

REVIEW ARTICLE

Role of BP230 autoantibodies in bullous pemphigoid

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ABSTRACT

Bullous pemphigoid (BP) is an autoimmune disease associated with subepidermal blistering due to autoantibodies directed against BP180 and BP230. BP180 is currently considered as the major pathogenic autoantigen. However, previous clinical findings suggested that anti-BP230 autoantibodies alone can cause skin lesions in animal models and many BP patients. The characteristics of BP230 and the pathogenic roles of anti-BP230 antibodies have been proposed. First, at the molecular level, BP230 mediates the attachment of keratin intermediate filaments to the hemidesmosomal plaque and interacts with other constituents of hemidesmosomes. Second, the presence of BP230 autoantibodies may correlate with specific clinical features of BP. The immunoglobulin (Ig)G autoantibodies from BP patients react mainly against the C-terminus of BP230, while the IgE autoantibodies are still inconclusive. Third, *in vivo*, autoantibodies against BP230 involved in the disease may not only induce the inflammatory response but also impair the structural stability of hemidesmosomes. This article reviews recently published work about the role of BP230 and its antibodies, including IgG and IgE, aiming to find clues of its clinical association and lay the foundation for the research on the pathogenicity of antibodies against BP230.

Key words: autoantibody, BP180, BP230, bullous pemphigoid, hemidesmosome.

INTRODUCTION

Bullous pemphigoid (BP) is an autoimmune blistering skin disease clinically characterized by itchy urticarial erythema and tense blisters. Autoantibodies in the sera of BP patients were found to be against hemidesmosomal target proteins BP180 (type XVII collagen, also termed BPAG2) and BP230 (BPAG1-e), which are involved in anchoring basal keratinocytes from the epidermis to the dermis.¹ Currently, most published work suggests that BP180-specific immunoglobulin (Ig)G is the main pathogenic autoantibody in BP and the injection of anti-BP180 IgG in animal models could lead to the key features of human BP. On the contrary, the function of the anti-BP230 IgG antibody in the pathogenesis of BP is largely unknown, but several evidences suggested that anti-BP230 IgG alone could cause the disease.^{2–8} These early studies investigating immune characteristics of BP focused on IgG reactivity. There is emerging evidence showing that IgE autoantibodies against BP antigens were detected in BP sera and may also play an important role in BP.^{9,10} and some researchers indicated that the anti-BP230 IgE was detected in the severe type of the BP patients.⁹ Thus, anti-BP230 IgE in BP patients may be an implication that they are severe and resistant to topical steroid therapy. This review will discuss the evidence regarding the pathogenic role of anti-BP230 IgG and IgE antibodies and provide insights on its association with clinical features of BP.

PHYSIOLOGICAL FUNCTION OF BP230

Molecular structure of BP230

In the early 1980s, Stanley *et al.*^{11,12} identified the 230-kDa epidermal form of bullous pemphigoid antigen 1 (BP230, also called BPAG1e), located in the hemidesmosomal inner plaque. BP230 is a member of the plakin family, which assists in the formation of a 3-D structure in cells and functions as a versatile cross-linking cytoskeleton. Regarding other plakins, BP230 contains a globular N-terminal domain, known as plakin domain (PD), which consists of spectrin repeats (SR) and an SH3 domain inserted in the central SR, a coiled-coil rod domain and a globular intermediate filament (IF) binding domain, which consists of two copies of the plakin repeat domain (PRD) (Fig. 1b).¹³ BP230 protein was one of the isoforms encoded by the dystonin gene (*DST*, also called *BPAG1*). Previous studies demonstrated that mice defective for the *DST* gene showed blisters in skin and neurodegeneration.¹⁴ Without BP230 anchoring IF to hemidesmosomes, keratin collapse and cytolysis of the basal cell layer occur. This process is distinct from the autoantibody-mediated immune reactions participating in the pathogenesis of BP, thus explaining the fact that no subepidermal blisters were observed in *DST* knockout mice.

