BP180 as the common autoantigen in blistering diseases with different clinical phenotypes

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Abstract. Bullous pemphigoid antigen 180 (BP180, type XVII collagen) is a transmembrane hemidesmosomal glycoprotein of basal keratinocytes that spans the lamina lucida of the dermal-epidermal junction. Five autoimmune subepidermal blistering diseases are associated with an immune response to BP180, including bullous pemphigoid (BP), pemphigoid gestationis (PG), cicatricial pemphigoid (CP), lichen planus pemphigoides (LPP), and linear IgA disease (LAD). The BP180 ectodomain consists of 15 interrupted collagen domains. The largest non-collagenous (NC) 16A domain is located next to the cell membrane. In BP, autoantibodies are directed to a tightly clustered set of epitopes located at the N-terminal 45 amino acids of the NC16A domain. However, some BP sera also react with other portions of the BP180 ectodomain or with the intracellular region of this protein. In PG, antibodies appear to exclusively recognize the immunodominant BP180 NC16A region. In CP, autoantibodies are directed to both the NC16A domain and the C-terminus of BP180 that projects into the lamina lucida/lamina densa interface of the dermal-epidermal junction. In LPP, autoantibodies react with an epitope located at the C-terminus of NC16A, that is not targeted by BP or PG sera. Finally, the epidermal 97 kDa and the keratinocyte-derived 120 kDa autoantigens of LAD (LABD97 and LAD-1, respectively) have recently been identified as portions of the BP180 ectodomain. These observations demonstrate that different clinical phenotypes are associated with an autoimmune response to the same autoantigen yet the immunoglobulin subclass of the autoantibody and the epitope that is recognized may be different. (Keio J Med 51 (1): 21–28, March 2002)

Key words: autoantigen, basement membrane zone, hemidesmosome, linear IgA disease, pemphigoid

Adhesion of basal keratinocytes to the underlying dermis is maintained by various structural proteins of hemidesmosomes, including the 230 kD bullous pemphigoid antigen (BP230), plectin, α6β4 integrin, BP180, and laminin 5. Directly or through other proteins these hemidesmosomal components associate with anchoring fibrils which originate in the lamina densa of the dermal-epidermal junction (DEJ) and extend into the subjacent connective tissue. The major component of anchoring fibrils is type VII collagen.1

Mutations for the different components of this anchoring complex as well as formation of autoantibodies against these structures result in split formation at the level of the DEJ. A major component of the dermal-epidermal anchoring complex and a major target of autoantibodies is BP180. BP180 is a member of the collagen protein family and is also referred to as type XVII collagen. It is a transmembrane glycoprotein with a type II orientation, i.e., the N-terminus localizes to the cytoplasm, whereas the C-terminal ectodomain consisting of about 1,000 amino acids projecting into the extracellular space.2,3 Rotary shadowing revealed that BP180 contains an intracellular globular head, an extracellular rigid rod corresponding to the 16th non-collagenous (NC) A and the 15th collagenous domain spanning the lamina lucida, and a flexible tail extending into the lamina densa of the DEJ (Fig. 1).2,3 The extracellular domain of BP180 forms a homotrimer.4 BP180 was identified and characterized independently using a biochemical approach5 and by screening an epi-

Presented at the 1190th Meeting of the Keio Medical Society in Tokyo, December 5, 2000.
Detlef Zillikens was supported by grants from the Wilhelm Sander-Stiftung (98.073.2) and the Deutsche Forschungsgemeinschaft (Zi 439/4-1).
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Fig. 1  Schematic diagram of BP180, soluble forms of its ectodomain, and recombinant fragments of the NC16A domain. BP180 is a transmembrane hemidesmosomal protein of basal keratinocytes with a type II orientation, i.e., the NH2 terminus localizes to the cytoplasm while the COOH terminus projects into the extracellular space. LABD97 and LAD-1 are generated by proteolytic cleavage of the BP180 ectodomain. The BP180 molecule consists of a series of alternating collagenous (C, light grey) and non-collagenous (NC, dark grey) domains. The extracellular portion of the 16th non-collagenous domain (NC16A) is the major target of autoantibodies in patients with BP and PG. NC16A has been subdivided into 6 fragments (NC16A1 through NC16A5) each approximately 15 amino acids in length (boxes). Amino acid residue numbers are shown above the boxes. C15, 15th collagenous domain of BP180; HP, hemidesmosomal plaque; MCW, Medical College of Wisconsin.

