

IgE autoantibodies against the intracellular domain of BP180

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Summary

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Conflicts of interest

None declared.

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Background Bullous pemphigoid (BP) is an autoimmune subepidermal blistering disease characterized by autoantibodies against the hemidesmosomal proteins BP180 (type XVII collagen) and BP230. BP not only involves IgG-mediated neutrophil activation, leading to blistering, but also IgE-dependent activation of mast cells and basophils. While IgG and IgE autoantibodies target the extracellular noncollagenous (NC) 16A domain of BP180, little is known whether other BP180 regions are targeted by these antibody classes.

Objectives To characterize IgE and IgG autoantibody binding to antigenic sites on the intracellular domain (ICD) of BP180 compared with BP180 NC16A.

Methods IgE/IgG autoreactivity against recombinant BP180 ICD and NC16A was determined by immunoblotting of sera from 18 patients with BP and 10 controls.

Results Total serum IgE was elevated in 16 of 18 BP sera. Most BP sera tested positive (15 of 18) to NC16A with both immunoglobulin classes. Additionally, 14 of 18 sera showed IgE reactivity with an epitope mapped to the ICD of BP180 (amino acid residues 103–266). Mapping of ICD antigenic sites revealed similar IgE and IgG reactivities for most regions except for greater IgE reactivity to amino acid residues 234–398 (11 of 18 BP sera) than IgG (five of 18). Control sera failed to display IgE reactivity to these antigens.

Conclusions The results indicate that BP180 NC16A is not the only antigenic determinant of IgE autoantibodies in BP and that additional, novel epitopes exist on different regions of the ICD of BP180. The heterogeneous autoimmune response against BP180 suggests intramolecular epitope spreading during disease progression.

Bullous pemphigoid (BP) is an autoimmune subepidermal blistering disease observed primarily in the elderly population. It is associated with autoantibodies against two hemidesmosomal proteins designated BP180 (type XVII collagen)¹ and BP230, the latter being localized to the intracellular hemidesmosomal plaque.² IgG autoantibodies against BP180 are particularly important for the loss of adhesion of the dermal-epidermal junction (DEJ) and primarily target a noncollagenous stretch of the ectodomain, NC16A.³ The development of lesions depends on complement activation and neutrophil recruitment, with subsequent release of elastase and gelatinase B at the lesional site.^{4,5} However, BP is also partially associated with Th2-type immune responses, including production of IgE autoantibodies.⁶ These may trigger mast cells and

basophils to release proinflammatory mediators, such as histamine. Mast cells have been shown to be a prerequisite for blister formation in experimental BP.⁷ In addition, IgE antibodies to the cutaneous basement membrane have been demonstrated to induce blisters at the histopathological level in SCID mice grafted with human skin.⁸ Indeed, subepidermal vesiculation in BP is often preceded by the development of urticarial plaques, with marked itching, further indicating possible involvement of IgE autoantibodies in the pathogenesis of BP by causing mast cell and basophil histamine release.

Previous studies have demonstrated that, in addition to the IgG autoantibodies, patients with BP also produce IgE autoantibodies against BP180 NC16A⁹ as well as to BP230.¹⁰ As the latter are directed against an intracellular antigen we asked

whether the intracellular domain (ICD) of BP180 may also be targeted by IgE autoantibodies. Our objectives were therefore comprehensively to characterize the intracellular IgE-reactive regions of BP180 in comparison with IgG.

Patients and methods

Serum samples were obtained from patients with BP ($n = 18$), before treatment, and from age-matched control donors (nine healthy and one diagnosed with pemphigus vulgaris). All samples were obtained following informed consent and approval of the local ethics committee. All patients with BP were characterized by (i) blistering skin disease, (ii) subepidermal cleavage by histopathology and at least one of the following diagnostics: (iii) linear deposits of IgG and/or C3 along the DEJ by direct immunofluorescence (IF), (iv) circulating antibodies against the epidermal side as revealed by indirect IF microscopy of 1 mol L^{-1} NaCl-split human skin and (v) autoantibodies against BP180 NC16A as determined by enzyme-linked immunosorbent assay (MBL, Nagoya, Japan). Serum IgE levels were determined by fluorescence enzyme immunoassay (UniCap 100; Phadia, Freiburg, Germany; normal range $< 100 \text{ kU L}^{-1}$).