BP230 binds IF and stabilizes cell integrity

Because BP230 serves as the key component of type I hemidesmosomes, its protein–protein interaction is critical to

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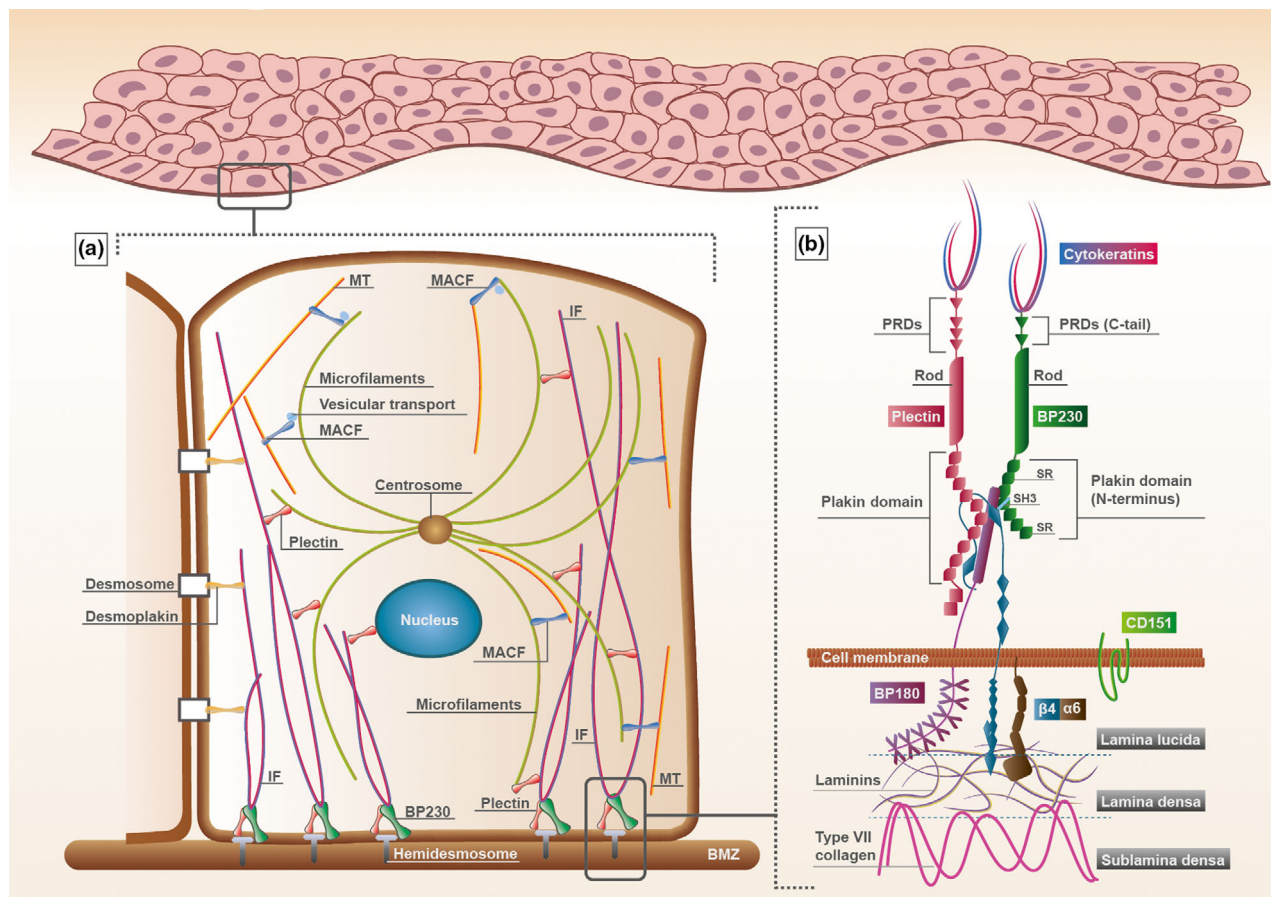


