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 emidesmosomal 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 charac-

terized 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]
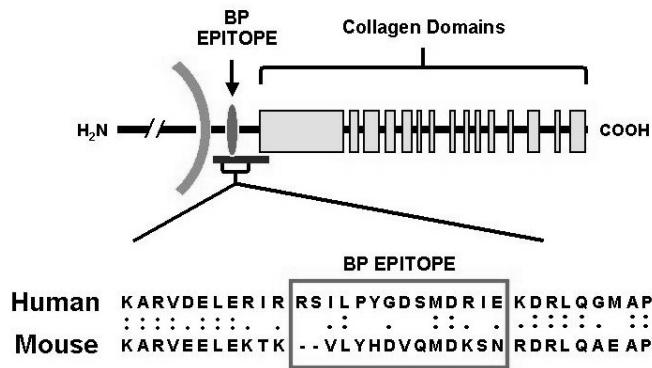
Fig. 1 Human and mouse BP180. A schematic representation of the structural organization of the human BP180 protein is shown at top. The orange arch designates the transmembrane domain. The red oval designates the NC16A antigenic site recognized by BP autoantibodies. The COOH-terminal extracellular region is made up of 15 interrupted collagen-like domains (yellow rectangles). The box (in red) at the bottom shows the amino acid sequence alignment of the human and murine BP180 within the BP epitope. Identical residues are designated by double dots and conservative substitutions are marked by a single dot. Sequence comparison of human and murine BP180 proteins reveals that an unusually high degree of sequence divergence is seen in the BP epitope region.

Fig. 2 Passive transfer of rabbit anti-mBP180 IgG. Neonatal BALB/c mice are injected intradermally with rabbit anti-murine BP180 IgG. After 24 h, gentle friction elicits persistent epidermal wrinkling, producing the "epidermal detachment" sign (panel a). Direct immunofluorescence analysis shows deposition of anti-BP180 IgG (panel b) and murine C3 (panel c) at the basement membrane zone. Histological examination of lesional skin reveals dermal-epidermal junction separation with an inflammatory infiltrate (panel d).

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 leukocytes, have been identified in lesional skin and/or blister fluids of BP patients, including a) granular proteins derived from degranulated leukocytes, such as eosinophil cationic protein (ECP), eosinophil major basic protein (MBP), and neutrophil-derived myeloperoxidase (MPO)[25–27] and b) chemoattractants and cytokines, such as C5a fragments, histamine, leukotriene B4, interleukin-1, -2, -4, -5, -6, -8, -15, TNF-α, IFN-γ, RANTES, and eotaxin.[28–32] Several proteinases are also found in BP blister fluid, including plasmin, collagenase, elastase, and 92-kD gelatinase.[33–40] These proteolytic enzymes are capable of degrading extracellular matrix proteins and thus, they may play a crucial role in subepidermal blister formation in BP.
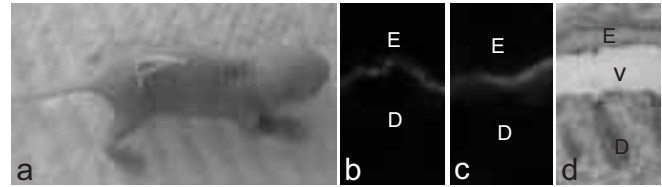
Development of an IgG Passive Transfer Model of BP