Table 1  Autoimmune Blistering Diseases Associated with Autoantibodies to BP180

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<td>Bullous pemphigoid</td>
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<td>Pemphigoid (herpes) gestationis</td>
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<td>Lichen planus pemphigoides</td>
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<td>Cicatricial pemphigoid</td>
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Bullous pemphigoid (BP) mainly affects the elderly and is by far the most common of the diseases associated with autoantibodies to BP180, followed by cicatricial pemphigoid, pemphigoid gestationis, linear IgA disease, and lichen planus pemphigoides. Although...
the incidence of BP in persons younger than 60 years is 0.7 new cases/1,000,000 people/year, this incidence increases to 158 new cases/1,000,000 residents/year in patients 90 years and older. Clinically, patients with BP have tense blisters arising on erythematous or normal skin. In the premonitory stage, for weeks, months, or even years the disease may present with erythematous macules, eczematous lesions, papules, and urticarial plaques. By direct immunofluorescence (IF) microscopy of perilesional skin biopsies, the patients reveal linear deposits of C3 and/or IgG along the DEJ (Fig. 2). By the use of 1 mol/L NaCl-split normal human skin as a substrate for indirect IF analysis, in about 90% of patients, circulating serum autoantibodies are detected that bind to the epidermal side of the artificial split (Fig. 3).

Through the use of immunoprecipitation and immunoblotting techniques, BP230 and BP180 were identified as targets of circulating autoantibodies in BP patients. Cloning of corresponding epidermal cDNAs revealed that BP230 and BP180 are products of distinct and unrelated genes. The BP230 gene maps to the short arm of the chromosome 6, locus 6p11–12. BP230 is a constituent of the hemidesmosomal plaque and belongs to the plakin family of proteins.

Whereas BP230 is located intracellularly, BP180 is a transmembrane protein. The NC16A domain adjacent to the cell membrane of basal keratinocytes has been identified as an immunodominant region of BP180 that is targeted by approximately 90% of BP sera (Fig. 4). Within this stretch of 76 amino acids, BP autoantibodies bind to four well-defined epitopes (MCW-0 through MCW-3) located at the N-terminal 45 amino acids (Fig. 1).

Based on these epitope mapping data, an ELISA system was developed utilizing a recombinant form of the entire BP180 NC16A domain as the target. This ELISA turned out to be highly sensitive and specific for the detection of autoantibodies to BP180 in patients.
with BP. No correlation has been reported between disease activity in BP and anti-DEJ antibody titers as detected by indirect IF microscopy. However, when we studied levels of autoantibodies to BP180 NC16A in patients with BP during the course of the disease, we detected a correlation between disease activity and serum levels of antibodies to NC16A. In addition, this study confirmed the lack of correlation of disease activity with titers of indirect IF microscopy which may be explained by the observation that IF staining predominantly reflects the activity with BP230 and to a lesser extent with BP180. Reactivity to BP180 NC16A was further found to be mediated mainly by IgG4 and IgE, and less frequently by IgG1, IgG2, or IgG3. Serum levels of IgE and IgG subclasses to BP180 NC16A also reflected disease activity, and no change was observed with regard to immunoglobulin subclass reacting predominantly with BP180 NC16A or the specific epitopes within this domain during the course of the disease.

Several investigations have been aimed at unequivocally demonstrating the pathogenic relevance of antibodies to BP180 in patients with BP. These attempts have been hindered by the fact that murine BP180 shows a lack of homology within the region that is homologous with the NC16A domain of human BP180. However, when neonatal mice were injected with antibodies from a rabbit that had been immunized with murine BP180, the skin of the mice developed an inflammatory blistering disease mimicking BP. Further studies demonstrated that, in addition to anti-BP180 antibodies, complement activation and neutrophil infiltration are prerequisites for disease induction in this experimental model. In another attempt, we recently grafted human skin onto the back of severe combined immunodeficient (SCID) mice. However, antibodies to desmoglein 1 and desmoglein 3 from patients with pemphigus foliaceus and pemphigus vulgaris reproduced the clinical, histological, and immunopathological findings of these diseases, antibodies to BP180 from patients with BP did not induce blisters.