Figure 1. Schematic representation of the interactions of cytoskeleton and dermal-epidermal anchoring complex in epithelial cells. (a) In a basal keratinocyte, BP230 and plectin anchor intermediate filaments (IF) to hemidesmosomes. Desmoplakin is involved in the anchorage of the IF system to desmosomes, where keratinocytes attach to the cell membrane in the epidermis. (b) Structural organization of BP230 and the molecular interactions involved in the assembly of hemidesmosomes are depicted. Hemidesmosomes belong to four protein families: plakins (BP230 and plectin), integrins ($\alpha 6 \beta 4$), collagens (BP180) and tetraspanins (CD151). The extracellular domains of these transmembrane structures link the basal keratinocytes to the basement membrane by binding to laminins, including laminin- $\gamma 1$ and laminin-332. BMZ, basement membrane zone; MACF, microtubule actin cross-linking factor; MT, microtubule; PRD, plakin repeat domain.

elucidate its role in the pathogenesis of BP. BP230 has been shown to interact with multiple proteins, including BP180, transmembrane protein $\beta 4$ integrin subunit, IF and ERBIN (Erb-B2 interacting protein).^{15,16} On the one hand, through its C-terminal tail containing PRD, BP230 specifically interacts with the IF network formed by keratins 5 and 14 and thereby connecting the cytokeratin network of basal keratinocytes to the extracellular matrix. On the other hand, through its N-terminal domains, BP230 binds to the $\beta 4$ subunit of $\alpha 6 \beta 4$ integrin and BP180, which are components of the anchoring filaments of the extracellular matrix.^{15,17} Additionally, plectin is associated with the keratin IF and the cell surface receptor $\alpha 6 \beta 4$ integrin (Fig. 1b). Furthermore, the N-terminal fragment of BP230 also binds to ERBIN, providing hemidesmosome assembly and Erb-B2 receptor signaling, although the function of this interaction is not yet fully elucidated.¹⁶ To date, there is no cytoplasmic protein identified interacting with the coiled-coil rod domain of BP230.

CIRCULATION AUTOANTIBODIES AGAINST BP230 IN BP

Production of autoantibodies is physiologically suppressed by self-tolerance.¹⁸ In pathological conditions, BP-specific autoantibodies may be generated secondary to failed self-tolerance which could be caused by drugs, ultraviolet (UV) light, infections or tissue damage.¹⁹

Anti-BP230 IgG

Although the NC16A of BP180 is the most common target of IgG found in nearly 90% of BP patients,²⁰ according to our published work review, approximately 50–80% of BP patients harbor BP230-specific IgG (Table 1).^{7,21–28} If anti-BP230 and BP180 IgG titers were combined as the diagnostic tool, the sensitivity to diagnose BP could reach 87–97.1%.^{26,29,30} IgG1 and IgG4 were the major subtypes of anti-BP230 IgG.³¹ Hamada *et al.*²² first examined full-length recombinant BP230

Table 1. Summary of autoantibodies IgG/IgE reactivity with BP230 fragments used for epitope mapping studies and assessing the association of BP230 autoantibodies with clinical features in BP