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
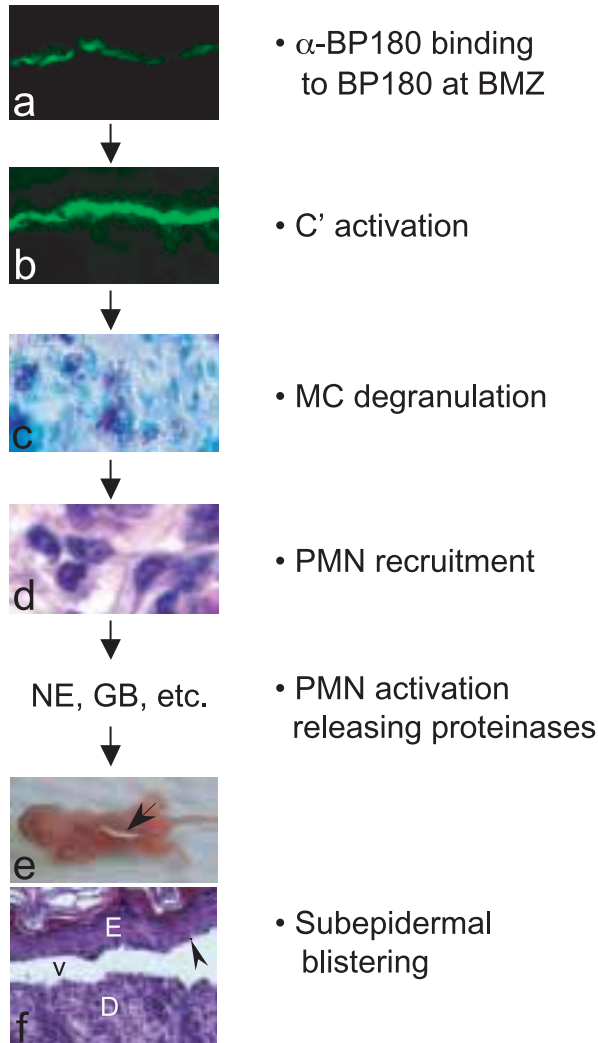
Fig. 3 Proposed mechanism of subepidermal blister formation in experimental BP. BP is an inflammatory autoimmune skin blistering disease with following key steps in the disease cascade: a) Anti-BP180 antibodies bind to the pathogenic epitope of BP180 antigen in basal keratinocytes of the basement membrane zone (BMZ); b) The Fc domain of anti-BP180 IgG bound to BMZ activates complement mainly via the classical pathway; c) Complement activation-derived fragments C3a and C5a activate mast cells to degranulated; d) Mast cells release proinflammatory cytokines recruiting neutrophils (PMN) into the skin site and activated PMN secrete proteinases including neutrophil elastase (NE), gelatinase B (GB) and others. These proteolytic enzymes work together to cause clinical (e) and histological (f) subepidermal blisters. E, epidermis; D, dermis; V, vesicle; Arrowhead, basal keratinocytes.

proteolytic enzymes. An immunopathological mechanism for BP is proposed and shown in Fig. 3.

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).[43,45] 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.[46] 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.[47] 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).[48]

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.[44] 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.[48,49] 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.[48]

Neutrophil infiltration is a prerequisite for experimental BP and the disease severity is directly correlated to the number of infiltrating neutrophils (Fig. 3).[50] 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.[51,52] 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.[53] Upon cell activation, these enzymes are secreted into the extracellular space.[53]

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.[54,55] *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).[38,56] 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.[43] 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 confirm 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;[57] b) *in vitro* DEJ separation induced by human BP autoantibodies specific for BP180NC16A depends on neutrophils;[58] 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.[40] 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 paucicellular 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 *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, herpes 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.[59–62]

References

1. Lever WF: Pemphigus. Medicine 1953; 32: 1–123
2. Stanley JR: Bullous pemphigoid. In: Fitzpatrick's Dermatology in General Medicine, Freedberg IM, Eisen AZ, Wolff K, Austen KF, Goldsmith LA, Katz SI, editors. McGraw-Hill, New York, NY, 1999; 666–671
3. Jordon RE, Beutner EH, Witebsky E, Blumental G, Hale WL, Lever WF: Basement zone antibodies in bullous pemphigoid. JAMA 1967; 200: 751–756

4. Stanley JR, Hawley-Nelson P, Yuspa SH, Shevach EM, Katz SI: Characterization of bullous pemphigoid antigen: a unique basement membrane protein of stratified squamous epithelia. Cell 1981; 24: 897–903

