

IgG autoantibody reactivity against bullous pemphigoid (BP) 180 and BP230 in elderly patients with pruritic dermatoses

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Summary

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Conflicts of interest

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Background Pruritic dermatoses of the elderly often pose a diagnostic and therapeutic challenge. Specifically, a prodromal phase of bullous pemphigoid (BP) has to be considered in patients with pruritic lesions of polymorphic appearance. These conditions frequently do not fulfil all the clinical, histological and immunopathological criteria for establishing the diagnosis of BP.

Objectives To investigate IgG reactivity against the autoantigens of BP, BP180 and BP230, by enzyme-linked immunosorbent assay, in elderly patients affected with various pruritic disorders who had never experienced clinically apparent blisters.

Methods The sera of 15 elderly patients with pruritic disorders (group I) were tested for IgG reactivity against BP180 and BP230. Also included were 30 patients with full-blown BP (group II) and 25 age-matched patients with immediate-type allergic reactions (group III).

Results Thirty-three per cent of the patients with pruritic disorders (group I) showed IgG against BP230 and/or BP180: four of 15 patients had IgG against BP230 while two of the 15 group I patients were BP180 reactive. All the BP sera (group II) showed IgG reactivity against BP180 and/or BP230. Notably, two of 25 control sera (group III) showed IgG reactivity against either BP180 or BP230. **Conclusions** The present findings suggest that IgG reactivity against BP230 (i.e. the COOH terminus), and to a lesser extent against BP180, is a common finding in pruritic disorders of the elderly with a wide clinical spectrum. IgG-mediated autoimmunity against the intracellular BP230 may facilitate a chronic, inflammatory response eventually leading to full-blown BP which is presumably associated with IgG against BP180.

Bullous pemphigoid (BP) is an autoimmune blistering disease, affecting particularly the elderly population. Clinically, it is characterized by the presence of tense blisters which arise preferentially on inflamed skin. Lesions normally predominate on the lower trunk, axillae, groins or flexor surfaces of the extremities. Less commonly (20–30% of cases), the mucous membranes are also affected. Particularly in the prodromal phase of BP, pruritus is a common feature.^{1,2}

In addition to the classical bullous phenotype, several atypical clinical variants have been described which are considered to represent prodromal phases of BP.^{3–5} These variants include generalized pruritus without any skin manifestation, urticarial or erythematous plaques, eczema-like or prurigo-like lesions presenting as pruritic papules or nodules and generalized multi-forme-like exanthema. These polymorphic clinical features

make it difficult to establish the diagnosis of BP in the differential diagnosis of pruritic disorders of the elderly. Eventually, based on characteristic immunopathological features, the diagnosis of BP may be considered or even established.

A hallmark of the pemphigoids, and particularly of BP, is the presence of IgG antibodies against two hemidesmosomal components of the epidermal basement membrane zone, a 180-kDa antigen (BP180, collagen XVII, BP antigen 2) and a 230-kDa antigen (BP230, BP antigen 1).² BP180 is a trans-membrane protein with a large extracellular domain (ECD), consisting of 15 interrupted collagen domains, that plays a pivotal role in cellular adhesion. In contrast, BP230 is a cytoplasmic protein, belonging to the plakin family, which mediates the interaction between intermediate filaments and the cell membrane. Because of its cytoplasmic localization, BP230

is presumably targeted by IgG autoantibodies as a secondary event, which is supported by recent experimental data.² In contrast, BP180 has been identified as the major antigenic target molecule of BP. Several immunological studies showed that the noncollagenous region 16A (NC16A), adjacent to the transmembranous portion of the BP180 ectodomain, is preferentially targeted by BP autoantibodies.^{6–8} The pathogenic significance of BP180-NC16A-specific IgG has been demonstrated both *in vivo* and *in vitro*.^{2,9,10} Furthermore, a recent study demonstrated that T and B cells of patients with BP preferentially recognize epitopes of the BP180 ectodomain in the active disease phase which supports the concept that autoimmunity against BP180 is the initial and critical step for the development of full-blown BP.¹¹

Several studies tried to correlate the autoantibody profile with the clinical phenotype and extent of BP. It has been shown that the titres of anti-BP180-NC16A IgG correlate with the clinical severity of BP, while IgG reactivity against the central portion of the BP180 ectodomain was associated with limited BP.^{12,13} On the other hand, IgG autoantibodies from BP patients with mucosal involvement seem preferentially to target the NH₂ and COOH terminal portions of the BP180 ectodomain. IgG against BP230, instead, was associated with a more limited phenotype of BP.^{13,14} All these studies were performed in patients with BP who presented clinically with the typical bullous phenotype.