Rabbit antibodies SA8009 were generated against recombinant glutathione S-transferase (GST) fusion proteins containing sequences of human BP180 (GST-NC16A2-4) as described previously.¹¹ BP180 cDNA fragments were cloned into linearized pGEX-6P-1 (Amersham Biosciences, Amersham, U.K.), resulting in the recombinant vectors pGEX-hCOL17-A, B, C and D. Expression in *Escherichia coli* NovaBlue and purification of BP180 ICD and NC16A1-5 was performed as previously reported.^{3,12} Recombinant proteins were fractionated by 15% sodium dodecyl sulphate-polyacrylamide gel electrophoresis, and electrophoretically transferred to nitrocellulose. Membranes were blocked overnight in 5% skimmed milk in TRIS-buffered saline with Tween 20 (TBST) [0.02 mol L^{-1} Tris-(hydroxymethyl)-aminomethane, 0.14 mol L^{-1} NaCl, 0.01% Tween 20, pH 7.5]. For IgG detection, blots were incubated with 1 : 50 diluted serum containing 1% bovine serum albumin in TBST for 3 h at room temperature. Bound antibodies were visualized enzymatically using 500-fold diluted rabbit antihuman IgG (heavy chain specific)-horse-radish peroxidase (HRP) (DakoCytomation, Glostrup, Denmark). For IgE detection, membranes were incubated with 1 : 2 diluted serum in TBST followed by 1000-fold diluted mouse antihuman IgE (heavy chain specific; Sigma, St Louis, MO, U.S.A.) and finally 1 : 500 diluted antimouse-HRP antibody (DakoCytomation). Blots were evaluated in a blinded fashion by two investigators. As the recombinant proteins were fused with GST, pure GST was included as a control in immunoblot analyses. Nonspecific tertiary antibody membrane binding was excluded by incubating blotted proteins with BP serum and antimouse antibody-HRP (no bands detected). Moreover, the pronounced IgG reactivity of a control serum against ICD B without corresponding bands in the IgE blot excluded cross-reactivity. The χ^2 test was employed for statistical analysis.

Results

cDNA sequences coding for four overlapping fragments of BP180 ICD (depicted in Fig. 1a) were successfully cloned into pGEX-6P-1 vector and expressed in *E. coli*. All ICD fragments, in addition to the full-length protein, showed bands of lower

