Lecture

Immunopathology of bullous pemphigoid, an autoimmune and inflammatory skin blistering disease

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Abstract. Bullous pemphigoid (BP) was first described by Lever in 1953 as a subepidermal blistering disease. Key immunohistological features of BP include dermo-epidermal junction (DEJ) separation, an inflammatory cell infiltrate in the upper dermis, and autoantibodies directed against two hemidesmosomal antigens, BP230 and BP180. In 1993, an IgG passive transfer mouse model of BP was developed by administering rabbit anti-murine BP180 antibodies to neonatal mice. This model recapitulates the key features of human BP. Systematic dissection of this BP model has revealed that subepidermal blistering is initiated by anti-BP180 antibodies and mediated by complement activation, mast cell degranulation, and neutrophil infiltration. Proteinases and reactive oxygen species released by infiltrating neutrophils work together to damage the basement membrane zone (BMZ), causing a subepidermal blister. Recently, another novel mouse model for BP has been developed by active immunization. C57BL/6J mice actively immunized with murine BP180 develop BP-like skin lesions. The IgG passive transfer and active models of BP provide us with invaluable in vivo systems not only for dissecting cellular and humoral responses in BP but also for developing effective therapies for this disease. (Keio J Med 52 (2): 128–133, June 2003)

Key words: animal model, bullous disease, hemidesmosome

BP is an Autoimmune and Inflammatory Disease

In 1953 Lever described the disease entity known as Bullous Pemphigoid (BP) as a subepidermal bullous dermatosis seen in the elderly.1 BP is characterized histologically by dermoepidermal junction (DEJ) separation with an inflammatory infiltrate and immunopathologically by in vivo deposition of autoantibodies and complement components along the basement membrane zone (BMZ). Electron microscopic studies reveal that the blister in BP is located in the lamina lucida.2

As an autoimmune disease, BP is characterized by circulating and tissue-bound autoantibodies. These autoantibodies bind to the BMZ and activate complement.3 These autoantibodies are directed against two major hemidesmosomal antigens of 230 kD (also referred to as BP230 or BPAG1) and 180 kD (also referred to as BP180, BPAG2, or type XVII collagen).4–7 Both BP180 and BP230 have been cloned and characterized at the molecular level.8–12 BP230 is an intracellular protein which localizes to the hemidesmosomal plaque, while BP180 is a transmembrane protein with a type II orientation (Fig. 1A).13–15 Its amino-terminal region localizes to the intracellular hemidesmosomal plaque, while its carboxyl-terminal portion projects into the extracellular milieu of the BMZ.12,15,16 The extracellular region consists of 15 collagen domains separated from one another by non-collagen sequences. BP180 as a transmembrane antigen makes it a preferred target for pathogenic autoantibodies in BP.

BP autoantibodies recognize multiple epitopes that cluster within the largest non-collagen domain of the BP180 antigen (referred to as NC16A, see Fig. 1).17,18 These BP180NC16A-specific autoantibodies are predominantly IgE and IgG isotypes and IgG1 and IgG4 subclasses.19–21 The serum levels of autoantibodies to BP180 NC16A are correlated with the severity of BP.20,22

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BP180-specific autoreactive T cells are present in BP and these T cells recognize epitopes located in the extracellular region of BP180, mainly in the NC16A domain.23,24 These T lymphocytes express CD4 memory T 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.

As an inflammatory disease, numerous inflammatory cells, including eosinophils, neutrophils, lymphocytes, and monocytes/macrophages are identified in the upper dermis and blister cavity, with eosinophils being the predominant cell type. Intact and degranulating eosinophils, neutrophils and mast cells are seen in the dermis, suggesting these cells have been activated locally. Indeed, various inflammatory mediators that can activate mast cells or leucocytes, such as histamine, leukotriene B4, interleukin-1, -2, -4, -5, -6, -8, -15, TNF-α, IFN-γ, RANTES, and eotaxin.25-32 Several proteinases are also found in BP blister fluid, including plasmin, collagenase, elastase, and 92-kD gelatinase.23,24 These proteolytic enzymes are capable of degrading extracellular matrix proteins and thus, they may play a crucial role in subepidermal blister formation in BP.