A few studies have occasionally detected IgG reactivity against the BP antigens, BP180 and BP230, in elderly patients with pruritic disorders who had never experienced blisters.^{5,15,16} Thus, the aim of the present study was to investigate comprehensively, utilizing various recombinant forms of BP180 and BP230, the presence of IgG autoantibodies against these BP autoantigens in elderly patients with clinically polymorphic, pruritic disorders without overt blisters compared with patients with full-blown BP and the characteristic bullous phenotype.

Materials and methods

Patients

The sera of 15 patients (aged 76 ± 6 years) with acute or chronic, mild to intense pruritus, who had never experienced blisters, were tested for IgG reactivity with BP180 and BP230 by enzyme-linked immunosorbent assay (ELISA) (group I). These patients presented with various clinical manifestations, including prurigo-like lesions ($n = 5$), pruritic erythema ($n = 5$) and pruritus of unknown aetiology ($n = 5$). These patients did not fulfil the clinical criteria for 'classic' BP based on clinical phenotype and direct and indirect immunofluorescence findings. Specifically, these patients did not show tissue-bound IgG despite the presence of circulating IgG against BP180 and/or BP230. Representative examples of the clinical appearance of the lesions are shown in Figure 1.

Also studied were the sera of 30 patients (aged 74 ± 16 years) with BP who (i) presented clinically with typical

tense blisters of the skin and, rarely, mucosal blisters/erosions, (ii) showed tissue-bound IgG at the dermal–epidermal junction by direct immunofluorescence and (iii) had serum IgG reactive with the epidermal part of saline-split human skin as determined by indirect immunofluorescence and IgG against BP180 and/or BP230 by ELISA (group II). Some of these patients presented with a prurigo-like phenotype with few or no blisters.

Group III consisted of 25 sex- and age-matched individuals (aged 78 ± 3 years) affected by immediate-type allergic disorders such as urticaria ($n = 5$), allergic rhinitis ($n = 13$), insect allergy ($n = 4$) and drug allergy ($n = 3$) who had never experienced bullous skin lesions.

Expression and purification of BP180 and BP230 recombinant proteins

The recombinant BP proteins were produced in a baculovirus expression system as glutathione-S-transferase (GST) and 6xHis-tagged fusion proteins as previously reported.¹⁷ BP180ex (baculoprotein containing the entire ECD of BP180; aa residues 486–1497), BP180-N (baculoprotein containing the NH₂ terminus of BP180; aa residues 490–812), BP180-C (baculoprotein containing the COOH terminus of BP180; aa residues 1048–1465), BP230-C (baculoprotein containing the COOH terminus of BP230; aa residues 1881–2649) and BP230-N (baculoprotein containing the NH₂ terminus of BP230; aa residues 1–1307) were fractionated by 10% sodium dodecyl sulphate–polyacrylamide gel electrophoresis and visualized by Coomassie staining and Western blot with a monoclonal mouse anti-GST antibody (1 : 2000, clone GST3-4C; Zymed, South San Francisco, CA, U.S.A.) to verify molecular weight and immunoreactivity (Fig. 2).

Enzyme-linked immunosorbent assay utilizing BP230 and BP180 recombinant proteins

ELISA procedures were performed as described recently.^{13,17} The affinity-purified BP230 and BP180 proteins were immobilized on to 96-well polystyrene plates (Maxisorb Immuno-plate; Nunc, Roskilde, Denmark) by coating each well with 0.5 µg of BP230-N, BP230-C, BP180ex, BP180-N and BP180-C, respectively, or a molar equivalent amount of the control protein GST/6xHis in 100 µL of 0.1 mol L⁻¹ bicarbonate buffer (pH 9.8), at 4 °C for 5 h. ELISA plates were then washed five times with TTBS [Tris-buffered saline (pH 7.5) with 0.05% Tween 20; Merck, Darmstadt, Germany] and blocked for 2 h with 100 µL of 5% skimmed milk powder in TTBS at room temperature. Sera were diluted 50-fold in blocking buffer and added to the wells in duplicate. After an incubation period of 12 h at 4 °C and consecutive washes, each well was reacted with alkaline phosphatase-labelled goat antihuman IgG (γ) (1/6000; Kirkegaard & Perry Laboratories, Gaithersburg, MD, U.S.A.). After another series of washes, 100 µL of p-nitrophenyl-phosphate (Kirkegaard & Perry Laboratories) was added to