5. Stanley JR, Woodley DT, Katz SI: Identification and partial characterization of pemphigoid antigen extracted from normal human skin. J Invest Dermatol 1984; 82: 108–111

6. Mutasim DF, Takahashi Y, Labib RS, Anhalt GJ, Patel HP, Diaz LA: A pool of bullous pemphigoid antigen(s) is intracellular and associated with the basal cell cytoskeleton-hemidesmosome complex. J Invest Dermatol 1985; 84: 47–53

7. Labib RS, Anhalt GJ, Patel HP, Mutasim DF, Diaz LA: Molecular heterogeneity of the bullous pemphigoid antigens as detected by immunoblotting. J Immunol 1986; 136: 1231–1235

8. Stanley JR, Tanaka T, Mueller S, Klaus-Kovtun V, Roop D: Isolation of complementary DNA for bullous pemphigoid antigen by use of patients' autoantibodies. J Clin Invest 1988; 82: 1864–1870

9. Diaz LA, Ratrie H 3rd, Saunders WS, Futamura S, Squiquera HL, Anhalt GJ, Giudice GJ: Isolation of a human epidermal cDNA corresponding to the 180-kD autoantigen recognized by bullous pemphigoid and herpes gestationis sera. Immunolocalization of this protein to the hemidesmosome. J Clin Invest 1990; 86: 1088–1094

10. Tanaka T, Parry DA, Klaus-Kovtun V, Steinert PM, Stanley JR: Comparison of molecularly cloned bullous pemphigoid antigen to desmoplakin I confirms that they define a new family of cell adhesion junction plaque proteins. J Biol Chem 1991; 266: 12555–12559

11. Sawamura D, Li K, Chu ML, Uitto J: Human bullous pemphigoid antigen (BPAG1). Amino acid sequences deduced from cloned cDNAs predict biologically important peptide segments and protein domains. J Biol Chem 1991; 266: 17784–17790

12. Giudice GJ, Emery DJ, Diaz LA: Cloning and primary structural analysis of the bullous pemphigoid autoantigen BP180. J Invest Dermatol 1992; 99: 243–250

13. Klatte DH, Kurpakus MA, Grelling KA, Jones JC: Immunochemical characterization of three components of the hemidesmosome and their expression in cultured epithelial cells. J Cell Biol 1989; 109: 3377–3390

14. Tanaka T, Korman NJ, Shimizu H, Eady RA, Klaus-Kovtun V, Cehrs K, Stanley JR: Production of rabbit antibodies against carboxy-terminal epitopes encoded by bullous pemphigoid cDNA. J Invest Dermatol 1990; 94: 617–623

15. Ishiko A, Shimizu H, Kikuchi A, Ebihara T, Hashimoto T, Nishikawa T: Human autoantibodies against the 230-kD bullous pemphigoid antigen (BPAG1) bind only to the intracellular domain of the hemidesmosome, whereas those against the 180-kD bullous pemphigoid antigen (BPAG2) bind along the plasma membrane of the hemidesmosome in normal human and swine skin. J Clin Invest 1993; 91: 1608–1615

16. Hopkinson SB, Riddelle KS, Jones JC: Cytoplasmic domain of the 180-kD bullous pemphigoid antigen, a hemidesmosomal component: molecular and cell biologic characterization. J Invest Dermatol 1992; 99: 264–270

17. Giudice GJ, Emery DJ, Zelickson BD, Anhalt GJ, Liu Z, Diaz LA: Bullous pemphigoid and herpes gestationis autoantibodies recognize a common non-collagenous site on the BP180 ectodomain. J Immunol 1993; 151: 5742–5750

18. Zillikens D, Rose PA, Balding SD, Liu Z, Olague-Marchan M, Diaz LA, Giudice GJ: Tight clustering of extracellular BP180 epitopes recognized by bullous pemphigoid autoantibodies. J Invest Dermatol 1997; 109: 573–579

