



IgE recognition of bullous pemphigoid (BP)180 and BP230 in BP patients and elderly individuals with pruritic dermatoses

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Abstract

Bullous pemphigoid (BP) is the most common autoimmune bullous disease of the elderly and is associated with IgG and IgE autoantibodies against the hemidesmosomal proteins, BP180 and BP230. The purpose of this study was to characterize the epitope specificity of IgE against defined regions of BP180 and BP230 in 32 BP patients and 21 elderly patients with pruritic disorders who did not yet fulfill all the criteria of BP by immunoblot (IB), ELISA and indirect immunofluorescence microscopy. Our findings show that IgE from BP sera preferentially targets the COOH-terminus of BP230 (IB: 16/32, ELISA: 12/32) and, to a lesser extent, the BP180-NC16A domain (IB: 11/32, ELISA: 9/32). Noteworthy, a subgroup of elderly patients with pruritic dermatoses also showed IgE recognition of BP180-NC16A (IB: 1/21, ELISA: 4/21) and less frequently of BP230 (IB: 2/21, ELISA: 2/21). Thus, IgE recognition of the BP autoantigens is presumably an early pathogenetic event in BP.

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1. Introduction

Bullous pemphigoid (BP) is the most common autoimmune bullous skin disease and has become of major clinical and socioeconomic importance because of its increasing prevalence in the aging population. Classical BP is characterized clinically by tense blisters on the limbs and trunk; in contrast, the prodromal phase or early clinical phases of BP are characterized by the presence of severe itch accompanied by a

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more polymorphic clinical phenotype including eczematous, papular, excoriated or urticarial lesions [1–3]. Establishing the diagnosis of BP in the prodromal phase may be difficult, also in light of recent observations that a subset of elderly patients who otherwise do not fulfill all the criteria for BP exhibits IgG reactivity against the BP autoantigens [4].

In BP, autoantibodies target components of the dermo-epidermal basement membrane zone (BMZ) of the skin and are mainly directed against two structural components of hemidesmosomes, BP180 (also termed collagen XVII or BP antigen 2) and BP230 (also termed BP antigen 1) [5–7]. IgG is the most common autoantibody subtype in BP even though IgA and IgE specific for BP180 and BP230 has been described [1,4]. IgG autoantibodies preferentially recognize epitopes of the BP180 ectodomain, *i.e.* the NH₂-terminal NC16a domain and the COOH-terminus [8–11]. Furthermore, IgG is directed against the NH₂- and COOH-termini of the cytoplasmatic BP230 [11,12]. There is evidence from *in vivo* and *in vitro* studies that IgG against the BP180 ectodomain is pathogenic while the clinical relevance of BP230-specific IgG is not clear yet [11,13].

A critical role of IgE autoantibodies in the pathogenesis of BP has been suggested based on several observations. The dermal inflammatory infiltrate of skin lesions in BP contains eosinophils and the early non-bullous phases of BP are frequently associated with eosinophilic spongiosis consisting of dermal edema associated with eosinophilic infiltration of the upper dermis [14,15]. Although IgE functions are commonly detected in allergic and parasitic diseases, this immunoglobulin subtype has been associated with autoimmune diseases such as Graves disease, Hashimoto thyroiditis and pemphigus vulgaris [16–18]. Although IgG autoantibodies are considered to play a key role in the pathogenesis of BP, injection of IgG specific for the BP180-NC16a domain does not reproduce all the key findings of BP such as the presence of an eosinophilic infiltrate [19]. Zone et al. [20] showed that the injection of a monoclonal IgE antibody reactive with the shed ectodomain of BP180 (LABD97) into human skin grafted onto SCID mice induced histological features reminiscent of BP. Fairley et al. [21] reported that injection of IgE from BP sera into human skin grafted onto athymic nude mice reproduced the early phase of BP lesions, including the presence of an eosinophilic infiltrate. Moreover, many patients with BP have elevated IgE serum concentrations which correlate with the clinical severity of BP [22]. In general, IgE autoantibodies target distinct regions of BP180 and BP230 known to bind the epitopes that are also recognized by IgG autoantibodies. Specifically, IgE targets the NH₂- and COOH-termini of BP230 [23–25] and extracellular and intracellular regions of BP180 [26–30].

In the present study, we examined serum IgE recognition of six major BP180 subdomains and the two major subdomains of BP230 in a cohort of patients with classical, *i.e.* bullous BP, prurigo-like BP and elderly patients with pruritic dermatoses who did not fulfill all the criteria of BP by indirect immunofluorescence (IIF) microscopy, immunoblot, or ELISA. We show by three different diagnostic measures that IgE against BP180 and BP230 is not only

present in patients with BP but also in elderly individuals with pruritic disorders who may represent a stage of pre-pemphigoid. These findings support the concept that IgE autoantibodies are critical in the immune pathogenesis of BP.

2. Materials and methods

2.1. Patients and control sera

Sera were obtained from patients with BP (n=32), with pruritic dermatosis (n=21) and age-matched control patients with immediate type allergic reactions (n=24). All patients gave written consent to participate in this study.

