

IgG, IgA and IgE autoantibodies against the ectodomain of BP180 in patients with bullous and cicatricial pemphigoid and linear IgA bullous dermatosis

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Summary

Background Bullous pemphigoid (BP), linear IgA bullous dermatosis (LABD) and cicatricial pemphigoid (CP) are clinically distinct autoimmune bullous skin diseases characterized by autoantibodies against components of the epidermal basement membrane. Like most patients with BP, a significant subgroup of patients with CP has circulating IgG specific for BP180, a transmembrane protein of hemidesmosomes. Moreover, sera of patients with LABD contain IgA autoantibodies reactive with a 97/120-kDa protein, LABD antigen 1, which is highly homologous to the extracellular portion of BP180. **Objectives** We aimed to determine whether, in these diseases, autoantibody reactivity to BP180 is restricted to distinct immunoglobulin subtypes.

Methods Utilizing a baculovirus-encoded form of the ectodomain of BP180, sera from patients with BP ($n = 10$), CP ($n = 9$), LABD ($n = 10$) and normal human control sera ($n = 10$) were analysed by immunoblot for IgG, IgA and IgE reactivity against BP180.

Results All of 10 BP sera displayed IgG, IgA and IgE reactivity with BP180. Six and seven of nine CP sera, respectively, contained IgG and IgA autoantibodies reactive with BP180, but none of nine sera contained BP180-specific IgE. Nine of 10 LABD sera contained IgA, and six of 10 IgG, which was reactive with BP180, but none of 10 sera showed IgE reactivity to BP180.

Conclusions The presence of IgG and IgA autoantibody responses to BP180 in patients with three clinically distinct autoimmune bullous diseases indicates that an autoimmune response to the same distinct adhesion protein may lead to different clinical manifestations. It is therefore conceivable that variable epitopes of BP180 are targeted by the different autoantibody isotypes, resulting in the distinct clinical pictures.

Key words: autoimmunity, B lymphocytes, BP180, bullous pemphigoid, cicatricial pemphigoid, epitope, hemidesmosomes, immunoglobulin subtypes, linear IgA bullous dermatosis

Bullous pemphigoid (BP) is an autoimmune blistering disorder characterized by subepidermal, generalized, tense blisters on normal or erythematous skin and, in some cases, blisters or erosions of the mucous membranes. A hallmark of BP is the presence of circulating autoantibodies (autoantibodies) directed against the basement membrane (BM) zone of stratified squamous epithelia.^{1,2} These autoantibodies react

predominantly with two components of hemidesmosomes, BP230 (also termed BP antigen 1) and BP180 (also termed BP antigen 2 or type XVII collagen).^{2–4} BP230, a cytoplasmic plaque constituent of hemidesmosomes, has been shown to associate with intermediate filaments.⁵ In contrast, BP180 is a type II transmembrane protein that may serve as a cell matrix receptor for ligands within the extracellular matrix, e.g. laminin-5.^{6–8} BP180-reactive autoantibodies are presumed to be critical in the pathogenesis of BP as rabbit IgG against a site of the extracellular domain (ECD) of murine BP180 induces subepidermal blisters that

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Table 1. Synopsis of clinical and immunological findings in patients with bullous pemphigoid (BP), cicatricial pemphigoid (CP) and linear IgA bullous dermatosis (LABD)