19. Bernard P, Aucouturier P, Denis F, Bonnetblanc JM: Immunoblot analysis of IgG subclasses of circulating antibodies in bullous pemphigoid. Clin Immunol Immunopathol 1990; 54: 484–494

20. Dopp R, Schmidt E, Chimanovitch I, Leverkus M, Brocker EB, Zillikens D: IgG4 and IgE are the major immunoglobulins targeting the NC16A domain of BP180 in Bullous pemphigoid: serum levels of these immunoglobulins reflect disease activity. J Am Acad Dermatol 2000; 42: 577–583

21. Laffitte E, Skaria M, Jaunin F, Tamm K, Saurat JH, Favre B, Borradori L: Autoantibodies to the extracellular and intracellular domain of bullous pemphigoid 180, the putative key autoantigen in bullous pemphigoid, belong predominantly to the IgG1 and IgG4 subclasses. Br J Dermatol 2001; 144: 760–768

22. Haase C, Budinger L, Borradori L, Yee C, Merk HF, Yancey K, Hertl M: Detection of IgG autoantibodies in the sera of patients with bullous and gestational pemphigoid: ELISA studies utilizing a baculovirus-encoded form of bullous pemphigoid antigen 2. J Invest Dermatol 1998; 110: 282–286

23. Budinger L, Borradori L, Yee C, Eming R, Ferencik S, Grosse-Wilde H, Merk HF, Yancey K, Hertl M: Identification and characterization of autoreactive T cell responses to bullous pemphigoid antigen 2 in patients and healthy controls. J Clin Invest 1998; 102: 2082–2089

24. Lin MS, Fu CL, Giudice GJ, Olague-Marchan M, Lazaro AM, Stastny P, Diaz LA: Epitopes targeted by bullous pemphigoid T lymphocytes and autoantibodies map to the same sites on the bullous pemphigoid 180 ectodomain. J Invest Dermatol 2000; 115: 955–961

25. Baba T, Sonozaki H, Seki K, Uchiyama M, Ikesawa Y, Toriisu M: An eosinophil chemotactic factor present in blister fluids of bullous pemphigoid patients. J Immunol 1976; 116: 112–116

26. Czech W, Schaller J, Schopf E, Kapp A: Granulocyte activation in bullous diseases: release of granular proteins in bullous pemphigoid and pemphigus vulgaris. J Am Acad Dermatol 1993; 29: 210–215

27. Borrego L, Maynard B, Peterson EA, George T, Iglesias L, Peters MS, Newman W, Gleich GJ, Leiferman KM: Deposition of eosinophil granule proteins precedes blister formation in bullous pemphigoid. Comparison with neutrophil and mast cell granule proteins. Am J Pathol 1996; 148: 897–909

28. Diaz-Perez JL, Jordon RE: The complement system in bullous pemphigoid. IV. Chemotactic activity in blister fluid. Clin Immunol Immunopathol 1976; 5: 360–370

29. Katayama I, Doi T, Nishioka K: High histamine level in the blister fluid of bullous pemphigoid. Arch Dermatol Res 1984; 276: 126–127

30. Kawana S, Ueno A, Nishiyama S: Increased levels of immunoreactive leukotriene B4 in blister fluids of bullous pemphigoid patients and effects of a selective 5-lipoxygenase inhibitor on experimental skin lesions. Acta Derm Venereol 1990; 70: 281–285

31. D'Auria L, Cordiali Fei P, Ameglio F: Cytokines and bullous pemphigoid. Eur Cytokine Netw 1999; 10: 123–134

32. Wakugawa M, Nakamura K, Hino H, Toyama K, Hattori N, Okochi H, Yamada H, Hirai K, Tamaki K, Furue M: Elevated levels of eotaxin and interleukin-5 in blister fluid of bullous pemphigoid: correlation with tissue eosinophilia. Br J Dermatol 2000; 143: 112–116