Fig 1. Polymorphic clinical phenotype of pruritic dermatoses of the elderly. Clinically, the patients with pruritic dermatoses presented with a generalized prurigo-like phenotype (a), diffuse pruritic erythema (b) or with pruritic papulovesicles on the extensor surfaces of the extremities (c).

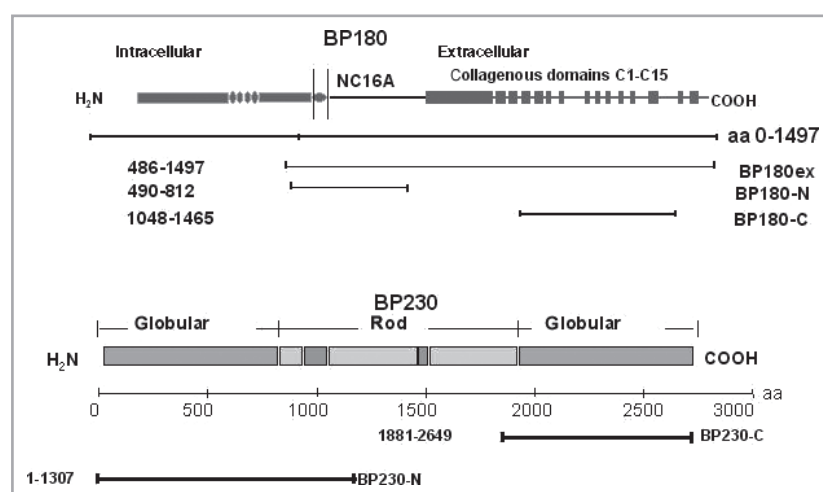


Fig 2. Baculovirus-derived BP180 and BP230 proteins. The recombinant bullous pemphigoid (BP) proteins were produced as glutathione-S-transferase (GST) and 6xHis-tagged fusion proteins in a baculovirus expression system. The recombinant proteins and the GST/6xHis control protein (generated by cotransfection of an empty pAcGHLT-A vector) were expressed in Sf21 cells and were purified by affinity chromatography over nickel-NTA agarose as previously described.¹⁷

the wells, and optical density (OD) was measured at 405 nm when a defined patient serum (positive reference control) reached an OD of 1.0 as determined by a Wallac 1420 microplate reader (PerkinElmer, Waltham, MA, U.S.A.). All extinction data were standardized based on the positive and negative internal standards, which were set to 1 and 0

OD units, respectively. Background reactivity of IgG to the control protein GST/6xHis was subtracted from the mean OD values obtained with BP180-N and BP180-C proteins. IgG serum reactivity of the patients against BP180 and BP230 was expressed as protein index value (PIV) in U mL⁻¹.

Results

IgG reactivity of the patients' sera by enzyme-linked immunosorbent assay

Sera from elderly patients with pruritic disorders (group I), classical BP with characteristic bullae (group II) and immediate-type allergy (group III) were tested against defined recombinant variants of the autoantigens of BP, BP180 and BP230, by ELISA. Specifically, IgG reactivity was determined against the BP180 ectodomain, the NH₂ terminal and COOH terminal portions of BP180 and against the COOH and NH₂ termini of BP230 (Fig. 2). IgG reactivity against distinct BP autoantigens was expressed as PIV, as described previously.^{13,17} The cut-off values for the detection of IgG reactivities were 20 for the ECD of BP180, 28 for BP180-N and BP180-C, 17 for BP230-N and 10 for BP230-C.