Patients with BP (69.9 ± 16.7 years) fulfilled all four diagnostic criteria: (i) characteristic clinical findings with erythema, urticarial plaques and/or tense blisters of the skin associated with pruritus; (ii) histopathological evidence of subepidermal blister formation with infiltrating eosinophils and neutrophils; (iii) linear IgG and/or C3 deposits at the dermo-epidermal junction of perilesional skin by direct immunofluorescence; and (iv) IgG serum reactivity with the epidermal side of saline split human skin (SSS) by indirect immunofluorescence.

While the majority (26/32) of the BP patients showed tense blisters, 6/32 BP patients showed a prurigo-like phenotype characterized by few or no blisters.

A total of 21 elderly patients with pruritic dermatoses (68.1 ± 11.5 years) were affected by acute pruritus, from mild to severe intensity, characterized by different clinical manifestations consisting of prurigo-like lesions (n=7), pruritic erythema (n=7), or pruritus sine materia (n=7). Four of these patients showed anti-BMZ IgG by indirect immunofluorescence and eleven had IgG against BP180 and/or BP230 by ELISA but did not yet fulfill all the diagnostic criteria of BP as defined above (Table 1).

Twenty-four age- and sex-matched control patients (64.7 ± 6.5 years) who suffered from immediate type allergic disorders were also studied. These patients presented with urticaria (n=5), allergic rhinitis (n=12), insect allergy (n=5) and urticarial drug exanthems (n=2).

2.2. Expression and purification of recombinant BP180 and BP230 proteins

Several recombinant BP180 and BP230 proteins including BP180ex (ectodomain of BP180; aa 485–1465, molecular weight (MW) 137 kilo Daltons (kDa)), BP180-N1 (aa 490–812, MW 38 kDa), BP180-NC16A (aa 467–567, MW 63 kDa), BP180-C1 (aa 1048–1465, MW 74 kDa), BP180-C2 (aa 1352–1465, MW 40 kDa), BP180-M (aa 809–1106, MW 60 kDa), BP230-C1 (aa 1881–2649, MW 113 kDa) and BP230-N (aa 1–1307, MW 173 kDa) are linked to glutathione-S-transferase (GST) and 6xHis tag and were produced in a baculovirus expression system as described previously [31]. In short, the recombinant BP180 and BP230 proteins and a GST/6xHis control protein (generated by cotransfection of an empty pAcGHLT-A vector) were expressed in Sf21 cells and were purified by affinity-chromatography over Nickel-NTA agarose (Qiagen, Hilden, Germany) using the batch method according to the manufacturer's protocol [32].

Table 1 Immunoserological characteristics of patients with classical bullous pemphigoid (BP), prurigo-like BP, pruritic dermatoses and age-matched allergic controls by direct and indirect immunofluorescence.

	DIF ^a	IIF ^b	Anti-BP230 IgG ^c	Anti-BP180 IgG ^d	Age	No (pts.)
Classical BP	26/26 (100%)	26/26 (100%)	19/26 (73%)	22/26 (84.6%)	69.7 ± 18.2	26
Prurigo-like BP	6/6 (100%)	6/6 (100%)	6/6 (100%)	3/6 (50%)	71.2 ± 9	6
Pruritic dermatoses	0/21 (0%)	4/21 (19%)	8/21 (38.1%)	5/21 (23.8%)	68.1 ± 11.5	21
Allergic disorders	—	—	1/24 (4.2%)	1/24 (4.2%)	64.7 ± 6.5	24

^a DIF, direct immunofluorescence microscopy (IgG).^b IIF, indirect immunofluorescence microscopy (IgG) utilizing saline-split human skin or monkey esophagus as a substrate.^c IgG against the NH₂-terminus and COOH terminus of BP230.^d IgG against the NH₂-terminus and COOH-terminus of BP180.

Purified proteins were finally dialyzed against PBS (pH 7.5) supplemented with 3 M urea. Affinity-purified BP proteins were stored in aliquots at -20 °C. Protein concentrations were determined by the DC Protein Assay (Bio-Rad, Munich, Germany) according to the manufacturer's protocol. The recombinant BP proteins were fractionated by 10% SDS-PAGE and visualized by Coomassie staining and western blot. By western blot, the proteins were detected via GST-Tag with a monoclonal mouse anti-GST Ab (1:2000, clone GST3-4C, Zymed, San Francisco, CA) and visualized by a HRP labeled monoclonal anti mouse Ab (1:2000) (DAKO, Glostrup, Denmark), following enhanced chemiluminescence (Millipore, Schwalbach/Ts, Germany) to verify the desired recombinant protein on the correct molecular size.

