

# Experimental models for the autoimmune and inflammatory blistering disease, Bullous pemphigoid

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**Abstract** Bullous pemphigoid (BP) is a subepidermal skin blistering disease characterized immunohistologically by dermal-epidermal junction (DEJ) separation, an inflammatory cell infiltrate in the upper dermis, and autoantibodies targeted toward the hemidesmosomal proteins BP230 and BP180. Development of an IgG passive transfer mouse model of BP that reproduces these key features of human BP has demonstrated that subepidermal blistering is initiated by anti-BP180 antibodies and mediated by complement activation, mast cell degranulation, neutrophil infiltration, and proteinase secretion. This model is not compatible with study of human pathogenic antibodies, as the human and murine antigenic epitopes are not cross-reactive. The development of two novel humanized mouse models for the first time has enabled study of disease mechanisms caused by BP autoantibodies, and presents an ideal *in vivo* system to test novel therapeutic strategies for disease management.

**Keywords** Autoimmune disease · Basement membrane · Hemidesmosome · Humanized animal model · Inflammation

## Etiology of bullous pemphigoid

In 1953, Lever [28] described bullous pemphigoid (BP) as a subepidermal blistering disorder primarily seen in the elderly. Lesional/perilesional skin of BP patients exhibits detachment of the basal keratinocytes of the epidermis from

the dermis at the level of the lamina lucida [55], resulting in tense, fluid-filled vesicles. BP is both an inflammatory disease and an autoimmune disease, characterized by an inflammatory infiltrate at the site of the dermal-epidermal junction separation and by the deposition of autoantibodies and complement components along the basement membrane zone (BMZ).

A number of inflammatory cells are present in the upper dermis and bullous cavity, including eosinophils (the predominant cell type), neutrophils, lymphocytes, and monocytes/macrophages. Both intact and degranulating eosinophils, neutrophils, and mast cells (MC) are found in the dermis. Local activation of these cells may occur via the multiple inflammatory mediators present in the lesional skin and/or blister fluids, including (a) granular proteins derived from degranulated leukocytes, such as eosinophil cationic protein (ECP), eosinophil major basic protein (MBP), and neutrophil-derived myeloperoxidase (MPO) [1, 4, 8] and (b) chemoattractants and cytokines, such as C5a fragments, histamine, leukotriene B<sub>4</sub>, interleukin-1, -2, -4, 5, -6, -8, -15, TNF- $\alpha$ , IFN- $\gamma$ , RANTES, and eotaxin [9, 10, 21, 22, 46, 47, 48, 58, 62]. Additionally, several proteinases are found in BP blister fluid, including plasmin, collagenase, elastase, and 92-kDa gelatinase [2, 14, 24, 27, 44, 45, 52, 57]. These proteolytic enzymes may play a crucial role subepidermal blister formation in BP via their ability to degrade extracellular matrix proteins.

BP patients generate a polyclonal repertoire of autoantibodies that bind to the BMZ and activate complement, as well as circulating autoantibodies [20]. These autoantibodies target two major hemidesmosomal antigens of 230 kD (BP230 or BPAG1) and 180 kD (BP180, BPAG2, or type XVII collagen) [25, 40, 56, 57]. BP230, a component of the hemidesmosomal plaque, is an intracellular protein, while BP180 is a type II transmembrane protein [19, 23, 56]. Like BP230,

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BP180's amino-terminal portion localizes to the intracellular hemidesmosomal plaque [15, 18, 19]. Its carboxyl-terminal region extends into the extracellular milieu of the BMZ, making it the preferred target for pathogenic BP autoantibodies. This antigenic extracellular region consists of 15 collagen domains separated from one another by non-collagen sequences. The largest of these non-collagen domains is referred to as NC16A. Epitope mapping studies indicate that BP autoantibodies of IgE and IgG isotypes and IgG1 and IgG4 subclasses recognize multiple epitopes that cluster within BP180 NC16A [3, 11, 16, 26, 63]. Serum levels of these autoantibodies are correlated with disease severity [11, 17, 49].

Most BP patients elicit a cell mediated autoimmune response in addition to the humoral response described. Autoreactive CD4+ T lymphocytes recognize epitopes within the extracellular region of BP180, primarily in the NC16A domain [5, 29]. These T cells express memory cell surface markers and exhibit a Th1/Th2 mixed cytokine profile. These studies suggest that BP is a T and B cell-dependent and antibody-mediated skin autoimmune disease.