Patient	Clinical status	Treatment	DIF	IIF	IgG ^a	IgA ^a	IgE ^a
BP1	Acute development of tense bullae on trunk and arms	Not commenced	IgG at BMZ	IgG at blister roof (SSS)	+	+	+
BP2	No blisters for 4 weeks; previously tense bullae on erythematous skin	Discontinued	IgG at BMZ	IgG at blister roof (SSS)	+	+	+
BP3	Blisters on the arms, eroded blisters on the trunk	Azathioprine 100 mg daily	IgG > IgA at BMZ	IgG ≥ IgA at blister roof (SSS)	+	+	+
BP4	Tense bullae on trunk, arms and thighs	Methylprednisone 40 mg daily	IgG at BMZ	IgG at blister roof (SSS)	+	+	+
BP5	Multiple tense bullae on upper trunk and arms, acute onset	Not commenced	IgG at BMZ	IgG at blister roof (SSS)	+	+	+
BP6	Tense bullae on arms and legs	Methylprednisone 60 mg daily	IgG at BMZ	IgG at blister roof (SSS)	+	+	+
BP7	Aggregated bullae on trunk and extremities	Prednisolone 36 mg daily	C3 at BMZ	BP180 ELISA +	+	+	+
BP8	Tense bullae on entire body, acute onset	Not commenced	IgG at BMZ	ND	+	+	+
BP9	Tense bullae on trunk	Not commenced	IgG at BMZ	IgG at blister roof (SSS)	+	+	+
BP10	Few bullae and crusty erosions on trunk and arms	Not commenced	IgG at BMZ	IgG at blister roof (SSS)	+	+	+
CP1	Erosions on conjunctivae/oral mucosa with mild scarring	Diaminodiphenylsulphone 100 mg daily	IgA at BMZ	– (SSS)	+	+	–
CP2	Erosions on conjunctivae/oral mucosa, tense blisters on trunk	Not commenced	IgG at BMZ	IgG at blister roof (SSS)	–	+	–
CP3	Erosions on oral mucosa with scarring of the palatine mucosa	Topical glucocorticoids	C3 at BMZ	– (SSS)	–	+	–
CP4	Presternal pustules and erosions	Diaminodiphenylsulphone	IgM at BMZ	– (SSS)	+	+	–
CP5	Tense blisters on arms, erosions on nasal/oral mucosa	Thalidomide 100 mg daily	C3 at BMZ	– (SSS)	+	+	–
CP6	Erosions of oral mucosa, conjunctivae, glans penis and thighs	Diaminodiphenylsulphone 100 mg daily, prednisone 40 mg daily, azathioprine 100 mg daily	– (oral mucosa); IgA, IgM, C3 at BMZ (skin)	– (SSS)	+	+	–
CP7	Bullae on head/trunk with early scarring alopecia	Prednisone 60 mg daily, azathioprine 100 mg daily	IgG > IgA, C3 at BMZ	– (SSS)	+	+	–
CP8	Chronic conjunctivitis with discrete symblepharon	Not commenced	IgG at BMZ (conjunctiva)	– (SSS)	+	–	–
CP9	Bullae and erosions on extremities with distinct scarring; previous episode of oral lesions	Tetracycline 200 mg daily, azathioprine 100 mg daily	–	– (SSS)	–	–	–
LABD1	Pruriginous papulovesicles on trunk	Diaminodiphenylsulphone 100 mg daily	IgA at BMZ	– (SSS)	–	+	–
LABD2	'Cluster of jewels'-like tense bullae on trunk	Prednisone 64 mg daily	–	– (SSS)	+	+	–
LABD3	Clustered bullae on the trunk, erosions on buccal mucosa	Diaminodiphenylsulphone 100 mg daily	IgA at BMZ	ND	+	+	–
LABD4	Acute onset of aggregated blisters on trunk	Not commenced	IgA at BMZ	– (SSS)	–	–	–
LABD5	Pruriginous papules and few vesicles on trunk and extremities	Not commenced	IgA at BMZ	– (SSS)	+	+	–

Table 1. *continued*

Patient	Clinical status	Treatment	DIF	IIF	IgG ^a	IgA ^a	IgE ^a
LABD6	Crusted erosions on trunk, initially tense bullae with centrifugal progression	Not commenced	ND	– (SSS)	+	+	–
LABD7	Urticarial, partly annular lesions on trunk	Diaminodiphenylsulphone 100 mg daily	IgA, C3 at BMZ	– (SSS)	–	+	–
LABD8	Extensive bullae and crusty erosions on entire skin	Diaminodiphenylsulphone 100 mg daily	IgA (IgM, C3) at BMZ	– (SSS)	+	+	–
LABD9	Bullous exanthem, initially on face, then on extremities	Diaminodiphenylsulphone 100 mg daily	IgA (IgM) at BMZ	– (SSS)	+	+	–
LABD10	Erosive pruriginous papules on shoulders, buttocks and arms	Diaminodiphenylsulphone 150 mg daily	IgA (IgM) at BMZ	– (SSS)	–	+	–

DIF, direct immunofluorescence of perilesional skin; BMZ, basement membrane zone; IIF, indirect immunofluorescence with patients' sera; BP180 enzyme-linked immunosorbent assay (ELISA) with BV13 protein or SSS (salt split normal human skin). ^aReactivity by immunoblot with recombinant BV13 protein (extracellular portion of BP180) (Fig. 1a–c). +, positive result; –, negative result; ND, not performed.

resemble BP, when injected into neonatal mice.⁹ BP180-reactive IgG targets primarily the NC16A domain of the ECD of BP180¹⁰ and regions located in the C-terminal end of the ECD of BP180.^{11,12}