2.3. Immunoblot analysis with recombinant BP180 and BP230 proteins

The affinity-purified BP230 and BP180 proteins were fractionated by 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis and blotted onto nitrocellulose membranes (Schleicher&Schuell, Einbeck, Germany). Nitrocellulose membranes were blocked for 4 h in 5% of skimmed milk powder (MP) in TBST (TBS pH 7.5 with 0.05% Tween 20; Merck, Nürnberg, Germany) at room temperature (RT). After three washes with TBST, the membranes were incubated with patients sera diluted at 1:25 in blocking-buffer overnight at 8 °C. After rinsing three times with TBST, the membranes were incubated 2 h at RT with a secondary HRP-labeled anti-human IgE antibody (Southern Biotechnology, Birmingham, AL), diluted at 1:1000 in blocking-buffer. After washes with TBST, the nitrocellulose membrane was incubated for 5 min with a commercial HRP substrate (Immobilon Western Chemiluminescent HRP substrate; Millipore, Schwalbach, Germany) and IgE binding was visualized by Chemi-smart 3000 (Pqlab, Erlangen, Germany).

2.4. ELISA with BP230 and BP180 recombinant proteins

Ninety-six well half area high binding microplates, (Micro-lon, Greiner Bio-One, Frickenhausen, Germany) were coated

with recombinant BP180 and BP230 proteins at a total of 0.5 µg protein or a molar equivalent amount of the control protein GST/6xHis in 50 µl of 0.1 M bicarbonate buffer, pH 9.8, at 4 °C overnight. After three washes with TBST (TBS pH 7.5 with 0.05% Tween 20 (Merck, Nürnberg, Germany)), ELISA plates were blocked for 2 h with 100 µl of a 5% skimmed milk powder (MP) solution in TBST at RT. Serum samples were diluted at 1:10 in blocking-buffer, added to the wells in duplicate and incubated overnight at 4 °C. ELISA plates were washed as above and wells were reacted with HRP labeled anti-human IgE at 1:1000 (Southern Biotechnology, Birmingham, AL) for 90 min. at RT. After another series of washes, 50 µl of the peroxidase substrate 2,2'-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) (Calbiochem, Schwalbach, Germany) was added to the wells. The optical density (OD) was measured at 405 nm as determined by sunrise microplate reader and Magellan V6 software (Tecan, Crailsheim, Germany).

To define an appropriate diagnostic threshold for IgE reactivity against the utilized recombinant BP180 and BP230 proteins, an 'optimal' threshold was chosen with respect to correctly classifying cases of IgE-reactive patients and controls. The subgroups of IgE-reactive patients and non-reactive controls were determined by IgE binding to the studied BP180 and BP230 recombinant proteins by immunoblot. The receiver operating characteristic (ROC) curve was compiled by utilizing SPSS (SPSS Inc., Chicago, IL, USA) to evaluate the diagnostic performance. The diagnostic threshold was determined as the OD value with the largest Youden index (YI=sensitivity+specificity-1), yielding a value of OD=0.1885, with a specificity of 95.6% and a sensitivity of 74.4%. Based on this calculation, with negative controls with an OD<0.1885 and positive controls with an OD>0.6, a protein index value (PIV) was calculated when the patient's serum reached a PIV>4. For semi-quantitative, longitudinal analysis, IgE serum reactivity against BP180 and BP230 of the patients was then expressed as PIV.

2.5. Indirect immunofluorescence microscopy (IIF)

IIF was performed on normal skin using polyclonal rabbit anti-human IgE/FITC (DAKO, Glostrup, Denmark). Skin sections were incubated with sera from patients with BP or pruritic dermatoses and from control patients (diluted at 1:20)

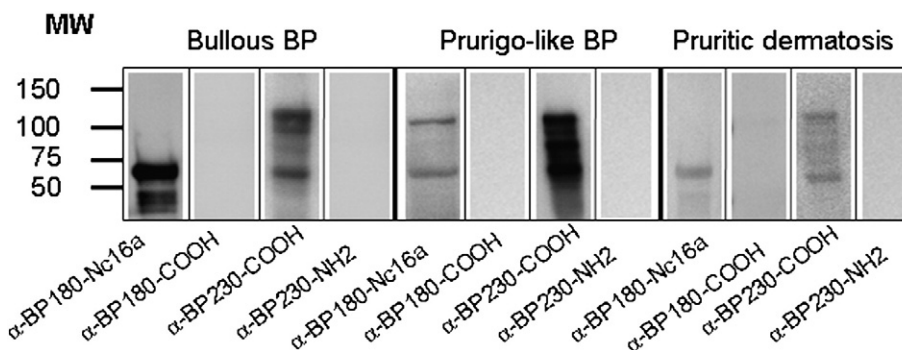


Figure 1 IgE reactivity against BP180 and BP230 in bullous pemphigoid (BP), prurigo-like BP and pruritic dermatoses by immunoblot analysis. Representative sera from a patient with bullous BP, prurigo-like BP, and a patient with a pruritic dermatosis were tested for IgE reactivity against two major subdomains of BP180 and BP230, respectively, by immunoblot. These subdomains are: BP180-NC16A (aa 467–567, MW 63 kDa), BP180-COOH (aa 1048–1465, MW 74 kDa), BP230-COOH (aa 1881–2649, MW 113 kDa) and BP230-NH2 (aa 1–1307, MW 173 kDa). The molecular weights (MW) in the figure are indicated by lines on the left.

for 30 min at RT. After three washes with PBS, the polyclonal rabbit anti-human IgE/FITC was added at a 1:20 dilution for 30 min.