#### Development of murine IgG passive transfer model of BP

The strong correlation between BP disease severity and serum BP180-specific autoantibody levels suggests that BP blister formation is mediated by autoantibodies. Early attempts to demonstrate the pathogenicity of patient autoantibodies via a passive transfer mouse model were unsuccessful because BP autoantibodies that react with an immunodominant and potentially pathogenic epitope in BP180-NC16A fail to cross-react with the murine form of this autoantigen (mBP180 NC14A) [30]. In 1993, Liu et al. [30] devised a strategy to overcome this difficulty and generated rabbit polyclonal antibodies raised against a cloned segment of mBP180 NC14A and passively transferred the purified rabbit anti-mBP180 IgG into neonatal BALB/c mice. The injected animals developed a disease that exhibited the following hallmarks of human BP: (a) clinical skin lesions; (b) *in vivo* deposition of rabbit IgG and mouse C3 at the basement membrane by direct IF; (c) dermal-epidermal separation and an extensive inflammatory cell infiltration by H&E staining [30]. This infiltrate includes neutrophils, lymphocytes, and monocytes/macrophages, with neutrophils being the predominant cells [7, 30].

#### Immunopathogenesis of experimental BP in the murine model

Development of an *in vivo* system to study an experimental BP model has allowed for great progress in defining the

etiopathogenesis of disease. Specifically, the roles of pathogenic antibodies, the complement system, inflammatory cells, and proteolytic enzymes have all been elucidated in the context of the murine IgG passive transfer model.

Injection of anti-mBP180 IgG initiated subepidermal blister formation, and the levels of circulating anti-mBP180 antibodies completely determine disease onset and severity [30, 34]. Epitope mapping studies demonstrated that pathogenic anti-BP180 antibodies recognize a 9–12 amino acid stretch within the murine BP180 NC14A region of the antigen [31]. This epitope overlaps the region of the human BP180 NC16A that contains the immunodominant epitopes recognized by human BP autoantibodies, supporting the relevance of this murine system as a model for human disease.

Using mice deficient in different inflammatory cell types, it has been demonstrated that mast cells, macrophages, and neutrophils, but not T and B lymphocytes, play a direct role in subepidermal blistering in experimental BP [7]. Anti-mBP180 autoantibodies trigger skin blistering in wild-type mice and mice deficient in T, B, or both T and B cells, but fail to induce lesions in mice deficient in mast cells, macrophages, or neutrophils. Quantification of disease severity in these mice demonstrated that mast cells and neutrophils play a key role in experimental BP.

Autoantibodies bind to basement membrane antigens and activate complement in human BP. In the rabbit anti-mBP180 IgG-induced BP, complement activation is absolutely required. Both BALB/c mice depleted of complement (by pretreatment with cobra venom) and C5-deficient mice are resistant to experimental BP, and reconstitution of the C5-deficient mice with C5a restores susceptibility [32]. F(ab')<sub>2</sub> fragments generated from the pathogenic anti-mBP180 IgG cannot induce subepidermal blisters in C5-sufficient mice [32]. Mice deficient in complement component C4 (specific for the classical and lectin pathways), but not in factor B (specific for the alternative pathway), are resistant to BP [41]. Wild-type mice depleted of complement component C1q (specific for the classical pathway) fail to develop BP skin lesions when injected with pathogenic IgG [41]. These data suggest that the classical pathway of the complement activation plays a major role in development of disease, namely through the generation of C5a that in turn activates MCs [6].

Mice injected with pathogenic anti-mBP180 antibodies exhibit extensive MC degranulation in the lesional skin, similar to that observed in human BP [6, 59]. MC-deficient mice are resistant to experimental BP, but reconstitution of these mice with MCs restores their susceptibility to disease. MC activation precedes neutrophil infiltration, and either the absence of MCs or the inhibition of MC degranulation prevents neutrophil infiltration and blister formation. However, MC-deficient mice reconstituted locally with neutrophils,

or injected locally with neutrophil chemoattractants IL-8 or TNF- $\alpha$  form blisters in response to anti-mBP180 IgG. These results suggest that mast cells release proinflammatory cytokines critical for neutrophil recruitment [6].

Experimental BP requires neutrophil infiltration, and there is a direct correlation between disease severity and number of infiltrating neutrophils [33]. Depletion of neutrophils, or disruption of the events upstream of neutrophil infiltration (complement activation and MC degranulation), renders mice resistant to BP blister formation. However, complement system and MC deficiencies only confer protection from the pathogenic effects of BP180 autoantibodies if neutrophil infiltration is blocked as a consequence; that is, complement activation and MC degranulation are only critical to disease progression in their roles of neutrophil recruitment. This suggests that infiltrating neutrophils are the cells that directly cause tissue injury in the dermal–epidermal junction, leading to BP skin blisters.