Cicatricial pemphigoid (CP) is a subepidermal autoimmune bullous disease which, in contrast to BP, mainly affects mucous membranes, such as the conjunctivae and oral, perianal and genital mucosa, leading to scarring of the affected tissue. In CP, circulating IgG autoantibodies have been shown to react with target various antigens within the epidermal BM, including BP180¹³ and the α -, β - and γ -chains of laminin-5.^{14,15}

Linear IgA bullous dermatosis (LABD) is a subepidermal autoimmune blistering disorder of the skin characterized by linear deposits of IgA at the dermoepidermal BM.^{16,17} Typically, patients with LABD suffer from characteristic pruritic vesicles and bullae that tend to progress by centrifugal cluster formation. Occasionally, LABD may also present with tense bullae reminiscent of BP or with oral erosions with scarring reminiscent of CP. The targeted autoantigens are heterogeneous.^{18–20} Most LABD sera recognize a 97-kDa protein located in the dermoepidermal BM that most probably corresponds to a proteolytic fragment of the ECD of BP180.²¹ In addition, LABD sera have been found to react with distinct regions of the ECD of BP180, namely the NC16A domain, which also harbours major epitopes recognized by sera from BP patients.^{22,23}

As most patients with BP, as well as a subset of patients with CP, have IgG reactive with BP180, and as a significant number of patients with LABD shows IgA reactive with BP180, the purpose of this study was to

investigate whether autoantibodies of a particular immunoglobulin isotype were restricted to a distinct clinical entity. Sera from patients with BP, CP and LABD were therefore screened by immunoblot (IB) analysis for IgG, IgA and IgE reactivity with a recombinant, baculovirus-derived protein representing the ECD of BP180. Our analyses demonstrate that BP180-reactive autoantibodies of various immunoglobulin subclasses are present in the sera of patients with the clinically distinct autoimmune bullous dermatoses BP, CP and LABD.

Materials and methods

Patients and controls

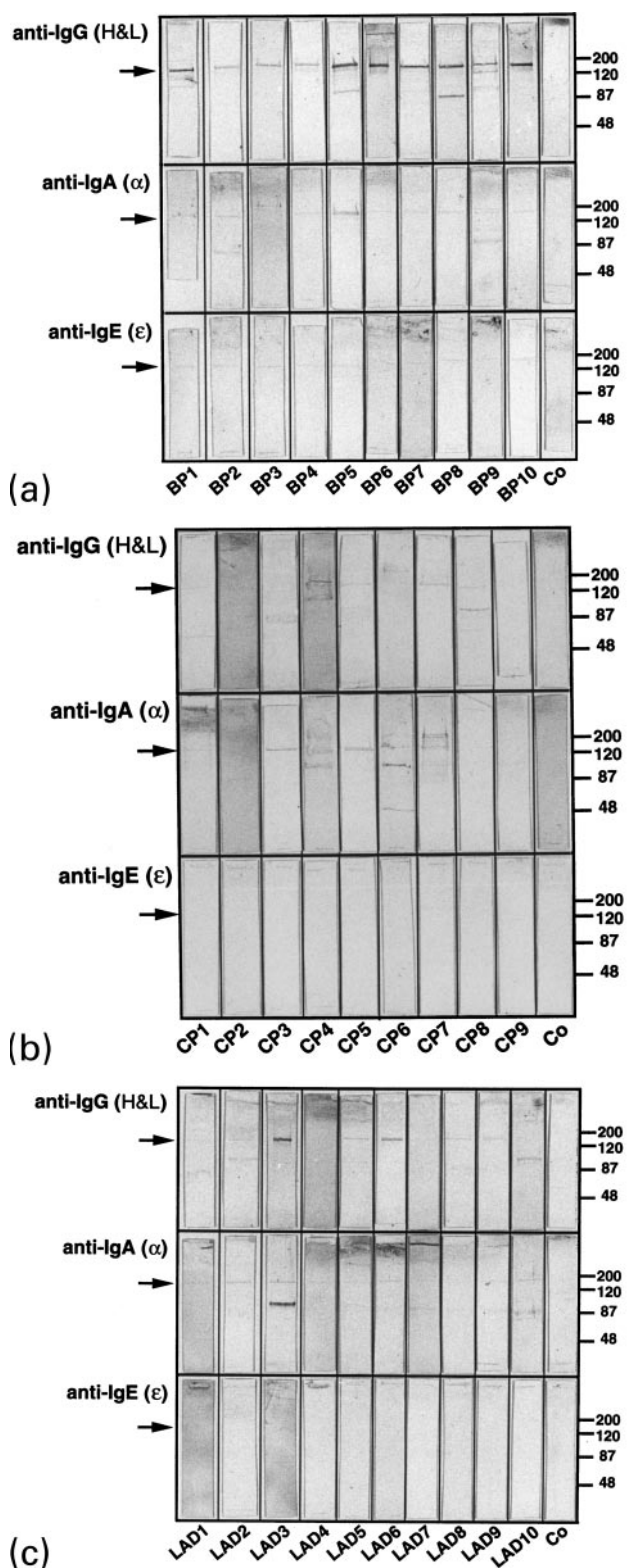
Serum samples were obtained from adult patients with generalized BP ($n = 10$), LABD ($n = 10$), CP ($n = 9$) and healthy volunteers ($n = 10$). The clinical diagnosis of BP was confirmed by: (i) subepidermal, generalized tense blisters on normal or erythematous skin predominantly on the trunk and extremities; (ii) IgG and/or C3 deposits at the dermal–epidermal junction of involved skin in direct immunofluorescence microscopy; and/or (iii) circulating IgG binding to the epidermal side of 1 mol L^{–1} NaCl separated normal human skin in indirect immunofluorescence microscopy (Table 1). The clinical diagnosis of CP was confirmed by: (i) subepidermal blisters and signs of dermal scarring predominantly at stratified mucous membranes; (ii) IgG and/or C3, or rarely IgA, deposits at the dermal–epidermal BM of involved skin in direct immunofluorescence microscopy; and/or (iii) circulating IgG binding to the epidermal side of 1 mol L^{–1} NaCl separated normal human skin in indirect