3. Results

3.1. Immunoblot analysis

Sera from 32 BP patients (classical BP, $n=26$; prurigo-like BP, $n=6$), 21 elderly patients with pruritic dermatoses and 22 patients with immediate type allergic reactions were analyzed for IgE reactivity against six defined regions of the BP180 ectodomain and two major subdomains of BP230 by immunoblot (Fig. 1). As shown in Table 2, the major antigenic region which was targeted by IgE autoantibodies was the COOH-terminus of BP230. In detail, 13/26 (50%) of the BP sera showed IgE reactivity against the COOH-terminal domain of BP230, so did 3/6 (50%) of the sera from patients with prurigo-like BP, and 2/21 (9.5%) of the patients with

pruritic dermatoses. In contrast, 0/22 of the allergic patients showed IgE reactivity to BP230.

Among the antigenic sites of BP180, the major autoantigen of BP, IgE autoantibodies preferentially recognized the NH2-terminus which includes the immunodominant BP180-NC16A domain. Specifically, this site was recognized by 10/26 (38.4%) of the classical BP sera, 1/6 (16.6%) of prurigo-like BP sera, and 1/21 (4.7%) sera from patients with pruritic dermatoses. Again, none of the allergic control patients showed IgE reactivity against the NH2-terminus or the BP180-NC16A domain of BP180.

Overall, 14/26 (53.8%) classical BP patients, 3/6 (50%) prurigo-like BP patients and 2/21 (9.5%) patients with pruritic dermatoses showed IgE reactivity against BP230. IgE against the entire BP180 ectodomain was identified in 12/26 (46.1%) classical BP patients, in 2/6 (33.3%) prurigo-like BP patients and in 2/21 (9.5%) pruritic dermatosis patients. Thus, in contrast to the preferential recognition of IgG autoantibodies of BP180 in BP, IgE autoantibodies more frequently targeted BP230 in the studied patients.

Table 2 IgE reactivity against BP230 and BP180 in patients with bullous pemphigoid (BP), prurigo-like BP, pruritic dermatoses and age-matched allergic controls by immunoblot.

	Anti-BP230 IgE ^a			Anti-BP180 IgE ^b						Anti-BP180 and/or BP230 IgE
	BP230 domains		Total	Domains					Total	
	NH2	COOH		NC16A	BP180 N1	BP180 C1	BP180 M	BP180 ex		
Classical BP	2/26 (7.6%)	13/26 (50%)	14/26 (53.8%)	10/26 (38.4%)	9/26 (34.6%)	3/26 (11.5%)	2/26 (7.6%)	3/26 (11.5%)	12/26 (46.1%)	16/26 (61.5%)
Prurigo-like BP	0/6 (0%)	3/6 (50%)	3/6 (50%)	1/6 (16.6%)	1/6 (16.6%)	1/6 (16.6%)	0/6 (0%)	2/6 (33.3%)	2/6 (33.3%)	3/6 (50%)
Pruritic	1/21 (4.7%)	2/21 (9.5%)	2/21 (9.5%)	1/21 (4.7%)	2/21 (9.5%)	0/21 (0%)	0/21 (0%)	1/21 (4.7%)	2/21 (9.5%)	2/21 (9.5%)
Allergic	0/22 (0%)	0/22 (0%)	0/22 (0%)	0/22 (0%)	0/22 (0%)	0/22 (0%)	0/22 (0%)	0/22 (0%)	0/22 (0%)	0/22 (0%)

^a BP230-NH2, NH2-terminus of BP230 (aa 1–1307); BP230-COOH, COOH-terminus of BP230 (aa 1881–2649).

^b NC16A, NC16A domain of BP180 (aa 467–567); BP180-N1, NH2-terminus of the BP180 ectodomain (aa 490–812); BP180-C1, COOH-terminus of the BP180 ectodomain (aa 1048–1465); BP180-M, mid portion of the BP180 ectodomain (aa 809–1106); BP180-ex, entire ectodomain of BP180 (aa 485–1465).

Table 3 IgE reactivity against BP230 and BP180 in patients with bullous pemphigoid (BP), prurigo-like BP, pruritic dermatoses and age-matched allergic controls by ELISA.