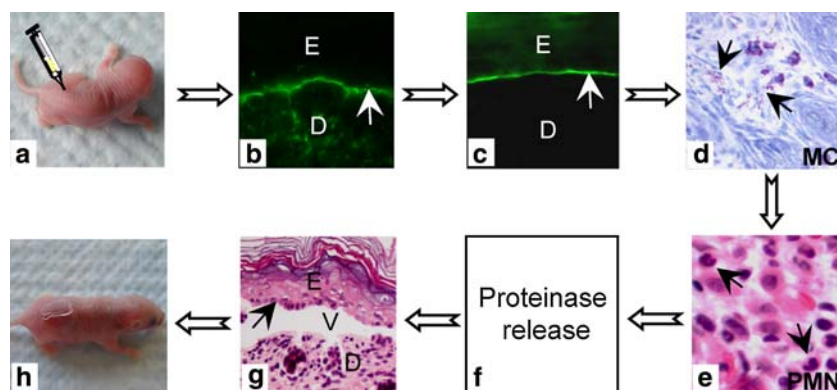
Upon activation through molecular interactions between the Fc $\gamma$ IIIR on the neutrophil cell surface and the Fc portion of pathogenic anti-mBP180 IgG [61], infiltrating neutrophils secrete proteolytic enzymes known to degrade the extracellular matrix, including neutrophil elastase (NE) and gelatinase B (GB). A deficit of either of these two enzymes blocks blister formation in mice [35, 36]. In vitro, although both GB and NE are capable of degrading the recombinant BP180 protein, only NE produces DEJ separation when incubated with skin sections [36, 52]. In vivo, the degradation of BP180 depends on NE activity. Taken together, these findings suggest that GB acts upstream of NE. Specifically, GB proteolytically inactivates the physiological inhibitor of NE ( $\alpha$ 1-proteinase inhibitor), which allows for

unmitigated NE cleavage of extracellular matrix proteins (including BP180), resulting in DEJ separation [37].

### Development of the humanized murine passive transfer model

While the rabbit anti-mBP180 IgG passive transfer model has provided invaluable insight to the key steps in BP disease development, it does not allow for experimentation with BP autoantibodies isolated from human clinical samples. A second animal model, in which rabbit antibodies directed against the extracellular domain of hamster BP180 (NC16A region) are transferred into neonatal hamsters, also lacks cross-reactivity with antibodies directed against human BP180 [59]. To assess the pathogenicity of human anti-BP180 autoantibodies, we generated a novel mouse strain in which the murine BP180NC14A was replaced with the homologous human BP180NC16A epitope cluster region [38]. The humanized NC16A (NC16+/+) mice injected with anti-BP180NC16A autoantibodies develop BP, and the disease development depends on complement, mast cells and neutrophils (Fig. 1).

Using the NC16A+/+ mice and BP patients' sera, we mapped the pathogenic epitope of BP180 in vivo [39]. IgG passive transfer experiments identified one pathogenic epitope (referred to as BP180NC16A2.5). BP180NC16A2.5-specific autoantibodies induced subepidermal blisters in the NC16A+/+ mice, and these blisters were blocked by pretreatment with recombinant BP180NC16A2.5. We also found that the NC16A2.5 pretreated mice had significantly reduced levels of BMZ-bound and circulating pathogenic



**Fig. 1** Proposed mechanism of subepidermal blister formation in humanized NC16A mouse model of BP. Subepidermal blistering is an inflammatory process involved in following steps: **a** anti-BP180 autoantibodies are injected into NC16A mice; **b** pathogenic antibodies bind to the pathogenic epitope of BP180 antigen in basal keratinocytes (BK); **c** the molecular interaction between BP180 antigen and anti-BP180 IgG activates the complement system (C') as evidenced by BMZ deposition of mouse C3; **d** C' activation products C3a and C5a

cause mast cells (MC) to degranulate; **e** MC activation leads to neutrophil (PMN) recruitment; **f** infiltrating PMNs bind to the BP180-anti-BP180 immune complex via the molecular interaction between Fc $\gamma$  receptors on neutrophils and the Fc domain of anti-BP180 IgG. Activated PMNs release proteolytic enzymes; **g** proteolytic enzymes degrade BP180 and other extracellular matrix proteins, leading to dermal–epidermal junction separation; **h** pathogenic antibody-injected mice develop clinical blisters

antibodies and showed reduced complement activation, mast cell degranulation and neutrophil infiltration. These results suggest that targeting the pathogenic epitope specifically could be a new therapeutic strategy to treat BP.