First author (reference)	Year	Study design	Sample	Recombinant protein of BP230 fragments	Method of measurement	Autoantibody detection	Outcome
Skaria <i>et al.</i> ²¹	2000	Cohort	25 BP, 6 PV patients and 12 healthy controls	BP230-A (aa 1–555), B (aa 663–1293), C (aa 1593–1891), D (aa 1946–2649), E (aa 2203–2649)	Immunoblot	IgG	IgG reactivity with A, B, C, D and E domain of BP230 was 8 (32%), 8 (32%), 1 (4%), 21 (84%) and 24 (64%) of 25 BP sera, respectively
Hamada <i>et al.</i> ²²	2001	Cohort	40 BP, 50 PV, 50 PF, 10 PNP, 11 HP patients and 20 healthy controls	Overlapping fragments: BP230-N (aa 1–979), M (aa 978–1946) and C (aa 1896–2649)	Immunoblot	IgG	IgG reactivity with N, M and C domain of BP230 was 15 (38%), 16 (40%) and 32 (80%) of 40 BP sera, respectively
Kromminga <i>et al.</i> ²⁴	2004	Cohort	56 BP, 10 PV, 10 EBA patients and 56 healthy controls	Overlapping fragments: BP230-N1 (aa 1–671), N2 (aa 607–1073), N3 (aa 1014–1758), C1 (aa 1710–2189) and C2 (aa 2137–2649)	Immunoblot and ELISA	IgG	IgG reactivity with N1, N2, N3, C1 and C2 domain of BP230 was 9 (28%), 5 (16%), 13 (41%), 22 (69%) and 12 (38%) of 32 BP sera, respectively. (Only 32 sera with reactivity to cell-derived BP230 were included by immunoblot)
Thoma-Uszynski <i>et al.</i> ²⁵	2004	Cohort	127 BP and 51 healthy controls and patients with unrelated diseases	BP230-N (aa 1–1307), C1 (aa 1881–2649) and C2 (aa 2077–2649); BP180ex (aa 485–1497)	ELISA	IgG, IgG1 and IgG4	Anti-BP230 IgG were detected in 105 of 127 BP sera. Sensitivity of IgG ELISA: N (0.724), C1 (0.654), C2 (0.685), BP180ex (0.953). Sensitivity of IgG1 ELISA: N (0.992), C1 (0.984), C2 (0.992), BP180ex (0.898). Sensitivity of IgG4 ELISA: N (0.992), C1 (1.0), C2 (0.890), BP180ex (1.0)
Yoshida <i>et al.</i> ²⁶	2006	Cohort	239 BP, 37 PF, 57 PV patients and 109 healthy controls	BP230-N (aa 1–979) + C (aa 1896–2649)	ELISA	IgG	IgG reactivity with N- and C-BP230 was 173 (72.4%) of 239 BP sera. Sensitivity and specificity of N- and C-BP230 ELISA were 72.4% and 99.5%, respectively
Ishiura <i>et al.</i> ⁷	2008	Case-control study	67 BP patients and 36 healthy controls	–	ELISA kits from MBL: BP180 (NC16A) and BP230 (N [aa 1–979] + C [aa 1896–2649])	IgG and IgE	Anti-BP230 IgG, anti-BP230 IgE and anti-BP180 IgE were found in 48 (72%), 45 (67%) and 20 (30%) of 63 BP sera, respectively. Anti-BP230 IgE showed a strong association with local EOS accumulation

Table 1. (continued)

First author (reference)	Year	Study design	Sample	Recombinant protein of BP230 fragments	Method of measurement	Autoantibody detection	Outcome
Tampoia <i>et al.</i> ²⁷	2009	Cohort	38 BP and 82 healthy controls	–	ELISA kits from MBL: BP180 (NC16A) and BP230 (N [aa 1–979] + C [aa 1896–2649])	IgG	IgG reactivity with BP230 and BP180 was 12 (60%) and 18 (90%) of 20 BP sera, respectively, in active stage, whereas none were positive for anti-BP230 IgG in remission stage
Roussel <i>et al.</i> ²⁹	2011	Tertiary care center retrospective study	190 BP patients and 78 controls with other AIBD	–	ELISA kits from MBL: BP180 (NC16A) and BP230 (N [aa 1–979] + C [aa 1896–2649]); IIF	IgG	Anti-BP230 IgG were detected in 59% of BP patients with moderate BP and in 62% of those with extensive disease. The sensitivity and specificity of ELISA of BP180 and BP230 were 87% and 88%, respectively, compared with 79% and 90% for BP180 ELISA, 61% and 96% for BP230 ELISA, and 81% and 63% for IIF
Charnaux <i>et al.</i> ²⁸	2011	Single-center retrospective Study	138 BP patients	–	ELISA kits from MBL: BP180 (NC16A) and BP230 (N [aa 1–979] + C [aa 1896–2649]); IIF	IgG	IgG reactivity with BP230 and BP180 was 81 (59%) and 119 (81%) of 138 BP sera, respectively; IIF were more frequently detected when the BP230 ELISA result was positive ($P < 0.001$)
Le Saché-de Peufelhoux <i>et al.</i> ³⁰	2012	Single-center retrospective and prospective study	74 BP patients	–	ELISA kits from MBL: BP180 (NC16A) and BP230 (N [aa 1–979] + C [aa 1896–2649]); IIF	IgG	Sensitivity of ELISA for combination of BP180 and BP230 was 95%, compared with 58% for BP230 ELISA and 92% for BP180 ELISA
Blöcker <i>et al.</i> ²³	2012	Cohort	118 BP patients and 50 PV, 170 RA and other inflammatory arthritides, 56 SLE, 483 healthy controls	Overlapping fragments: BP230-N1 (aa 2–443), N2 (aa 432–864), N2a (aa 432–587), N2b (aa 568–725), N2c (aa 705–864), R1 (aa 846–1297), R2 (aa 1286–1724) C1 (aa 1710–2033), C2 (aa 2024–2342) and C3 (aa 2326–2649)	Immunoblot and ELISA	IgG	Among all the fragments, BP230-C3 provided the best efficiency in serologically diagnosing BP by immunoblot. Sensitivity and specificity BP230-C3 ELISA was 56.8% and 97.6%, respectively
Hayakawa <i>et al.</i> ⁵	2016	Retrospective study	153 BP patients	Overlapping fragments: BP230-N (aa 1–979), M (aa 978–1946) and C (aa 1896–2649)	Immunoblot and ELISA of BP230 RP, ELISA kit	IgG	41 of 38 (92.7%) BP230-BP sera showed clear positive reactivity with BP230-C, while only 8 (19.5%) and 19 (46.3%) sera