	Anti-BP230 IgE ^a			Anti-BP180 IgE ^b					Anti-BP180 and/or BP230 IgE	
	Domains		Total	Domains						Total
	NH2	COOH		NC16A	BP180 N1	BP180 C1	BP180 M	BP180 ex		
Classical BP	3/26 (11.5%)	10/26 (38.4%)	11/26 (42.3%)	9/26 (34.6%)	1/26 (3.8%)	2/26 (7.6%)	3/26 (11.5%)	5/26 (19.2%)	10/26 (38.4%)	13/26 (50%)
Prurigo-like BP	0/6 (0%)	2/6 (33.3%)	2/6 (33.3%)	0/6 (0%)	0/6 (0%)	1/6 (16.6%)	0/6 (0%)	1/6 (16.6%)	1/6 (16.6%)	2/6 (33.3%)
Pruritic	1/21 (4.7%)	2/21 (9.5%)	2/21 (9.5%)	4/21 (19.0%)	2/21 (9.5%)	1/21 (4.7%)	2/21 (9.5%)	2/21 (9.5%)	6/21 (28.6%)	6/21 (28.6%)
Allergic	1/24 (4.2%)	2/24 (8.3%)	2/24 (8.3%)	4/24 (16.7%)	2/24 (8.3%)	1/24 (4.2%)	4/24 (16.7%)	1/24 (4.2%)	5/24 (20.8%)	5/24 (20.8%)

^a BP230-NH2, NH2-terminus of BP230 (aa 1–1307); BP230-COOH, COOH-terminus of BP230 (aa 1881–2649).

^b NC16A, NC16A domain of BP180 (aa 467–567); BP180-N1, NH2-terminus of the BP180 ectodomain (aa 490–812); BP180-C1, COOH-terminus of the BP180 ectodomain (aa 1048–1465); BP180-M, mid portion of the BP180 ectodomain (aa 809–1106); BP180-ex, entire ectodomain of BP180 (aa 485–1465).

3.2. Enzyme linked immunosorbent assay (ELISA)

By ELISA, the COOH-terminus of BP230 was the most frequently targeted antigenic domain of IgE autoantibodies. As shown in Table 3, 10/26 (38.4%) classical BP sera, 2/6 (33.3%) prurigo-like BP sera, and 2/21 (9.5%) pruritic dermatosis patients' sera showed IgE reactivity against the COOH-terminus of BP230. Noteworthy, 2/24 (8.3%) sera from allergic controls also showed IgE reactivity against this domain.

With regard to IgE reactivity against BP180 by ELISA, IgE reactivity against the BP180-NC16A domain was found in 9/26 (34.6%) classical BP patients and 4/21 (19%) pruritic dermatosis patients, and 4/24 (16.7%) allergic control donors. By ELISA, none of the 6 prurigo-like BP patients showed IgE reactivity against BP180.

In the group of elderly patients with pruritic skin diseases, IgE autoantibodies recognized BP180 in 6/21 (28.6%) cases and BP230 in 2/21 (9.5%) cases. In this group of patients, IgE preferentially targeted the BP180-NC16A domain (*i.e.* in 4/21 cases).

Furthermore, IgE and IgG reactivity against the BP180 ectodomain and BP230 was also investigated in the patients BP, pruritic disorders and allergic controls by ELISA. Overall, in 32 BP patients, IgE autoantibodies against BP230 and BP180 were detected in 13 and 11 sera, respectively (Table 4). Moreover, 25/32 patients showed IgG against BP230 and BP180. Noteworthy, in most of the BP patients, IgE and IgG autoantibodies targeted the identical domains of BP180 and BP230 in individual patients. In contrast, the sera from patients with pruritic dermatoses, showed a disparate pattern of IgE and IgG autoreactivity, *i.e.* some patients showed only IgE reactivity while others showed exclusively IgG reactivity against BP180 and/or BP230 (Table 5).

3.3. Indirect immunofluorescence analysis

IgE autoantibodies against the dermo-epidermal basement membrane zone were detected by indirect immunofluorescence microscopy utilizing human skin as a substrate in 7/

26 (26.9%) classical BP patients, in 1/6 (16.6%) prurigo-like BP patients and in 1/21 (4.7%) pruritic dermatosis patients (Fig. 2). None of the 22 allergic control patients showed IgE antibodies by indirect immunofluorescence (Table 6).

4. Discussion

This is the first study that analyzes IgE in patients with BP and elderly individuals with pruritic dermatoses (who do not yet fulfill all the BP criteria) utilizing three different immunoserological techniques. We found that nearly 20% of elderly patients with pruritic dermatoses showed IgE against BP180-NC16a and almost 10% against the COOH-terminus of BP230 and NH2-terminus of BP180, respectively. In patients with pruritic dermatoses, IgE was more frequently detected against BP180 (6/21 sera) than BP230 (2/21 sera). In this view, IgE may be an effector molecule in the early

Table 4 Correlation of IgE and IgG recognition of BP230 and BP180 by ELISA in classical bullous pemphigoid (BP), prurigo-like BP, elderly patients with pruritic dermatoses and age-matched patients with immediate type allergy.

	Anti-BP230 ^a IgE	Anti-BP230 ^a IgG	Anti-BP180 ^b IgE	Anti-BP180 ^b IgG
Classical BP	11/26 (42.3%)	19/26 (73.9%)	10/26 (38.5%)	22/26 (84.6%)
Prurigo-like BP	2/6 (33.3%)	6/6 (100%)	1/6 (16.7%)	3/6 (50%)
Elderly with pruritic dermatoses	2/21 (9.5%)	8/21 (38.0%)	3/21 (14.3%)	5/21 (23.8%)
Immediate type allergy	2/24 (8.3%)	1/24 (4.1%)	3/24 (12.5%)	1/24 (4.2%)

^a IgE or IgG against the NH2- and COOH-terminus of BP230.