Various cytokines and mediators have been implicated in the inflammatory reaction in BP patients. In blister fluid of these patients, elevated levels of several cytokines and chemokines, including TNFα, IL-1α, IL-1β, IL-4, IL-5, IL-6, IL-8, and IL-10 have been detected compared to levels in suction blisters induced in healthy controls. To investigate the functional relevance of anti-BP180 autoantibodies, we recently tested the release of various cytokines from cultured human keratinocytes after treatment with IgG to BP180 purified from the serum of BP patients. We detected a dose- and time-dependent secretion of IL-6 and IL-8, but not of TNFα, IL-1α, IL-1β, IL-10, or MCP-1 on stimulation with IgG from patients compared to healthy controls.

This effect was abolished by depleting immunoreactivity to the N-terminal 30 amino acids of BP180 NC16A. Upregulation of IL-6 and IL-8 after incubation of keratinocytes with IgG from patients was also found at the mRNA level. These data demonstrate that BP-associated autoantibodies to well-defined epitopes on the human BP180 ectodomain may trigger a signal transduction event that leads to the expression and secretion of IL-6 and IL-8 from human keratinocytes. Treatment of the cells with dapsone inhibited the release of IL-6 and IL-8 from cells incubated with human IgG, but did not affect the mRNA levels for these two inflammatory mediators. The effect of dapsone on the secretion of IL-6 and IL-8 by keratinocytes stimulated with antibodies to BP180 may well contribute to improvement of the skin disease in patients treated with this drug.

**Pemphigoid Gestationis**

Pemphigoid gestationis (PG), formerly referred to as herpes gestationis, most commonly presents during the second or third trimester of pregnancy or in the immediate post-partum period, often as a non-bullous disease. Skin lesions include erythematous papules as well as urticarial, eczematous, and erythema multiforme-like lesions. Less frequently, papulo-vesicles and blisters are encountered in PG (Fig. 5). The autoimmune response in PG is directed against BP180 and, less frequently, BP230. Like in BP, the NC16A domain has been identified as the major target of autoantibodies and is recognized by more than 80% of PG sera (Fig. 4). Recent epitope mapping studies identified two well-defined antigenic sites within a 22 amino acid segment of BP180 (NC16A2 and NC16A2.5) as the major antigenic targets of PG sera. In contrast to findings in BP, reactivity to NC16A is dominated by IgG1 and IgG3 in PG patients. As IgG3 and IgG1 are the IgG subclasses with the strongest complement-fixing properties, these observations may well explain complement deposition at the DEJ which is the most consistent immunopathological finding in the skin of PG patients.

**Lichen Planus Pemphigoides**

In contrast to bullous lichen planus, in which blisters are confined to lichen planus lesions, in lichen planus pemphigoides (LPP), blister formation usually follows the appearance of papules and plaques associated with LP and may arise on both lichen planus lesions and unaffected skin. In addition, the finding of linear deposits of C3 and IgG along the DEJ by direct IF microscopy differentiates LPP from bullous lichen planus. Several observations speak against a simple association of lichen planus and BP in LPP patients. LPP is seen in
younger patients and the clinical course is less severe compared to BP. This concept is substantiated by recent findings that autoantibodies in LPP sera react with BP180 NC16A4 (MCW-4), an epitope that is not recognized by antibodies in BP. Therefore, LPP can be distinguished from BP not only clinically but also immunologically.