33. Oikarinen AI, Zone JJ, Ahmed AR, Kiistala U, Uitto J: Demonstration of collagenase and elastase activities in the blister fluids from bullous skin diseases. Comparison between dermatitis herpetiformis and bullous pemphigoid. J Invest Dermatol 1983; 81: 261–266

34. Lauharanta J, Salonen EM, Vaheri A: Plasmin-like proteinase associated with high molecular weight complexes in blister fluid of bullous pemphigoid. Acta Derm Venereol 1989; 69: 527–529

35. Baird J, Lazarus GS, Belin D, Vassalli JD, Busso N, Gubler P, Jensen PJ: mRNA for tissue-type plasminogen activator is present in lesional epidermis from patients with psoriasis, pemphigus,

or bullous pemphigoid, but is not detected in normal epidermis. J Invest Dermatol 1990; 95: 548–552

36. Gissler HM, Simon MM, Kramer MD: Enhanced association of plasminogen/plasmin with lesional epidermis of bullous pemphigoid. Br J Dermatol 1992; 127: 272–277

37. Kramer MD, Reinartz J: The autoimmune blistering skin disease bullous pemphigoid. The presence of plasmin/alpha 2-antiplasmin complexes in skin blister fluid indicates plasmin generation in lesional skin. J Clin Invest 1993; 92: 978–983

38. Ståhle-Bäckdahl M, Inoue M, Guidice GJ, Parks WC: 92-kD gelatinase is produced by eosinophils at the site of blister formation in bullous pemphigoid and cleaves the extracellular domain of recombinant 180-kD bullous pemphigoid autoantigen. J Clin Invest 1994; 93: 2022–2030

39. Schaefer BM, Jaeger C, Drepper E, Kramer MD: Plasminogen activation in bullous pemphigoid immunohistology reveals urokinase type plasminogen activator, its receptor and plasminogen activator inhibitor type-2 in lesional epidermis. Autoimmunity 1996; 23: 155–164

40. Verraes S, Hornebeck W, Polette M, Borradori L, Bernard P: Respective contribution of neutrophil elastase and matrix metalloproteinase 9 in the degradation of BP180 (type XVII collagen) in human bullous pemphigoid. J Invest Dermatol 2001; 117: 1091–1096

41. Anhalt GJ, Labib RS, Voorhees JJ, Beals TF, Diaz LA: Induction of pemphigus in neonatal mice by passive transfer of IgG from patients with the disease. N Engl J Med 1982; 306: 1189–1196

42. Roscoe JT, Diaz L, Sampaio SA, Castro RM, Labib RS, Takahashi Y, Patel H, Anhalt GJ: Brazilian pemphigus foliaceus autoantibodies are pathogenic to BALB/c mice by passive transfer. J Invest Dermatol 1985; 85: 538–541

43. Liu Z, Diaz LA, Troy JL, Taylor AF, Emery DJ, Fairley JA, Giudice GJ: A passive transfer model of the organ-specific autoimmune disease, bullous pemphigoid, using antibodies generated against the hemidesmosomal antigen, BP180. J Clin Invest 1993; 92: 2480–2488

44. Chen R, Fairley JA, Zhao ML, Giudice GJ, Zillikens D, Diaz LA, Liu Z: Macrophages, but not T and B lymphocytes, are critical for subepidermal blister formation in experimental bullous pemphigoid: macrophage-mediated neutrophil infiltration depends on mast cell activation. J Immunol 2002; 169: 3987–3992

45. Liu Z, Roopenian DC, Zhou X, Christianson GJ, Diaz LA, Sedmak DD, Anderson CL: Beta2-microglobulin-deficient mice are resistant to bullous pemphigoid. J Exp Med 1997; 186: 777–783

46. Liu Z, Diaz LA, Swartz SJ, Troy JL, Fairley JA, Giudice GJ: Molecular mapping of a pathogenically relevant BP180 epitope associated with experimentally induced murine bullous pemphigoid. J Immunol 1995; 155: 5449–5454