^b IgE or IgG against the NH2 and COOH-terminus of the BP180 ectodomain.

Table 5 Synopsis of IgE and IgG reactivity against the NH2- and COOH-terminus of BP230 and the NH2- and COOH-terminus of the BP180 ectodomain in elderly patients with pruritic dermatoses by ELISA.

	Anti-BP230 ^a				Anti-BP180 ^b					
	NH2		COOH		COOH		NH2		BP180 ex	
	IgE	IgG	IgE	IgG	IgE	IgG	IgE	IgG	IgE	IgG
PR 1	+	—	+	+	—	—	+	—	+	—
PR 2	—	—	—	—	—	+	—	+	—	+
PR 3	—	+	—	—	—	—	—	—	+	—
PR 4	—	+	—	+	—	+	—	+	—	+
PR 5	—	+	+	+	—	—	—	—	—	—
PR 6	—	+	—	—	—	—	—	—	—	—
PR 7	—	+	—	—	—	—	—	—	—	—
PR 8	—	—	—	—	—	—	—	+	—	—
PR 9	—	+	—	—	—	—	—	—	—	—
PR 10	—	+	—	—	—	+	—	+	—	+
PR 11	—	—	—	—	—	+	—	+	—	+
PR 12	—	—	—	—	—	—	—	—	—	—
PR 13	—	—	—	—	—	—	—	—	—	—
PR 14	—	—	—	—	—	—	—	—	—	—
PR 15	—	—	—	—	+	—	+	—	—	—
PR 16	—	—	—	—	—	—	—	—	—	—
PR 17	—	—	—	—	—	—	—	—	—	—
PR 18	—	—	—	—	—	—	—	—	—	—
PR 19	—	—	—	—	—	—	—	—	—	—
PR 20	—	—	—	—	—	—	—	—	—	—
PR 21	—	—	—	—	—	—	—	—	—	—

^a BP230-NH2, NH2-terminus of BP230 (aa1-1307); BP230-COOH, COOH terminus of BP230 (aa1881-2649).

^b COOH-terminus of the BP180 ectodomain (aa1048-1465), NH2-terminus of the BP180 ectodomain (aa490-812), BP180-ex, full ectodomain of BP180 (aa485-1465).

pathogenetic event preferentially targeting the major BP autoantigen, BP180. Epitope spreading in addition to IgE-mediated activation of mast cells and basophils may lead to recognition of the intracellular protein BP230 and an amplification of the inflammatory response later on. Indeed, it has been experimentally demonstrated that mast cell degranulation and release of tryptase are associated with separation of the epidermo-dermal junction [33]. Moreover, activation of mast cells and basophiles may induce symptoms characteristic of BP such as itching, erythema and urticaria. These symptoms are common in unrelated IgE-mediated diseases such as urticaria and atopic eczema.

With regard to the different clinical manifestations of BP, there was no significant difference among the studied groups in detecting IgE reactivity against BP230 or BP180 even though IgE reactivity against the COOH-terminus of BP230 was found more frequently by immunoblot (IB) and ELISA. Specifically, we identified IgE against the COOH-terminal domain of BP230 in 16/32 (IB) and in 12/32 (ELISA) BP sera. In contrast, 11/32 and 9/32 BP sera showed IgE reactivity against BP180-NC16A by IB and ELISA, respectively. Similar data using ELISA were published by Ishiura et al. [25].

Recently Dresow et al. [30] studied IgE reactivity against recombinant BP180 intracellular domain (ICD) by IB in BP patients. They reported that IgE reactivity against the ICD of BP180 is slightly lower than IgE reactivity against BP180-NC16a.

In the present study, IgE reactivity against BP230 and BP180 was less frequently detected in prurigo-like BP than

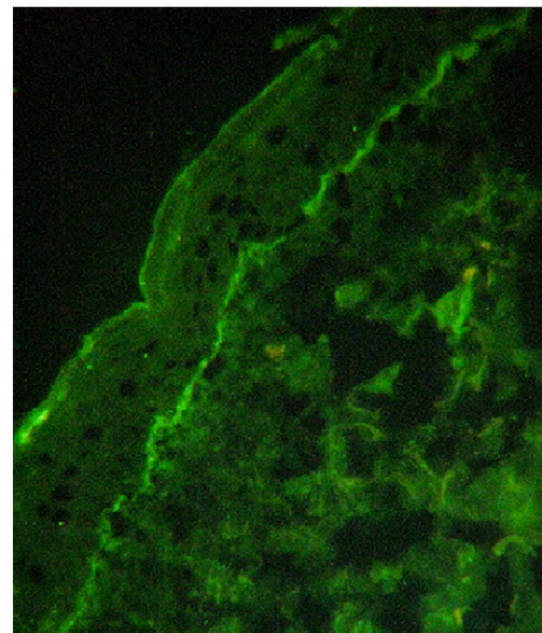


Figure 2 IgE reactivity against the dermo-epidermal basement membrane zone (BMZ) by indirect immunofluorescence microscopy. Shown is the IgE reactivity with the BMZ of normal human skin of sera from a patient with bullous pemphigoid (BP), prurigo-like BP and a pruritic dermatosis. Serum IgE binding to the BMZ was visualized by a FITC-labeled anti-human IgE antibody.

