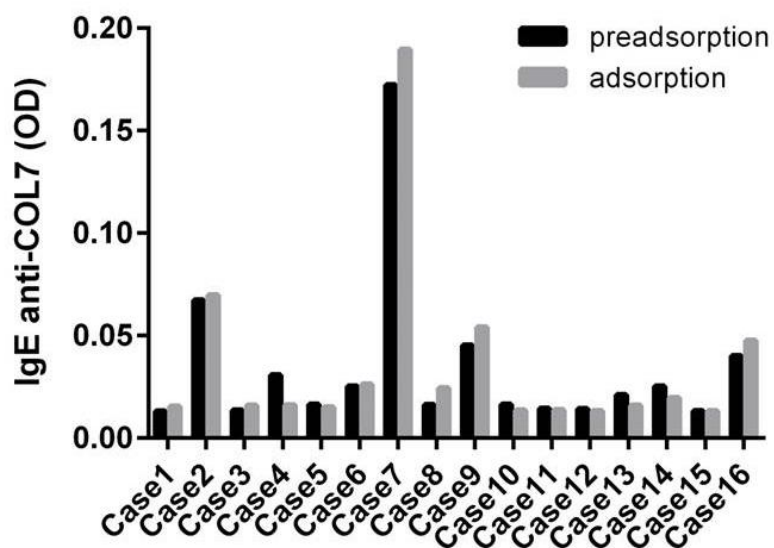


Figure S2. IgE COL7 ELISA with/without adsorption by Protein G.

Randomly selected 16 sera before (preadsorption) and after adsorption (adsorption) from patients with EBA were tested with IgE COL7 ELISA.

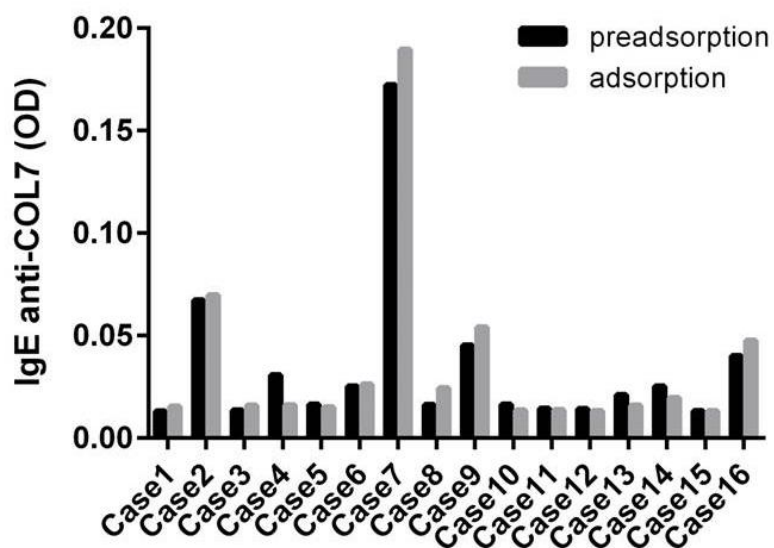


Figure S2. IgE COL7 with/without adsorption by Protein G.

Randomly selected 16 sera before (preadsorption) and after adsorption (adsorption) from patients with EBA were tested with IgE COL7 ELISA.