Table 1. (continued)

First author (reference)	Year	Study design	Sample	Recombinant protein of BP230 fragments	Method of measurement	Autoantibody detection	Outcome
Gornowicz-Porowska <i>et al.</i> ⁵⁸	2017	–	82 BP patients, including 20 BP patients with ND (BP + ND) and 62 BP patients without ND (BP – ND)	–	from MBL and Immune EM ELISA kits from Euroimmune: BP180 (NC16A) and BP230 (C3 [aa 2326–2649]); IIF	IgG	showed weak reactivity with BP230-N and BP230-M, respectively. 8 of 14 BP230-BP patients showed clinical features of rare subgroups of BP Prevalence of ND among BP patients was 24.4%. There were no statistically significant differences in autoantigens profiles between BP + ND and BP – ND groups
Delaporte <i>et al.</i> ⁹	1996	Cohort	19 BP, 3 PV, 3 linear IgA diseases, 3 AD patients and 3 healthy controls	55-kDa recombinant protein (rBP55) (C-terminal region of BP230)	Immunoblot	IgE	Anti-rBP55 IgE were found in 12 of the 19 BP sera and only in patients with a severe form of the disease
Iwata <i>et al.</i> ³¹	2008	Retrospective case series analysis	37 BP, 6 PV, 5 PF patients and 26 healthy controls	–	ELISA kits from MBL: BP180 (NC16A) and BP230 (N [aa 1–979] + C [aa 1896–2649])	IgG, IgG1, IgG2, IgG3, Ig4 and IgE	Anti-BP230 IgG and anti-BP230 IgE and anti-BP180 IgE were detected in 26, 8 and 8 of 37 BP sera, respectively. Anti-BP230 subtype of IgG: 16 (44%) were positive for IgG1, 5 (14%) were positive for IgG2, 5 (14%) were positive for IgG3, and 26 (70%) were positive for IgG4. IgG1 and IgG4 were the major isotypes of both anti-BP180 and anti-BP230 Ab
Fania <i>et al.</i> ³³	2012	Cohort	32 BP, 21 pruritic dermatoses and 24 allergy patients	BP230-C1 (aa 1881–2649) and N (aa 1–1307); BP180-ex (aa 485–1465), N1 (aa 490–812), NC16A (aa 467–567), C1 (aa 1048–1465), C2 (aa 1352–1465), M (aa 809–1106)	Immunoblot, ELISA and IIF	IgG and IgE	Anti-BP230 IgG, anti-BP230 IgE and anti-BP180 IgE were found in 25, 13 and 11 of 32 BP sera, respectively. IgE from BP sera preferentially targets the C-terminus of BP230 (IB, 16/32; ELISA, 12/32) and, to a lesser extent, the BP180-NC16A domain (IB, 2/32; ELISA, 3/32) and the BP180-NC16A domain (IB, 11/32; ELISA, 9/32)

Table 1. (continued)