A second humanized animal model has been developed by Nishie et al. [42, 43] that replaces the mouse BP180 (COL17) with the human analogue. Upon injection of human anti-BP180 autoantibodies, these mice reproduce the DEJ separation at the lamina lucida, the deposition of human IgG along the DEJ, and an inflammatory cell infiltrate consisting of neutrophils and eosinophils seen in human disease. The pathogenic effects of the autoantibody injection are ablated by pretreatment with a 77-amino acid peptide fragment of COL17 NC16A, referred to as R1. These studies further support the therapeutic potential of employing decoy peptides to block the pathogenic epitope.

### Relevance of murine and humanized passive transfer models to human BP

Both in vitro and in vivo data demonstrate that BP180 is the target for pathogenic autoantibodies in BP (Table 1). While clinical human BP and experimental murine and humanized murine BP closely mimic each other at the clinical, histological, and immunological levels, the IgG passive transfer models do not reflect the large number of eosinophils typically found in the inflammatory infiltrate of human BP lesional skin. Some patients do exhibit neutrophil-rich pemphigoid, indicating that the neutrophil-mediated blistering observed in these mouse models may be one of several disease mechanisms that contribute to the formation and persistence of subepidermal blisters. The presence of a neutrophil-rich inflammatory infiltrate in the two humanized mouse models, as well as in the aforementioned hamster model, lend credence to a neutrophil-mediated disease mechanism. Furthermore, findings that in vitro DEJ separation induced by human BP autoantibodies specific for BP180NC16A depends on neutrophils [51] and neutrophil-derived elastase and gelatinase B [50], and that BP180 degradation by human BP blister fluid depends on neutrophil elastase activity [57] support this theory. Taken together, these findings strongly suggest that like the IgG passive transfer model of BP, neutrophils may be responsible for subepidermal blister formation in human BP, at least in those patients who show neutrophil infiltration in their lesional/perilesional skin. The humanized mouse models do not yet offer insight to an eosinophil-mediated mechanism of disease progression, leaving the role of these inflammatory cells, if there is any, yet to be discovered. It is possible that these established mouse models, without additional experimental manipulations, may not be able to fully duplicate human BP pathology related to eosinophil infiltration;

**Table 1** In vitro and in vivo evidence of pathogenicity of anti-BP180 antibodies

System	Antibodies used	Reference
In vitro		
Human skin section	BP sera	[13]
	Anti-BP180NC16A autoantibodies	[51]
	Rabbit anti-BP180NC16A IgG	
In vivo		
Wild-type mice	Rabbit anti-murine BP180 IgG	[30]
Hamster	Rabbit anti-hamster BP180 IgG	[60]
Humanized BP180 mice	Anti-BP180NC16A autoantibodies	[38, 39]
Humanized NC16A mice	Anti-BP180NC16A autoantibodies	[42, 43]

therefore, they may not be appropriate to study the role of eosinophils in BP.

Zone et al. [64] reported that IgE hybridoma to LABD97 antigen, a component of the shed ectodomain of BP180, when injected subcutaneously in SCID mice with engrafted human skin, induced eosinophil infiltration and histological blisters in engrafted human skin. Fairley et al. [12] also reported that total IgE isolated from BP sera, when injected into human skin grafted onto athymic nude mice, triggered a dermal infiltrate composed of neutrophils, eosinophils, and histological separation of the epidermis from the dermis. These findings implicate eosinophils in BP; however, a direct link between eosinophil recruitment and blistering in these human skin graft models needs to be established.

### Conclusions

The murine IgG passive transfer model of BP developed by Liu et al. [30] has provided an invaluable tool for the dissection of the mechanism of disease progression. The development of humanized murine models of BP now provides unprecedented insight to the pathogenic role of the autoantibodies that mediate disease. Initial observations with these humanized models indicate that humanized mouse BP exhibits phenotypic and immunological similarities with murine and human BP. Additionally, these humanized models enabled the first identification of pathogenic epitopes in human disease. Blocking of the pathogenic epitope with decoy peptides, if they do not enhance the autoimmune responses, presents a novel clinical therapeutic strategy that may be very effective in abolishing the pathogenic effects of disease.

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