immunofluorescence microscopy (Table 1). The clinical diagnosis of LABD was confirmed by: (i) subepidermal blisters that tended to form clusters; (ii) predominantly IgA deposits at the dermal–epidermal junction of involved skin in direct immunofluorescence microscopy; and/or (iii) circulating IgA binding to the epidermal side of 1 mol L⁻¹ NaCl separated normal human skin in indirect immunofluorescence microscopy (Table 1).

Immunoblot analysis with recombinant bullous pemphigoid 180 protein

BV13 protein was produced in an eukaryotic baculovirus expression system and was purified over Nickel-NTA, as previously described.²⁴ BV13 is a baculoprotein which contains the ECD of BP180 linked to glutathione-S-transferase and 6 × His tag. This region of BP180 has been to shown to harbour the major autoantibody epitopes.^{10,11} Two micrograms of purified BV13 were run on a 10% Tris–glycine gel (BioRad, Hercules, CA, U.S.A.) by sodium dodecyl sulphate–polyacrylamide gel electrophoresis and were then blotted on to nitrocellulose (BioRad), as previously described.²⁵ Afterwards, the nitrocellulose membrane was incubated overnight at room temperature with BP, LABD and CP sera uniformly diluted at 1 : 50 in phosphate-buffered saline (PBS) with 5% fat-free milk powder (MP) and 0.05% Tween 20 (all from BioRad). After three washes with PBS/0.05% Tween 20, the blotted BV13 protein was incubated for 2 h at room temperature with a series of second antibodies diluted at 1 : 1000 in PBS/5% MP/0.05% Tween 2. These were: (i) an alkaline phosphatase (AP)-labelled goat antihuman IgG (H&L); (ii) an AP-conjugated goat antihuman IgA (α); and (iii) an AP-labelled goat antihuman IgE (ε) (all from Zymed, San Francisco, CA, U.S.A.). Binding of the second antibody was visualized by incubation of the nitrocellulose membrane with 5-bromo-4-chloro-3-indolyl-phosphate and nitroblue tetrazolium as a substrate (Kirkegaard and Perry, Gaithersburg, MD, U.S.A.) for 10 min.

Figure 1. Immunoblot analysis of sera from patients with (a) bullous pemphigoid (BP), (b) cicatricial pemphigoid (CP), (c) linear IgA bullous dermatosis (LAD) and healthy controls (Co) utilizing a baculovirus-derived recombinant BP180 protein. A representative blot of each tested serum and a normal human control serum is shown. Reactivity to BP180 (extracellular domain) is visible as a specific band of approximately 130 kDa (arrow).



Results

Bullous pemphigoid 180 reactivity of bullous pemphigoid sera

As shown in Table 1, all sera showed strong IgG as well as variable, but significant, IgA reactivity to BV13 protein. In addition, all BP sera also contained detectable IgE reactivity with BV13. Results representative of two consecutive experiments are shown (Fig. 1a).