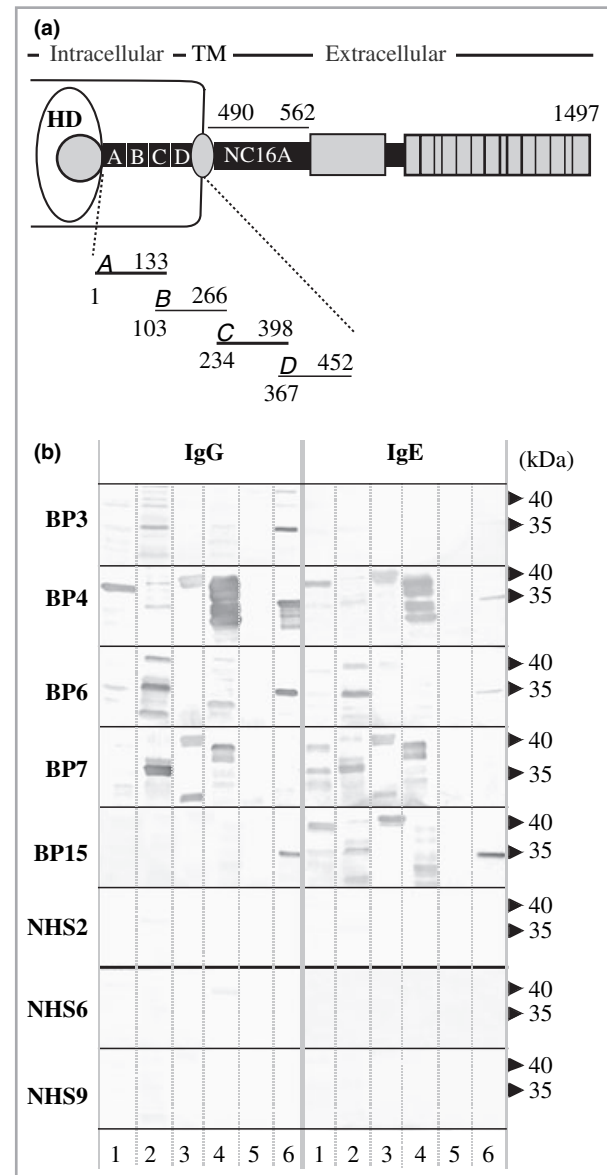


Fig 1. (a) Schematic organization of human type XVII collagen (BP180) and recombinant peptides of BP180 intracellular domain (ICD) used in this study. Ultrastructurally, BP180 has a globular head (intracellular), a central rod and a flexible tail within the lamina densa of the dermal-epidermal junction.¹⁷ The ectodomain of BP180 consists of a series of alternating collagenous (grey) and noncollagenous (NC; black) domains.¹⁸ HD, hemidesmosome; TM, transmembrane region. (b) Representative immunoblots. Lane 1, ICD A; lane 2, ICD B; lane 3, ICD C; lane 4, ICD D; lane 5, glutathione S-transferase; lane 6, NC16A1-5. NHS, normal human sera. Migration positions of molecular weight markers are shown on the right.

Table 1 IgE reactivity of 18 bullous pemphigoid (BP) sera against four overlapping intracellular domain (ICD) fragments and the noncollagenous (NC) 16A domain of BP 180

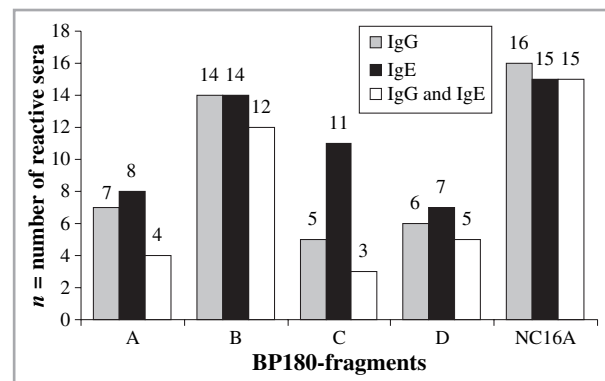
BP sera	ICD				ECD	Total IgE levels (kU L ⁻¹)
	ICD A (1–133)	ICD B (103–266)	ICD C (234–398)	ICD D (367–452)	NC 16A (490–562)	
BP1						2178
BP2						14
BP3						9430
BP4						154
BP5						4349
BP6						1046
BP7						3138
BP8						790
BP9						2785
BP10						842
BP11						658
BP13						1482
BP15						417
BP16						742
BP17						20835
BP18						1734
BP19						70
BP20						1929
Positive sera ^a	8/18	14/18	11/18	7/18	15/18	2922 ^b
NH sera ^a	0/10	0/10	0/10	0/10	0/10	67 ^b

ICD A–D, recombinant fragments A–D, amino acid range in brackets; ECD, extracellular domain; NH sera, normal human sera; dark shading represents a positive immunoblot. ^aNumber of sera reactive/total number of sera tested. ^bMean IgE level.

molecular weight when stained by antihuman BP180-GST antibody (not shown), indicating some degradation products. IgE and IgG autoreactivities of 20 BP sera and 10 negative controls were studied against four epitopes on the BP180 ICD (ICD A, B, C and D) and extracellular NC16A1–5 by epitope mapping. In addition to the 18 patients with BP included in this study two patients with BP showed IgG and/or IgE antibodies against GST, and were therefore not evaluated. Representative blots are shown in Figure 1b.