First author (reference)	Year	Study design	Sample	Recombinant protein of BP230 fragments	Method of measurement	Autoantibody detection	Outcome
Hashimoto <i>et al.</i> ³⁵	2017	Retrospective study	36 BP, 10 AD patients and 35 healthy controls	–	ELISA kits from MBL: BP180 (NC16A) and BP230 (N [aa 1–979] + C [aa 1896–2649])	IgG and IgE	Anti-BP230 IgG, anti-BP230 IgE and anti-BP180 IgE were found in 22, 18 and 21 of 36 BP sera, respectively

aa, amino acid; Ab, antibodies; AD, atopic dermatitis; AIBD, autoimmune bullous disease; BP, bullous pemphigoid; ELISA, enzyme-linked immunosorbent assay; EOS, eosinophile; HP, herpetiformis pemphigus; IB, immunoblot; IF, indirect immunofluorescence; Ig, immunoglobulin; ND, neurodegenerative disorders; PF, pemphigus foliaceus; PNP, paraneoplastic pemphigus; PV, pemphigus vulgaris; RA, rheumatoid arthritis; SLE, systemic lupus erythematosus.

for epitope mapping, and they found that IgG autoantibodies reacted with the C-terminus of BP230 in 32 (80%) of the 40 BP patients. It is worth mentioning that anti-BP230 IgG titers carry prognostic value, as the high titer was often seen in patients with an extended course of disease and the lower titer was observed during the remission phase.^{7,27} In a recent study,⁵ there were 41 BP patients whose sera only reacted with BP230 and these patients were defined as “BP230-type BP”. They also demonstrated that the BP230-type BP sera preferentially targeted the C-terminal domain of BP230. Among the 14 BP230-type BP patients, eight showed variable clinical features of BP, including the pretibial localized pemphigoid, dysidrosiform pemphigoid, eczematous pemphigoid, pemphigoid nodularis and Brunsting–Perry pemphigoid. These rare phenotypes of BP were considered to be associated preferentially with BP230.

Anti-BP230 IgE

Previous studies show that more than 70% of BP patients presented elevated circulating total IgE,³² and approximately 38–68% of BP patients have BP230-specific IgE. IgE preferentially bound to BP230 rather than BP180,^{7,33,34} though some studies show no difference of IgE reactivity between BP180 and BP230 or that it even responds more to BP180 (Table 1).^{31,35} A subgroup of elderly patients with pruritic dermatoses also showed IgE recognition to BP180 and BP230, which was presumed as an early event (severely itchy and erythematous lesions without bullae) in BP.³³ Several studies also analyzed the BP-specific IgE and the relationships to clinical features. One study showed that anti-BP230 IgE autoantibodies positively correlated with nodular phenotype,³⁵ and Delaporte *et al.*⁹ demonstrated that IgE autoantibodies against the C-terminus of BP230 were found only in patients with severe BP. All of these patients were treated with systemic steroids. This suggests that anti-BP230 IgE may be a marker implying resistance to topical steroid therapy in BP patients. However, there are some studies stating that, compared with anti-BP180 IgE autoantibodies, the anti-BP230 IgE autoantibodies did not correlate with disease severity of BP patients.^{31,35} The relationship between anti-BP230 IgE autoantibodies and clinical features remains controversial, which should be determined in future studies with larger cohorts of BP patients.

There is no clear evidence regarding the source of BP230 IgE. Most studies of BP230 IgE used commercial kits that only detect antibodies against the N- and C-termini of BP230, and there was a possibility that IgE autoantibodies against other domains could not be detected. Further IgE-specific epitope mapping will reveal the antigen site and shed light on the function of BP230 IgE. Both IgG and IgE react with the C-terminus of BP230 in BP patients. It is possible that anti-BP230 IgE may be generated from IgG immunoglobulin class switch.

IN VIVO STUDIES OF AUTOANTIBODIES AGAINST BP230

Animal models have shown that IgG autoantibodies against BP180 are pathogenic; however, the pathogenic role of