47. Liu Z, Giudice GJ, Swartz SJ, Fairley JA, Till GO, Troy JL, Diaz LA: The role of complement in experimental bullous pemphigoid. J Clin Invest 1995; 95: 1539–1544

48. Chen R, Ning G, Zhao ML, Fleming MG, Diaz LA, Werb Z, Liu Z: Mast cells play a key role in neutrophil recruitment in experimental bullous pemphigoid. J Clin Invest 2001; 108: 1151–1158

49. Wintroub BU, Mihm MC Jr, Goetzl EJ, Soter NA, Austen KF: Morphologic and functional evidence for release of mast-cell products in bullous pemphigoid. N Engl J Med 1978; 298: 417–421

50. Liu Z, Giudice GJ, Zhou X, Swartz SJ, Troy JL, Fairley JA, Till GO, Diaz LA: A major role for neutrophils in experimental bullous pemphigoid. J Clin Invest 1997; 100: 1256–1263

51. Jordon RE, Kawana S, Fritz KA: Immunopathologic mechanisms in pemphigus and bullous pemphigoid. J Invest Dermatol 1985; 85 (1 Suppl): 72s–78s

52. Gammon WR: Immune complex and complement-mediated leukocyte recruitment in bullous pemphigoid. Immunol Ser 1989; 46: 509–525

53. Weiss SJ: Tissue destruction by neutrophils. N Engl J Med 1989; 320: 365–376

54. Liu Z, Shipley JM, Vu TH, Zhou X, Diaz LA, Werb Z, Senior RM: Gelatinase B-deficient mice are resistant to experimental bullous pemphigoid. J Exp Med 1998; 188: 475–482

55. Liu Z, Shapiro SD, Zhou X, Twining SS, Senior RM, Giudice GJ, Fairley JA, Diaz LA: A critical role for neutrophil elastase in experimental bullous pemphigoid. J Clin Invest 2000; 105: 113–123

56. Liu Z, Zhou X, Shapiro SD, Shipley JM, Twining SS, Diaz LA, Senior RM, Werb Z: The serpin alpha1-proteinase inhibitor is a critical substrate for gelatinase B/MMP-9 *in vivo*. Cell 2000; 102: 647–655

57. Yamamoto K, Inoue N, Masuda R, Fujimori A, Saito T, Imajoh-Ohmi S, Shinkai H, Sakiyama H: Cloning of hamster type XVII collagen cDNA, and pathogenesis of anti-type XVII collagen antibody and complement in hamster bullous pemphigoid. J Invest Dermatol 2002; 118: 485–492

58. Sitaru C, Schmidt E, Petermann S, Munteanu LS, Brocker EB, Zillikens D: Autoantibodies to bullous pemphigoid antigen 180 induce dermal-epidermal separation in cryosections of human skin. J Invest Dermatol 2002; 118: 664–671

59. Morrison LH, Labib RS, Zone JJ, Diaz LA, Anhalt GJ: Herpes gestationis autoantibodies recognize a 180-kD human epidermal antigen. J Clin Invest 1988; 81: 2023–2026

60. Bernard P, Prost C, Durepaire N, Basset-Seguin N, Didierjean L, Saurat JH: The major cicatricial pemphigoid antigen is a 180-kD protein that shows immunologic cross-reactivities with the bullous pemphigoid antigen. J Invest Dermatol 1992; 99: 174–179

61. Tamada Y, Yokochi K, Nitta Y, Ikeya T, Hara K, Owaribe K: Lichen planus pemphigoides: identification of 180 kd hemidesmosome antigen. J Am Acad Dermatol 1995; 32: 883–887

62. Zone JJ, Taylor TB, Meyer LJ, Petersen MJ: The 97 kDa linear IgA bullous disease antigen is identical to a portion of the extracellular domain of the 180 kDa bullous pemphigoid antigen, BPAg2. J Invest Dermatol 1998; 110: 207–210