Table 6 IgE reactivity against BP230 and BP180 in bullous pemphigoid (BP), prurigo-like BP (P-L BP) and pruritic dermatoses (PR) by immunoblot (IB), ELISA, and indirect immunofluorescence (IIF) ^a.

Patients	Anti-BP230 IgE ^a				Anti-BP180 IgE ^a						IgE IIF ^c
	NH2		COOH		NH2		NC16A		COOH		
	IB ^b	ELISA	IB	ELISA	IB	ELISA	IB	ELISA	IB	ELISA	
BP 1	—	—	+	+	+	—	+	—	—	—	+
				(43.3)							
BP 2	—	—	—	—	—	—	—	—	—	—	+
BP 3	—	—	—	—	—	—	—	—	—	—	—
BP 4	—	—	+	+	+	—	+	—	+	+	—
				(127.7)						(5.0)	
BP 5	—	—	+	—	—	—	—	—	—	—	—
BP 6	—	—	+	+	—	—	—	—	—	—	—
				(380.1)							
BP 7	+	—	+	+	+	—	+	+	—	—	+
				(406.9)				(23.3)			
BP 8	+	+	—	—	+	—	+	+	—	—	—
		(21.2)						(10.0)			
BP 9	—	—	—	—	—	—	—	—	—	—	—
BP 10	—	—	—	—	—	—	—	—	—	—	—
BP 11	—	—	+	+	+	+	+	+	—	—	—
				(30.7)		(113.5)		(209.5)			
BP 12	—	—	—	—	—	—	—	—	—	—	—
BP 13	—	—	—	—	+	—	—	—	—	—	+
BP 14	—	—	+	+	+	—	+	+	—	—	—
				(143.9)				(10.1)			
BP 15	—	—	—	—	—	—	—	—	—	—	—
BP 16	—	—	—	—	—	—	—	—	—	—	—
BP 17	—	—	+	—	+	—	+	—	—	—	—
BP 18	—	—	—	—	—	—	—	+	—	—	—
								(5.9)			
BP 19	—	—	+	+	—	—	—	+	—	—	+
				(239.4)				(7.2)			
BP 20	—	—	—	—	—	—	—	—	—	—	—
BP 21	—	+	+	+	—	—	—	+	—	—	+
		(22.1)		(45.9)				(37.8)			
BP 22	—	—	—	—	—	—	—	—	—	—	—
BP 23	—	+	+	+	—	—	—	—	—	—	+
		(6.9)		(21.6)							
BP 24	—	—	+	+	—	—	+	+	+	+	—
				(207.8)				(4.5)		(40.4)	
BP 25	—	—	—	—	—	—	+	+	—	—	—
								(13.3)			
BP 26	—	—	+	—	+	—	+	—	+	—	—
P-L BP 1	—	—	+	+	—	—	—	—	+	+	—
				(26.0)						(10.5)	
P-L BP 2	—	—	+	—	—	—	—	—	—	—	—
P-L BP 3	—	—	+	+	+	—	+	—	—	—	+
				(163.1)							
P-L BP 4	—	—	—	—	—	—	—	—	—	—	—
P-L BP 5	—	—	—	—	—	—	—	—	—	—	—
P-L BP 6	—	—	—	—	—	—	—	—	—	—	—
PR 1	+	+	+	+	+	+	+	+	—	—	+
		(46.5)		(746.2)		(46.1)		(171.7)			
PR 2	—	—	—	—	—	—	—	—	—	—	—
PR 3	—	—	—	—	—	—	—	—	—	—	—
PR 4	—	—	+	—	+	—	—	—	—	—	—

Table 6 (continued)

Patients	Anti-BP230 IgE ^a				Anti-BP180 IgE ^a						IgE IIF ^c
	NH2		COOH		NH2		NC16A		COOH		
	IB ^b	ELISA	IB	ELISA	IB	ELISA	IB	ELISA	IB	ELISA	
PR 5	—	—	—	+	—	—	—	+	—	—	—
				(10.8)				(22.8)			
PR 6	—	—	—	—	—	—	—	—	—	—	—
PR 7	—	—	—	—	—	—	—	—	—	—	—
PR 8	—	—	—	—	—	—	—	+	—	—	—
								(4.3)			
PR 9	—	—	—	—	—	—	—	—	—	—	—
PR 10	—	—	—	—	—	—	—	—	—	—	—
PR 11	—	—	—	—	—	—	—	—	—	—	—
PR 12	—	—	—	—	—	—	—	—	—	—	—
PR 13	—	—	—	—	—	—	—	—	—	—	—
PR 14	—	—	—	—	—	—	—	—	—	—	—
PR 15	—	—	—	—	—	+	—	—	—	+	—
						(24.2)				(9.0)	
PR 16	—	—	—	—	—	—	—	—	—	—	—
PR 17	—	—	—	—	—	—	—	—	—	—	—
PR 18	—	—	—	—	—	—	—	—	—	—	—
PR 19	—	—	—	—	—	—	—	—	—	—	—
PR 20	—	—	—	—	—	—	—	—	—	—	—
PR 21	—	—	—	—	—	—	—	+	—	—	—
								(7.5)			