Autoantibody profile of elderly patients with pruritic dermatoses (group I)

Thirty-three per cent (five of 15) of the elderly patients with pruritic disorders showed IgG reactivity with at least one of

the employed BP antigens (Tables 1 and 2). Specifically, three of five (60%) sera reacted with BP230 only, one of five (20%) sera with BP180 only, and one of five (20%) sera showed IgG reactivity with BP180 and BP230 (Table 1). The two sera that were reactive with the BP180 ectodomain recognized the NH₂ terminal and COOH terminal domain of BP180. Of the four sera that showed IgG reactivity against BP230, three sera reacted with the COOH terminus and two sera reacted with the NH₂ terminus of BP230 (Table 2). Overall, the COOH terminus of BP230 was preferentially targeted by the sera of these patients (Table 3). The polymorphous clinical spectrum of these patients ranged from diffuse erythema to pruritic papules and nodules which responded well to topical treatment with glucocorticoids, ultraviolet (UV) B radiation and systemic antihistamines over a follow-up period of 4–31 months.

Autoantibody profile of the patients with full-blown bullous pemphigoid (group II)

All of the 30 BP sera showed IgG reactivity against BP180 and/or BP230 (Table 1). Specifically, IgG against BP180 and BP230 was found in 14 of 30 patients with BP, IgG reactivity against BP180 only in nine of 30 patients, and IgG against

IgG reactivity	Patient group		
	Group I (pruritic disorders)	Group II (full-blown pemphigoid)	Group III (immediate-type allergy)
BP180 only	1/15 (7%)	9/30 (30%)	1/25 (4%)
BP230 only	3/15 (20%)	7/30 (23%)	1/25 (4%)
BP180 + BP230	1/15 (7%)	14/30 (47%)	0/25 (0%)
Neither	10/15 (67%)	0/30 (0%)	23/25 (92%)
Total	n = 15	n = 30	n = 25

Table 1 IgG reactivity against BP180 and BP230 in elderly patients with pruritic dermatoses and bullous pemphigoid, and age-matched controls

Table 2 Clinical and immunoserological characteristics of five elderly patients with pruritic disorders with autoantibodies against components of the dermal–epidermal basement membrane zone (BMZ)

Anti-BMZ antibodies									
Patient	DIF	IIF	Anti-BP180 IgG ^a			Anti-BP230 IgG ^a		Clinical phenotype ^b	Follow-up period (months)
			BP180ex	BP180-N	BP180-C	BP230-N	BP230-C		
1	—	+	—	—	—	—	+	Erythematous plaques	31
2	—	+	+	+	+	—	—	Pruritic papules	17
3	—	+	—	—	—	+	—	Prurigo nodules on the extensor surfaces of the arms	10
4	—	+	+	+	+	+	+	Generalized itchy erythema	10
5	—	+	—	—	—	—	+	Itchy diffuse erythema	4

^aBP180ex, BP180 ectodomain (aa486–1497); BP180-N, NH₂ terminus of BP180 (aa490–812); BP180-C, COOH terminus of BP180 (aa1048–1465); BP230-N, NH₂ terminus of BP230 (aa1–1307); BP230-C, COOH terminus of BP230 (aa1881–2649). ^bNone of the patients developed blisters during the observation period. DIF, direct immunofluorescence microscopy; IIF, indirect immunofluorescence microscopy utilizing normal human split skin or monkey oesophagus as substrate.

^aBP180ex, BP180 ectodomain (aa486–1497); BP180-N, NH₂ terminus of BP180 (aa490–812); BP180-C, COOH terminus of BP180 (aa1048–1465); BP230-N, NH₂ terminus of BP230 (aa1–1307); BP230-C, COOH terminus of BP230 (aa1881–2649). ^bNone of the patients developed blisters during the observation period. DIF, direct immunofluorescence microscopy; IIF, indirect immunofluorescence microscopy utilizing normal human split skin or monkey oesophagus as substrate.

Table 3 Autoantibody profile of elderly patients with pruritic disorders and patients with bullous pemphigoid

Patient group	Anti-BP180 IgG				Anti-BP230 IgG		
	Anti-BP180 ± anti-BP230 IgG	Total	Domain		Total	Domain	
			NH ₂	COOH		NH ₂	COOH
Group I (pruritic dermatoses)	5/15 (33%)	2/15 (13%)	2/15 (13%)	2/15 (13%)	4/15 (27%)	2/15 (13%)	3/15 (20%)
Group II (bullous pemphigoid)	30/30 (100%)	23/30 (77%)	16/30 (53%)	13/30 (43%)	21/30 (70%)	8/30 (27%)	17/30 (57%)
Group III (immediate-type allergy)	2/25 (8%)	1/25 (4%)	0/25 (0%)	1/25 (4%)	1/25 (4%)	1/25 (4%)	0/25 (0%)

BP230 only in seven of 30 patients. Of the 23 BP180-reactive sera, 16 (70%) reacted against BP180-N while 13 (57%) reacted with the COOH terminus of BP180. Of the 21 BP230-reactive sera, eight (38%) reacted with the NH₂ terminus and 17 (81%) with the COOH terminus of BP230 (Table 3).