Development of an IgG Passive Transfer Model of BP

The notion that serum levels of autoantibodies to BP180 antigen are closely correlated with disease severity strongly suggests that BP is an autoantibody-mediated skin blistering disorder. Diaz, Anhalt, and their co-workers were the first to use IgG passive transfer models to demonstrate the pathogenic activity of antiepidermal autoantibodies associated with pemphigus vulgaris and pemphigus foliaceus.41,42 However, previous attempts with similar approaches to demonstrate the pathogenicity of patient autoantibodies had been unsuccessful. BP autoantibodies that react with an immunodominant and potentially pathogenic epitope in NC16A of BP180 fail to crossreact with the murine form of this autoantigen and thus cannot be assayed for pathogenicity in a conventional passive transfer mouse model.43 To overcome this difficulty, we cloned a segment of the murine BP180 protein homologous with the human BP180 NC16A (mBP180 NC14A), generated rabbit polyclonal antibodies against this recombinant antigen, and passively transferred the purified rabbit anti-mBP180 IgG into neonatal BALB/c mice. The injected animals developed a blistering disease that closely mimicked human BP (Fig. 2) including the following immunohistological features: a) clinical skin lesions; b) in vivo deposition of rabbit IgG and mouse C3 at the basement membrane demonstrated by direct IF; c) DEJ separation with an extensive inflammatory cell infiltrate demonstrated by H&E staining.43 This infiltrate includes neutrophils, lymphocytes, and monocytes/macrophages, with neutrophils being the predominant cell type.43,44

Immunopathogenesis of BP

With this IgG passive transfer model available, a systematic dissection of the pathogenesis of BP has been carried out so as to define the role of pathogenic antibodies, complement system, inflammatory cells, and
Role of anti-BP180 antibodies: Subepidermal blister formation in experimental BP is initiated by anti-mBP180 IgG and disease onset and severity is totally dependent on the levels of anti-mBP180 antibodies in circulation (Fig. 3). Epitope mapping studies revealed that pathogenic anti-BP180 antibodies recognize a 9–12 amino acid stretch within the murine BP180 NC14A region of the antigen. Significantly, this epitope overlaps the region of the human BP180 NC16A which contains the immunodominant epitopes recognized by human BP autoantibodies.

Role of the complement system: It has been well-documented that human BP autoantibodies bind to basement membrane antigens and activate complement. To determine whether the complement system is involved in experimental BP, we used molecular and immunological approaches and demonstrated: 1) C5-deficient mice are resistant to experimental BP; 2) BALB/c mice pretreated with cobra venom factor to deplete complement are resistant to experimental BP; 3) F(ab')2 fragments generated from the pathogenic anti-mBP180 IgG cannot induce subepidermal blisters in C5-sufficient mice; and 4) C5-deficient mice reconstituted with C5a become susceptible to experimental BP. Using mice deficient in complement component C4 (the classical pathway-specific) and factor B (the alternative pathway-specific), we recently found that the classical pathway of complement activation plays a major role in disease development (Carter, et al., unpublished observation). Further studies reveal that the major function of complement activation is to generate C5a that in turn activates mast cells (Fig. 3).

Role of mast cells, macrophages, and neutrophils: Using mice deficient in different inflammatory cell types, we demonstrated that mast cells, macrophages, and neutrophils, but not T and B lymphocytes, play a direct role in subepidermal blistering in experimental BP. Pathogenic anti-mBP180 antibodies trigger BP skin disease in wild-type mice and mice deficient in T or B, or both T and B cells, but fail to induce skin lesions in mice deficient in mast cells, macrophages, or neutrophils. Quantification of disease severity in these mice revealed that mast cells and neutrophils play a major role in experimental BP.