In all, 15 of the 18 BP sera displayed IgE autoantibodies against NC16A1–5. For the first time we demonstrate substantial IgE reactivity to the N-terminal ICD of BP180 with the same number of sera reacting to at least one of these regions (Table 1). ICD B was recognized most frequently by both IgE and IgG (14 of 18) whereas ICD C was recognized more frequently by IgE (11 of 18) than IgG (five of 18). No IgE reactivity to ICD, NC16A1–5 or GST was found in the non-BP controls. The relative reactivities of IgE and IgG in all 18 patients against the subdomains of BP180 are summarized in Figure 2. Additionally, we observed that serum IgE levels were significantly elevated in 16 of 18 patients with untreated BP, with a mean IgE level of 2922 kU L⁻¹, in contrast to only 67 kU L⁻¹ in the non-BP controls. In 16 of 18 sera, IgG reactivity against NC16A1–5 could be detected and in 14 of 18 against BP180 ICD, the majority of these reacting to ICD B.

The correlation of IgE and IgG autoantibodies against the different epitopes was studied using a χ^2 test for significance. A significant correlation was found for ICD B, ICD D and

**Fig 2.** Summary of the number of bullous pemphigoid sera with IgG and/or IgE reactivity against different fragments of BP180.

NC16A1–5 ($P < 0.05$). However, there was no correlation between total serum IgE levels and IgE autoantibodies to the ICD fragments.

Discussion

The extracellular NC16A domain of BP180 is widely accepted to be the main target for BP autoantibodies. In recent studies, additional IgG autoantibody-binding sites were described on the intracellular region of this protein, where the central region contains the immunodominant epitopes.^{13,14} This was confirmed in the present study where IgG autoantibodies in

16 of 18 sera reacted against recombinant ICDs. Most of the antibodies reacted with a central portion of the ICD (amino acid residues 103–266).

For the first time we show the existence of IgE autoantibodies against BP180 ICD in BP sera. Fine mapping of antigenic sites within the ICD and NC16A1–5 revealed similar reactivity patterns for IgG and IgE. This is in good agreement with Döpp *et al.* who described a correlation for IgG and IgE antibodies against NC16A1–5.⁹ Serum from one patient with IgE reactivity against ICD showed no IgE reactivity with NC16A. This observation indicates that additional epitopes other than NC16A may be targeted by IgE antibodies.

Previous studies have shown that autoantibodies can penetrate living cells, reach their intracellular targets, and affect their function.¹⁵ The sites on the BP180 ICD, targeted by IgE and IgG autoantibodies, include sequences critical for the incorporation of the protein into the hemidesmosomes.¹⁶ Thus, it is possible that autoantibodies against the BP180 ICD have pathogenic significance in dermal–epidermal separation by impairing the interaction of BP180 with other constituents of the hemidesmosome. This impairment could be elicited by both IgE and IgG autoantibodies. In addition, an IgE-mediated activation of mast cells and basophils is feasible, which may govern certain BP symptoms including itching, erythema and urticaria. Although the nature of epitope spreading and the relative levels of various immunoglobulin isoclasses and subclasses in BP still require further evaluation, especially in terms of clinical outcomes, the present data underline the potential role of BP180-specific IgE in BP.