**Linear IgA Disease**

Linear IgA disease (LAD) is characterized by linear IgA deposits at the DEJ. Clinically, considerable variations are seen with respect to the age of disease onset, morphology of bullous lesions, and mucosal involvement. LAD of childhood, also referred to as chronic bullous disease of childhood, is the most common autoimmune bullous disorder in children (Fig. 6). By immunoelectron microscopy, most LAD sera bind within the lamina lucida, others label the lamina densa, and still others react with both locations. Similarly, by indirect IF microscopy on salt-split skin, most LAD sera label the epidermal side of the split, some show dermal binding, and others stain both sides. Different target antigens of the lamina lucida-type of LAD have been reported, including a 97 kD protein (LABD97) extracted from epidermis and a 120 kD polypeptide (LAD-1) secreted into the medium of cultured human keratinocytes. Based on biochemical studies and peptide sequence analyses, it now appears that LABD97 and LAD-1 are generated as proteolytic cleavage products of the BP180 ectodomain. Some LAD sera also contain autoantibodies to full-length BP180 as detected by immunoblotting of epidermal or keratinocyte extracts. When we tested 50 LAD sera for reactivity with BP180 NC16A, the immunodominant region of BP180 in BP and PG, antibodies to this domain were found in 20% of LAD patients. However, when reactivity to NC16A was abolished, the sera continued to react with LAD-1 or BP180. These results suggest that LAD sera, in contrast to the majority of BP sera, target epitopes on the entire BP180 ectodomain.

In another subset of patients, a combined IgA and IgG response was detected. For this disease, the term linear IgA/IgG bullous dermatosis was proposed. Interestingly, in addition to LAD patients, a large number of BP sera has been found to react with the 120 kD soluble ectodomain of BP180 (LAD-1). In a recent study, we investigated the hypothesis that there is a significant overlap in the autoimmune isotype profiles associated with BP and LAD. For this study, several new recombinant forms of BP180 were generated in the baculovirus expression system, including the full-length protein. IgG reactivity with full-length BP180 or portions of it was detected in 39 of 40 (98%) of BP sera; interestingly, 88% of BP sera also contained IgA
anti-BP180 autoantibodies. Similarly, anti-BP180 reactivity in LAD sera was also attributed to both an IgA (68%) and IgG (76%) autoantibody response. IgA and IgG autoantibodies to the intracellular portion of BP180 were found in 14% and 28% of BP sera, respectively, and 8% of LAD sera (same percentage for both isotypes). These findings demonstrate that both BP and LAD patients have a dual IgG and IgA autoimmune response to BP180 which is directed not only to the ectodomain but also to the intracellular portion of this protein.59

Cicatricial Pemphigoid

Cicatricial pemphigoid (CP) is a chronic blistering disease of the mucous membranes and skin. It may involve the oral, ocular, nasal, pharyngeal, laryngeal, esophageal, and anogenital mucous membranes (Fig. 7). Skin lesions appear in about 30% of CP patients. CP is a heterogeneous disease with respect to the clinical site of involvement, the isotype of autoantibodies (IgA, IgG, or the combination of IgA and IgG), the site of autoantibody binding by indirect IF microscopy on salt-split skin (epidermal, dermal, or both), and the target antigens (BP180, laminin 5, laminin 6, β4 integrin, type VII collagen).60 About 30% of patients with the clinical phenotype CP reveal autoantibodies to laminin 5.61,62 In a recent study on 26 patients with the clinical phenotype CP, we found reactivity with laminin 5 in 7 sera and with BP180 in the remaining 19 patients. Thirty-two percent of the BP180 reactive sera only showed IgA reactivity to this protein. By the combined use of the soluble BP180 ectodomain (LAD-1) and recombinant BP180 NC16A, 84% of the BP180 reactive sera were positive. IgG reactivity was preferentially found against NC16A, whereas IgA antibodies predominantly recognized LAD-1. Thirty-two percent of the BP180 reactive sera revealed reactivity with the intracellular domain of this protein. These findings demonstrate that autoantibodies in CP target epitopes on both the extra- and intracellular domains of BP180 and underline the importance of testing for both IgG and IgA reactivity in CP sera.62

Conclusion

The observations outlined above demonstrate that five autoimmune diseases with different clinical phenotypes are associated with an autoimmune response to the same autoantigen, yet the immunoglobulin subclass and the sites on the autoantigen that are targeted are different. Future studies will have to identify the pathogenically relevant epitopes on BP180 that mediate subepidermal blister formation in these diseases.

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Fig. 7 Conjunctivitis and synechiae of the conjunctivae of the left eye in a patient with cicatricial pemphigoid.


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