Like human BP, extensive mast cell degranulation is seen in the lesional skin of mice injected with pathogenic anti-mBP180 antibodies. Mast cell activation precedes neutrophil infiltration (Fig. 3). Without mast cells or inhibition of mast cell degranulation, neutrophil infiltration is impaired and subsequent blister formation is abolished. Mast cell-deficient mice, which are resistant to experimental BP, when reconstituted locally with neutrophils or injected locally with neutrophil chemoattractants IL-8 or TNF-α, became susceptible to the pathogenic activity of anti-mBP180 IgG. These results suggest that mast cells play a critical role in recruiting neutrophils by releasing some key proinflammatory cytokines.
Neutrophil infiltration is a prerequisite for experimental BP and the disease severity is directly correlated to the number of infiltrating neutrophils (Fig. 3). Neutrophil infiltration depends on complement activation and subsequent mast cell degranulation. Lack of a functional complement system or mast cells resulted in lack of neutrophil recruitment and subsequent BP blister formation. Mice depleted of neutrophils are resistant to experimental BP. Therefore, infiltrating neutrophils are the cells that directly cause tissue injury in the DEJ, leading to BP skin blisters.

Role of proteolytic enzymes: Proteinases and reactive free radicals from infiltrating inflammatory cells, acting either alone or synergistically, have been implicated as effector molecules contributing to tissue damage in BP lesions. Neutrophil granules contain a variety of proteolytic enzymes, including elastase, cathepsin G, collagenase, and gelatinase B, which are known to degrade specific elements of the extracellular matrix. Upon cell activation, these enzymes are secreted into the extracellular space.

In experimental BP, levels of several proteolytic enzymes are significantly increased including neutrophil elastase (NE) and gelatinase B (GB). Mice genetically deficient in NE or GB are resistant to experimental BP. In vitro, although both GB and NE are capable of degrading recombinant BP180 protein, only NE produces DEJ separation when incubated with skin sections (Fig. 3). These findings demonstrate that proteolytic enzymes released from neutrophils damage the BMZ directly, causing DEJ separation.

Is the IgG Passive Transfer Model of BP Relevant to Human BP?

Experimental BP shares key characteristics with human BP at the clinical, histological, and immunological levels. Using this IgG passive transfer model of BP, we have identified key steps in the process of disease development. However, one significant difference between human and mouse BP exists: the majority of biopsies from human patients with BP show large numbers of eosinophils in their lesional skin, while in the IgG passive transfer mouse model, neutrophils are the predominant inflammatory cell type. Some human patients may have neutrophil-rich or cell-poor biopsies. These different pathological features in human BP indicate that BP is a heterogeneous disease and subepidermal blister formation can be initiated and sustained by more than one disease mechanisms. Therefore, it is quite possible that the neutrophil-mediated subepidermal blistering triggered by rabbit anti-mBP180 antibodies represents one of the pathogenic mechanisms. Indeed, recent studies directly confirmed some key findings from the BP mouse model: a) as in the rabbit anti-mBP180 IgG passive transfer model, rabbit antibodies directed against the extracellular domain of hamster BP180 (NC16A region) trigger BP-like subepidermal blisters in neonatal hamsters; b) in vitro DEJ separation induced by human BP autoantibodies specific for BP180NC16A depends on neutrophils; c) human BP blister fluids contain high levels of both neutrophil elastase and gelatinase B, but BP180 degradation by blister fluid depends on neutrophil elastase activity. 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. Whether eosinophils are directly involved in BP and if so, what the exact role these cells play in BP both need to be determined. What causes blisters in pauci-cellular BP is another interesting arena for future investigation.

Conclusions

Based on recent experimental evidence from both human BP studies and studies of animal model systems, we believe that human BP and mouse BP share not only a common immunohistological characteristics, but also a common immunopathogenesis underlying the disease development. Therefore, these animal models of BP have proved and continue to be invaluable in vivo systems in dissecting the anti-BP180 IgG-mediated inflammatory cascade in BP and should be very useful to test new therapeutic strategies for BP. Since BP180 is also an autoantibody target in several other subepidermal blistering diseases including cicatricial pemphigoid, herpetic gestationis, linear IgA bullous dermatosis, and lichen planus pemphigoides, findings from the IgG passive transfer models of BP should have significant implications for these skin autoimmune disorders.

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