Autoantibody profile of the patients with immediate-type allergy (group III)

Among the age-matched control subjects with immediate-type allergies, we found that 2 of 25 sera (8%) showed IgG reactivity against either BP180 or BP230 (Table 1). Specifically, one serum reacted against the COOH terminal domain of BP180 and one serum with the NH₂ terminus of BP230 (Table 3). Both individuals had allergic rhinitis and had not recently experienced skin rashes or bullous lesions.

Discussion

In the present study, we detected IgG autoantibodies against the autoantigens of BP, BP180 and BP230, in a significant portion (33%) of 15 elderly patients with pruritic dermatoses who did

not fulfil all the criteria to establish the diagnosis of BP. Notably, the intracellular BP230 antigen was recognized by the majority (80%) of the reactive sera while the transmembranous BP180 antigen was recognized by only 40% of these sera. In these individuals, IgG autoantibodies preferentially recognized the COOH terminal domain of BP230, which is in line with previous studies. These findings extend previous observations showing occasional IgG recognition of sera from patients with chronic pruritic dermatoses against BP180 and/or BP230 (Table 4). The preferential detection of anti-BP230 IgG in the described cases of pruritic dermatoses which may be considered as 'prepemphigoid' challenges our current understanding of the pathogenesis of BP, that IgG against the transmembrane BP180, but not against the intracellular BP230, is critical for the initial steps in the pathogenesis of BP. Notably, none of the five patients with pruritus of unknown aetiology had IgG against BP180 or BP230, suggesting that the presence of exanthematous pruritic lesions was more likely to be associated with autoantibodies. The autoantibody titres (expressed as PIV values) of the BP180/BP230-reactive sera from the five patients with pruritic disorders were comparable with those of the 30 BP sera (data not shown).

Table 4 Overview of reported cases with pruritic, nonbullous dermatoses and IgG reactivity against the bullous pemphigoid (BP) antigens, BP180 and BP230

Patients enrolled		Reactivity against BP antigens				References
		Characterization of IgG reactivity (%)				
		Total, n (%)	Anti-BP180	Anti-BP180 + anti-BP230	Anti-BP230	
n	Inclusion criteria					
97	Patients with pruritic disorders	24 (25)	33	20	47	Rieckhoff-Cantoni et al. (1992) ²²
32	Elderly subjects with itchy skin	3 (9)	0	0	100	Hachisuka et al. (1996) ¹⁵
25	Elderly patients with pruritus	3 (12)	100	ND	ND	Hofmann et al. (2003) ¹⁶
16	Pemphigoid patients without bullous lesions	16 (100)	12.5	12.5	75	Wever et al. (1995) ²⁴ Tamada et al. (1995) ²⁵ Powell et al. (2002) ²⁶ Schmidt et al. (2002) ²⁷ Tashiro et al. (2005) ²⁸
15	Elderly patients with pruritic dermatoses	5 (33)	20	20	60	Present study
ND, not determined.						

ND, not determined.

There is evidence for a proinflammatory role of IgG against BP230 in the pathogenesis of BP. Hall *et al.*¹⁸ showed in a rabbit model of BP that injection of 18-amino acid, BP230-derived peptides (which had previously been identified as immunodominant epitopes) led to an enhanced inflammatory response to UVB irradiation, which was associated with a neutrophilic and eosinophilic infiltrate and linear deposits of IgG and C3 at the dermal–epidermal junction. The BP230 peptide-injected animals developed an IgG response against these peptides but did not show any clinical evidence of a bullous phenotype mimicking BP. An independent study confirmed these findings by demonstrating that the passive transfer of rabbit polyclonal antibodies raised against an antigenic sequence of human BP230 into mice induced an inflammatory cutaneous response.¹⁹ Guo *et al.*²⁰ generated a mouse which was deficient for BP230 gene expression that developed skin fragility and neurological abnormalities but no evidence for bullous lesions. In summary, these findings strongly suggest that IgG-driven autoimmunity against BP230 supports rather than initiates the clinical manifestation of BP.