Bullous pemphigoid 180 reactivity of cicatricial pemphigoid sera

As shown in Table 1, six of nine sera contained IgG and seven of nine contained IgA reactive with BV13. In contrast, none of nine sera contained IgE reactive with BV13. Except in three sera (CP2, CP3 and CP8), results for both autoreactive IgA and IgG were identical. Results representative of two consecutive experiments are shown (Fig. 1b).

Bullous pemphigoid 180 reactivity of linear IgA bullous dermatosis sera

As shown in Table 1, nine of 10 sera displayed detectable IgA reactivity with BV13. In addition, six of 10 sera also contained IgG against BV13. In contrast, none of 10 sera showed IgE reactivity with BV13. Results representative of two consecutive experiments are shown (Fig. 1c).

Bullous pemphigoid 180 reactivity of normal control sera

None of 10 normal control sera diluted at 1 : 50 showed IgG, IgA or IgE reactivity with BV13 (Fig. 1a–c).

Discussion

We have assessed, by IB analysis utilizing a baculovirus-encoded recombinant BP180 protein, the reactivity of sera from patients with three distinct autoimmune bullous skin disorders: (i) BP, the most frequent autoimmune bullous disease, which is presumably mediated by circulating autoantibodies against BP180;^{9–12} (ii) CP, a rare blistering disease, which primarily affects stratified mucous membranes and in a subset of patients is associated with IgG reactivity to BP180;¹³ and (iii) LABD, a clinically distinct autoimmune bullous disease of children or adults characterized by circulating autoantibodies

mainly against a 97-kDa constituent of the BM, which is highly homologous to the ECD of BP180.^{17,21}

The aim of this study was to determine whether BP180 reactivity in these disorders is restricted to the presence of distinct immunoglobulin isotypes. IgG reactivity to BP180 was detected in 100% of the sera from adult patients with BP, which is in line with previous observations from our laboratory²⁴ and others.^{10–12} IgG reactivity to BP180 was also detected in six of nine sera from the patients with CP, which extends previous observations.¹³ Surprisingly, six of 10 LABD sera contained IgG reactive with BP180, a finding described so far only in a subset of patients with a clinical picture resembling LABD but exhibiting deposits of both IgG and IgA at the BM of lesional skin.^{25,26} This condition has therefore been classified as linear IgA/IgG bullous dermatosis (LAGBD).^{25,26} The specificity of IgG and IgA autoantibodies from LAGBD patients appears to be heterogeneous. These sera predominantly immunoblotted proteins of approximately 97 kDa, 180 kDa or 230 kDa, as well as additional, as yet uncharacterized epidermal and dermal antigens.^{25–27} However, some of these sera showed IgG and IgA reactivity against a single antigen, most frequently the 97-kDa antigen of LABD.²⁵

IgA autoreactivity, the hallmark of LABD, was also investigated in sera of patients with BP and CP. BP180-reactive IgA autoantibodies were detected not only in most of the tested LABD sera but also in 10 of 10 BP sera and seven of nine CP sera (Table 1). The observed IgA reactivity to BP180 in LABD is in line with the recent identification of LABD-1 as a homologue of the ECD of BP180²¹ and the identification of the NC16A domain of BP180 as a site that harbours epitopes that may be recognized by both IgG and IgA autoantibodies.^{10,23} The detection of BP180-reactive IgA in most CP sera in this study is interesting as the IgA subtype is predominantly associated with mucous membranes. This finding is in line with the recent identification of BP180-reactive IgA in a patient with CP.²⁸ Sera of BP patients with severe disease, including mucous membrane involvement, have recently been shown to contain both IgG and IgA reactive with components of the dermoepidermal BM.^{29,30} However, Kirtschig and Wojnarowska did not find a direct correlation between IgA reactivity to 180- and 230-kDa BM proteins and the occurrence of mucosal lesions in BP.³¹

The detection of BP180-reactive IgE autoantibodies in all BP sera studied is interesting in light of the identification of IgE reactive with BP230 in patients

with severe BP.³² Nine of 10 BP patients had active skin disease and one had a recent episode of a bullous eruption (Table 1).

In conclusion, this study provides additional evidence that BP180 autoreactivity *per se* is not an immunological feature specific for BP, CP or LABD. It thus remains to be elucidated whether differential epitope recognition and/or subclasses of circulating autoantibodies are critical for the evolving clinical phenotype of these distinct autoimmune bullous skin disorders.

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