^a The detection of IgE reactive with BP180 or BP230 is expressed as "+", the absence of IgE reactivity as "-". The cut-off for ELISA reactivity was set as a PIV of 4.

^b Immunoblot.

^c IgE Indirect Immunofluorescence.

in classical, bullous BP. IgE against BP180-NC16A was identified in only 1/6 (IB) and 0/6 (ELISA) sera from patients with a prurigo-like phenotype, whereas in classical BP, 10/26 (IB) and 9/26 (ELISA) sera showed IgE reactivity against BP180-NC16A.

The control group consisted of sex-matched patients with immediate type allergic disorders. In this group, we did not detect IgE against BP230 or BP180 by IB. In contrast, IgE against BP180 and BP230 was detected in 5/24 sera from allergic patients by ELISA. This unexpected finding needs to be substantiated in a larger patient cohort.

By IF assay, we found anti-BMZ IgE preferentially in classical bullous BP (7/26 sera), while one serum from a prurigo-like BP patient and an elderly patient with pruritus, respectively, was also positive. Except for one BP patient, IgE was detected by IIF only in those cases that were also reactive by ELISA or IB. Thus, IIF was less sensitive than ELISA and IB and may be linked to a greater degree of BMZ damage, as suggested by Jedlikova et al. [34]. Noteworthy, Yayli and co-workers have shown in a recent study that 41% of patients with BP have linear IgE deposits at the dermo-epidermal basement membrane zone [35].

Furthermore, we studied a potential correlation between the presence of IgE and IgG autoantibodies against the two main subdomains of BP230 and three immunodominant epitopes of BP180. IgG autoantibodies were detected more frequently than IgE autoantibodies, as shown in Table 4.

There was a good correlation between IgE and IgG autoantibodies in the BP patients as determined by ELISA, while a similar correlation was not found in the 21 pruritic patients. In elderly patients with pruritic skin disorders who may represent a subset of patients with early BP, IgE autoantibodies were preferentially directed against BP180-NC16A. In contrast, as shown in a recent study from our group [36], these patients have IgG autoantibodies mainly against the COOH-terminus of BP230.

Once fullblown BP is overt, IgE and IgG autoantibodies seem to target the same or similar epitopes of BP230 and BP180 which is reflected by the good correlation of IgE and IgG autoreactivity in classic BP.

Fairley et al. [27] explained the fine specificity profiles of IgE and IgG autoantibodies reactive with BP180 by a clonal selection of autoreactive B cells. Initially, plasma cells produce initial IgM and later on, via the help of T cells, IgG, IgE and IgA with the same antigen specificity. Epitope spreading which may involve IgG and IgE recognition of multiple epitopes of BP180 and BP230 is linked to a more severe clinical course of BP as shown in a recent European multicenter prospective study [37].

Regarding reactivity by IB and ELISA, these two techniques show a differential correlation for the detection of IgE and IgG autoantibodies depending on the targeted autoantigen. As shown in Table 6, there is a good correlation for IgE recognition of the COOH-terminal domain of BP230 by IB

(16/32) and ELISA (12/32). In the majority, *i.e.* 12/32 of the IgE reactive BP sera, IgE recognition of BP230 was detected by IB and ELISA.

In contrast, there was not a good correlation for IgE reactivity against BP180-NC16A by IB and ELISA. Even though, 11/32 BP sera showed IgE against BP180-NC16A by IB and 9/32 by ELISA, there was only an overlap of IgE reactivity against BP180-NC16A in 6/32 sera by both techniques.

A recent work by Fairley et al. [35] provided some evidence that targeting of IgE autoantibodies may be therapeutically exploited in BP. They therapeutically used omalizumab, a humanized monoclonal antibody against human IgE in a refractory BP patient who showed significant clinical improvement upon treatment. This was the first *in vivo* demonstration in support of a critical role of IgE autoantibodies in the pathogenesis of BP.

5. Conclusions

Our data show that IgE autoantibodies react mainly against the COOH-terminus of BP230 and, to a lesser extent, against BP180-NC16A in patients with BP. In contrast, elderly patients with pruritic dermatoses show preferential IgE recognition of the BP180-NC16A. We consider both, IgE and IgG autoantibodies to be critical in the pathogenesis of BP. Further data are necessary to explain how these autoantibody subtypes induce dermo-epidermal separation and the characteristic phenotype of BP.

Conflict of interest statement

None of the authors has any potential financial conflict of interest related to this manuscript.

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