References

- Labib RS, Anhalt GJ, Patel HP *et al.* Molecular heterogeneity of the bullous pemphigoid antigens as detected by immunoblotting. *J Immunol* 1986; **136**:1231–5.
- Tanaka T, Korman NJ, Shimizu H *et al.* Production of rabbit antibodies against carboxy-terminal epitopes encoded by bullous pemphigoid cDNA. *J Invest Dermatol* 1990; **94**:617–23.
- Zillikens D, Rose PA, Balding SD *et al.* Tight clustering of extracellular BP180 epitopes recognized by bullous pemphigoid autoantibodies. *J Invest Dermatol* 1997; **109**:573–9.
- Kasperkiewicz M, Zillikens D. The pathophysiology of bullous pemphigoid. *Clin Rev Allergy Immunol* 2007; **33**:67–77.
- Shimanovich I, Mihai S, Oostingh GJ *et al.* Granulocyte-derived elastase and gelatinase B are required for dermal–epidermal separation induced by autoantibodies from patients with epidermolysis bullosa acquisita and bullous pemphigoid. *J Pathol* 2004; **204**:519–27.
- Sitaru C, Zillikens D. Mechanisms of blister induction by autoantibodies. *Exp Dermatol* 2005; **14**:861–75.
- Chen R, Ning G, Zhao ML *et al.* Mast cells play a key role in neutrophil recruitment in experimental bullous pemphigoid. *J Clin Invest* 2001; **108**:1151–8.
- Zone JJ, Tayler T, Hull C *et al.* IgE basement membrane zone antibodies induce eosinophil infiltration and histological blisters in engrafted human skin on SCID mice. *J Invest Dermatol* 2007; **127**:1167–74.
- Döpp R, Schmidt E, Chimanovitch I *et al.* IgG4 and IgE are the major immunoglobulins targeting the NC16A domain of BP180 in bullous pemphigoid: serum levels of these immunoglobulins reflect disease activity. *J Am Acad Dermatol* 2000; **42**:577–83.
- Ghohestani RF, Cozzani E, Delaporte E *et al.* IgE antibodies in sera from patients with bullous pemphigoid are autoantibodies preferentially directed against the 230-kDa epidermal antigen (BP230). *J Clin Immunol* 1998; **18**:202–9.
- Sitaru C, Schmidt E, Petermann S *et al.* Autoantibodies to bullous pemphigoid antigen 180 induce dermal–epidermal separation in cryosections of human skin. *J Invest Dermatol* 2002; **118**:664–71.
- Olaru F, Mihai S, Petrescu I *et al.* Generation and characterization of monoclonal antibodies against the intracellular domain of hemidesmosomal type XVII collagen. *Hybridoma (Larchmt)* 2006; **25**:158–62.
- Perriard J, Jaunin F, Favre B *et al.* IgG autoantibodies from bullous pemphigoid (BP) patients bind antigenic sites on both the extracellular and the intracellular domains of the BP antigen 180. *J Invest Dermatol* 1999; **112**:141–7.
- Di Zenzo G, Grosso F, Terracina M *et al.* Characterization of the anti-BP180 autoantibody reactivity profile and epitope mapping in bullous pemphigoid patients. *J Invest Dermatol* 2004; **122**:103–10.
- Alarcon-Segovia D, Llorente L, Ruiz-Arguelles A. The penetration of autoantibodies into cells may induce tolerance to self by apoptosis of autoreactive lymphocytes and cause autoimmune disease by dysregulation and/or cell damage. *J Autoimmun* 1996; **9**:295–300.
- Borradori L, Koch PJ, Niessen CM *et al.* The localization of bullous pemphigoid antigen 180 (BP180) in hemidesmosomes is mediated by its cytoplasmic domain and seems to be regulated by the beta4 integrin subunit. *J Cell Biol* 1997; **136**:1333–47.
- Nonaka S, Ishiko A, Masunaga T *et al.* The extracellular domain of BPAG2 has a loop structure in the carboxy terminal flexible tail in vivo. *J Invest Dermatol* 2000; **115**:889–92.
- Zillikens D, Giudice GJ. BP180/type XVII collagen: its role in acquired and inherited disorders of the dermal–epidermal junction. *Arch Dermatol Res* 1999; **291**:187–94.