Previous studies, conducted in patients with classical BP, identified the transmembrane BP180 antigen as the major target of IgG autoantibodies in the sera of patients with BP.^{2,8,13} Moreover, the passive transfer of anti-BP180-NC16A IgG into mice induces a phenotype with subepidermal bullae which is highly characteristic of BP.^{9,10} Di Zenzo *et al.*²¹ also identified intracellular epitopes of BP180 that were recognized by IgG autoantibodies early on which strongly suggests that IgG recognition of intracellular epitopes of BP180 and BP230 is not a late event in the BP pathogenesis. A previous study from our group in 127 patients with classical BP showed a significant association of IgG autoantibodies against BP230 and a limited clinical phenotype of BP.¹³

BP is the most common autoimmune bullous disorder of the elderly, with a peak at the age of 60–80 years. Tense blisters are the typical manifestation of BP. In an unestablished percentage of cases, blisters can be preceded by mild to intractable pruritus persisting for several months prior to development of full-blown BP, a condition known as ‘prepemphigoid’ or ‘early BP’. As recently reviewed by Lamb *et al.*,⁵ cases of prepemphigoid manifest as urticaria-like plaques, eczema-like lesions and dermatitis herpetiformis-like lesions. They suggested in their reported cases that atypical BP rarely develops into classical BP and may just be a less aggressive form of full-blown BP. In the present study, none of the patients with pruritic skin lesions reactive with BP230 and/or BP180 developed blisters during a follow-up period of 4–31 months.

Pruritus is a hallmark of many inflammatory skin disorders and also represents the most common neurocutaneous disorder in elderly patients. Thus, a loss of tolerance against BP230 and BP180 may be a consequence of chronic pruritus. Previously, Hachisuka *et al.*¹⁵ showed that autoantibodies against components of the dermal–epidermal junction (particularly against BP230) are fairly common in elderly patients with itchy dry skin. Rieckhoff-Cantoni *et al.*²² analysed 97 patients with pruritic dermatoses including BP. As expected,

they found serum and tissue-bound autoantibodies against BP180 and BP230 in bullous BP. In contrast, patients with chronic inflammatory dermatoses such as prurigo, eczema and lichen planus occasionally had serum IgG against components of the dermal–epidermal junction (mainly anti-BP230 IgG) while tissue-bound IgG autoantibodies were not detected by direct immunofluorescence.²² Notably, none of the latter patients developed the classical phenotype of BP within a follow-up period of 18 months. Recently, Hofmann *et al.*¹⁶ investigated retrospectively the humoral response to BP180 by a commercially available BP180-NC16A ELISA in 25 elderly patients with pruritus, and found that 12% of the subjects showed IgG reactivity against the NH₂ terminus of the BP180 ectodomain (Table 4). Finally, a series of case reports detected the same autoantibody profile in patients with pruritic prepemphigoid compared with full-blown BP with tense blisters which in some cases developed later on.^{23–28} Notably, the present study also detected IgG against BP180 and BP230 in two elderly individuals with immediate-type allergy who had experienced neither pruritic nor bullous skin lesions.

The findings of the present study suggest that pruritic dermatoses of the elderly are associated with anti-BP230 IgG (and to a lesser extent with anti-BP180 IgG) which has been shown to exert proinflammatory activity in animal models of BP. Additional, not yet identified, factors are presumably necessary to develop the full-blown bullous phase of BP including IgG autoantibodies against the BP180-NC16a domain. In general, agents implicated in the development of clinically apparent autoimmunity include infection, drugs, physical factors such as UV radiation and burns, and tumours. Several of these factors have been associated mainly in single cases with the outcome of BP. The relative role of inter- and intramolecular epitope spreading in IgG recognition of BP180 and BP230 epitopes and the evolving clinical phenotype needs to be defined in further studies. The availability of highly sensitive specific ELISAs with defined BP180 and BP230 recombinants will be extremely helpful in defining the molecular mechanisms leading from autoimmunity against the BP autoantigens to full-blown BP.

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