anti-BP230 antibodies is unclear.^{3,36} Several attempts to develop animal models using anti-BP230 IgG revealed inconsistent results. Rabbits immunized with BP230-derived peptides developed inflammatory responses following epidermal damage with IgG and C3 deposition at the basement membrane zone (BMZ) after exposure to UV-B irradiation.² It suggested that the damage to basal cell allowed the access of anti-BP230 antibodies and the fixation of complement with inflammation at the BMZ. Kiss *et al.*³ showed subepidermal blister formation with intensive granulocyte infiltration after s.c. injection of high-titer BP230 polyclonal rabbit IgG into neonatal mice. It was the first evidence that an antibody against BP230 could produce the typical clinical, immunopathological and histopathological signs of BP, suggesting the pathogenic role of anti-BP230 IgG. Recently, Haeberle *et al.*^{4,37} and Muramatsu *et al.*^{4,37} also successfully illuminated the pathogenic function of anti-BP230 IgG autoantibodies. They demonstrated that in scurfy mice whose regulatory T cells (Treg) were defective (*Foxp3* mutation), there was spontaneous production of IgG1 autoantibodies to BP230. Haeberle *et al.*^{4,37} found that the monoclonal antibody 20B12 from scurfy mice could recognize the murine BP230 and induce subepidermal blisters in neonatal mice. However, this model did not fully recapitulate BP pathology, because there was no eosinophil infiltration at the BMZ. Additionally, a case also described how BP developed in a 7-year-old boy with immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome, who had loss-of-function mutations in *Foxp3*.³⁸ These studies indicated that Treg regulated the production of autoantibody targeting BP antigens by inhibiting autoreactive T cells and thereby preventing the development of autoimmune bullous disease. However, some publications argue against the pathogenic role of anti-BP230 in BP. Feldrihan *et al.*³⁶ demonstrated that no clinical change was found in neonatal mice after injection of IgG against the C-terminus of BP230, though they observed a dose-dependent binding of transferred IgG without complement deposition and inflammatory reaction in the murine skin. One of the possibilities was that autoantibodies could not bind readily to BP230 and a potent trigger to expose the antigen was needed. Furthermore, BP230-specific antibodies may lack the capacity to activate the recruitment of immune cells and complement system. Such antibodies may need a much longer administration time *in vivo* to reach the threshold needed for the activation of immune pathways. Nevertheless, antibodies against BP230 might trigger disease through a subtle and non-inflammatory mechanism, including the depletion of the autoantigens from keratinocytes as anti-BP180 antibodies did.³⁹

Immunoglobulin E plays an important role in BP disease course as omalizumab has proven effective in treating the symptoms in BP patients.¹⁰ Some studies also found that anti-BP230 IgE levels showed a strong correlation with local eosinophil accumulation.⁷ However, the pathogenicity of BP230 IgE has not been studied. The pathogenic role of anti-BP230 IgE may be elucidated by injection of anti-BP230 IgE into the humanized IgE receptor eosinophil model as suggested for anti-hNC16A IgE-induced BP in the future.⁴⁰

MECHANISM OF ANTI-BP230 AUTOANTIBODY PRODUCTION

Epitope spreading

Epitope spreading (ES) has expanded our knowledge of autoimmune disease pathogenesis in recent years.⁴¹ ES is a process by which “secondary” epitopes are specifically targeted by autoantibodies.

Epitope spreading is the probable explanation for the clustering of autoantibody recognition at the BMZ. Antibodies directed against other extracellular epitopes of the BMZ may lead to tissue injury with consequent exposure to BP230.^{21,42,43} Anti-BP230 autoantibodies may be sequentially produced during clinical courses. In addition, the ES phenomenon occurred after BP230 was exposed to the immune system by damage to keratinocytes. Multicenter prospective studies have demonstrated that ES events in BP preferentially occurred at an early stage of the disease and seem to be correlated with disease severity.^{42,44} In anti-BP180 and anti-BP230 IgG double-positive BP patients, anti-BP230 IgG seems to be produced through intermolecular ES. In “BP230-type BP”, however, anti-BP230 IgG are considered to be produced by different mechanisms.⁵ Furthermore, consistent with the BMZ structure, anti-BP230 antibodies are also detected in other autoimmune bullous diseases, such as paraneoplastic pemphigus (PNP). Autoantibodies from patients with PNP can interact with several plakins, including desmoplakin, plectin, periplakin and envoplakin (Fig. 1a).⁴¹ Intracellularly localized BP230 recognition is thought to occur secondarily after initial tissue damage.

Given the above evidence, the N-terminus of BP230 seems critical for its localization to the hemidesmosome, by containing binding sites critical to its interaction with the intracellular domain of BP180 and $\beta 4$ integrin subunit.^{15,17} Moreover, according to a multicenter prospective study, in one of 17 BP patients with ES, IgG reactivity to the N-terminus of BP230 was preceded by recognition of the C-terminus of BP230.⁴⁴ However, several epitope mapping studies showed that sera from BP patients prefer to bind to the C-terminus than the N-terminus of BP230.^{21–23,45} Thus, we suspect that the N-terminus of BP230 may be the first epitope to be recognized in the early disease phase after basal cell membrane disruption, then the epitope spread to the C-terminus and became the major epitope in BP. Further studies are needed to clarify how ES in BP230 by monitoring the development of autoantibody reactivity against BP230 epitopes during clinical courses.

BP230 leaks out of basal cell after damage

In some particular conditions, intracellular molecules were exposed extracellularly.^{46,47} Several studies demonstrated that IgG bind to intracellular epitopes of BP180 and/or BP230 in the early course of BP.^{25,42,48,49} Inoue *et al.*⁶ recently reported a patient with mucous membrane pemphigoid who only had anti-BP230 IgG and did not show reactivity to either laminin-332 or the ectodomain of BP180 during disease progression. The patient only developed ulcers on the oral mucosa and hard palate without conjunctiva and skin involvement. Ishiura *et al.*⁷

reported a patient who only had anti-BP230 IgG and IgE autoantibodies at the onset of the disease, and later showed increase of anti-BP180 IgG levels as the skin lesions spread. These findings suggested that the intracellular-localizing BP230 may leak out of basal cells, then anti-BP230 antibodies are generated and bind extracellularly, thus initiating the complement system and causing inflammation. Further investigations are needed to determine the binding location of BP230 and anti-BP230 autoantibodies.

Damage to basal cell allows access of anti-BP230 antibodies

An *in vivo* study indicated that exposure to UV-B light could allow access of anti-BP230 antibodies,² or even antibodies directly penetrating intact cells,³ then binding to the target antigen and contributing to subepidermal blister formation. Ohata *et al.*⁸ found that BP had often developed in patients with psoriasis. Of 145 patients, one only had anti-BP230 IgG and two had both anti-BP230 and anti-laminin- γ 1 autoantibodies. The damage to the BMZ in patients with psoriasis may induce the accessibility of anti-BP230 autoantibodies to the basal cell. Moreover, autoantibodies against BP230 may access intact basal keratinocytes.⁵⁰ Previous electron microscopy studies even showed that intracellular plaques of the hemidesmosome had disappeared and anchoring filaments inside basal keratinocytes with degenerative alterations in non-inflammatory bullae of BP.⁵¹

Association of BP with neurological diseases: dystonin expression in the nervous system

Several researches indicated that BP patients typically have an increased risk to concomitantly suffer from neurological diseases, such as Parkinson's disease, dementia, epilepsy and multiple sclerosis.^{52–54} On the other hand, patients with neurological diseases appear to be at risk of developing BP.^{55,56} The *DST* gene encodes distinct isoforms of not only BP230 (epithelial form) but also BPAG1a (neuronal isoform, anchoring neural IF to the actin cytoskeleton) and BPAG1b (muscular isoform) by multiple alternate splicing variants.⁵⁷ Thus, cross-reaction between BP230 and the neural dystonin, which share antigen recognition, may explain the intriguing phenomenon of the relationship between BP and neurological diseases.⁵⁸

CONCLUSION

BP230 is one of the major autoantigens in BP patients and the autoantibodies against BP230 may be produced by several possible mechanisms. The presence of BP230 autoantibodies implied several particular clinical features of BP patients, such as pretibial localized pemphigoid and pemphigoid nodularis. Currently, a plethora of clinical studies indicate that an autoantibody against BP230 alone could contribute to the pathogenesis of BP and that the anti-BP230 autoantibody may interfere with the assembling of the hemidesmosomes *in vivo*. However, the exact pathogenicity of anti-BP230 autoantibodies remains unclear. Further studies are needed to determine the different functions of the different antibody subtypes, and their

relationship with clinical features of BP. Better understanding of the interaction between BP230 and BP230-specific autoantibodies may lead to more targeted therapy for BP patients, especially for the BP230-type BP patients.

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