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Understanding The Physiological Mechanisms Of Bullous Pemphigoid

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Abstract: An autoimmune response against two hemidesmosomal proteins in the dermal–epidermal junction, BP180 and BP230, is the characteristic of bullous pemphigoid (BP), a blistering dermatosis. BP180 is an extracellular domain-bearing transmembrane glycoprotein, while BP230 is intracellularly bound and associated with the hemidesmosomal plaque. Most BP patients possess autoantibodies that bind to the noncollagenous 16A domain (NC16A) of BP180, an immunodominant region of BP180 that is extracellularly located adjacent to the protein's transmembrane domain. Autoreactive T and B cell responses to BP180 in BP patients have been reported. In mice, a bullous skin disease with a very close analog of human blood pressure is induced by passive antibody transfer to the mouse BP180 ectodomain. In this model of animals, the formation of lesions relies on mast cell degranulation, complement activation, and neutrophil and eosinophil recruitment. Dermal–epidermal separation occurs in cryosections of human skin when autoantibodies against BP180 are co-incubated with leukocytes. Granulocytes secrete proteinases that cause loss of cell-matrix adhesion. The development of new treatment strategies for BP may be facilitated by our increasing knowledge of its pathophysiology.

Index Terms **- Autoimmunity . Bullous pemphigoid . BP180 . BP230 . Hemidesmosome**

HUMORAL IMMUNE RESPONSE IN BULLOUS PEMPHIGOID

In BP, the humoral response is usually polyclonal and involves many BP antigen epitopes. Autoantibodies to the NC16A domain, the immunodominant region of BP180, are found in most BP patients.[1] Recent studies have demonstrated that memory B lymphocytes specific for the NC16A domain are present and can be activated to produce autoantibodies in vitro. [2]These BP180 NC16Aspecific autoantibodies are mainly representative of the IgE and IgG isotypes and the IgG1 and IgG4 subclasses.[3-4] Clinical observations and experimental data have now well established the autoimmune etiology of BP. These include: (1) patients have autoantibodies and autoreactive T cells to well-characterized self-antigens; (2) tissue damage is seen when antibody-antigen complexes are present; and[5,6] (3) in vitro models using human skin and in vivo animal models recapitulate the critical role of pathogenic autoantibodies in disease induction.

(3) Autoantibodies from women suffering from gestational pemphigoid can transfer transplacentally to the f etus, with a resulting, temporary bullous eruption; [7-14](4) specific HLA genotypes are associated with the disease; [15](5) the severity of BP correlates inversely with blood levels of autoantibodies to BP180 NC16A;[3,19] (6) the disease shows a response to immunosuppressive treatment. The first passive transfer investigations on the blister-inducing

potential of autoantibodies in BP were conducted by Sams and Gleich. These experiments were made in Rhesus monkeys that received plasma from patients with BP for transfusion. Although they transfused a great amount of BP plasma, it was not possible to reproduce essential features of BP in any one of the recipients.[20] Similarly, injection of IgG from BP patients did not cause subepidermal blisters, while pemphigus autoantibodies cause acantholytic blisters when IgG autoantibodies are injected into neonatal mice isolated from sera of patients.[21] However, if IgG was isolated from the sera of BP patients and was injected into the rabbit corneas, then there was a local inflammatory reaction; this did not occur in rabbit skin. Consequently, one-third of injected rabbits developed subepidermal blisters.[22] In experimental animals, pathogenic BP autoantibodies failed to cross-react with the homologous target antigens, a fact that can explain why all previous attempts at demonstrating the harmfulness of the patient autoantibodies have been unsuccessful. Alternatively, Liu et al [5] prepared rabbit polyclonal antibodies directed against a sequence of the murine BP180 protein that shares homology to the human BP180 NC16A (mBP180 NC14A). They then administered the purified rabbit anti-mBP180 IgG passively into newborn Balb/c mice. A severe disease that resembled closely human BP developed in the injected animals. Similarly, autoantibodies generated from BP patients which target BP180 elicit subepidermal blister formation when administered to human skin cryosections.[11] In contrast, autoantibodies directed against the cytoplasmic protein BP230 merely induce an inflammatory response in rabbits following additional disruption of their epidermis.[25] Moreover, in animal models, passive transfer of anti-BP230 antibodies has not caused any pathogenic changes similar to those induced by BP. [26]Altogether, these studies have suggested the hypothesis that autoantibodies against the extracellular domain of BP180 are pathogenetically significant while autoantibodies against intracellular antigenic epitopes on BP230 (and the intracellular domain of BP180) are a secondary process. [27] On the contrary, it was identified that the in situbound antibodies isolated from the skin lesions of BP targeted BP230 in particular. Amazingly, Kiss et al. recently found that unlike previous studies, newborn mice injected with rabbit antibodies to BP230 displayed subepidermal splits. This important finding does lend evidence to the proposal that anti-BP230 autoantibodies are part of the disease mechanism of BP as it shows them to indeed gain access to their putative intracellular target antigen. [28] However, more elucidation needs to be provided to address the issue of the pathogenic importance of autoantibodies to BP230 and the contributory role of autoantibodies of varying specificities for the induction of blisters in BP.

CELLULAR RESPONSE IN BULLOUS PEMPHIGOID

Not a lot of mechanisms on how the immunological response is developed in BP is known. It should be so that peripheral resistance to BP antigens breaks down resulting into BP. Blisters would develop as the autoantibodies production that would result. T- and B-cell reactivities against BP180 are almost invariably detectable in BP patients, whereas BP230 reactivity is not, and differential epitope identification of BP180 seems to be associated with different clinical severity: Although restricted BP is more often associated with the middle region of the BP180 ectodomain, large BP is associated with T- and B-cell reactivity with the NH2-terminal portion.[29] Normal individuals also had these autoreactive cells. Allele DQβ1*0301 MHC II restricts the response to the BP180 ectodomain. [20,29]The cytokine profile of the T cells was a mixed Th1/Th2 profile. [21]These T cells carried the cell surface markers for memory CD4 T cells. In BP patients, antibodies anti-BP180 and anti-BP230 belonged to the isotype IgG1, IgG4, and IgE, showing both autoreactive Th1 and Th2 involvement in the control of the BP target antigens' response. This is because Th1 cytokines, like IFNγ, can induce the secretion of IgG1 and IgG2, while Th2 cytokines, like IL-4, IL-5, and IL13, have been demonstrated to regulate the secretion of IgG4 and IgE. [31,32]Many Th1 and Th2 cytokines have been shown to have elevated serum levels that correlate with disease activity in people with high BP.[33] Th2 cytokines were produced by most T cell clones that were specific to BP180. Since patients' peripheral blood contains CD4 positive, primarily Th2-like, autoreactive T-cells that recognize the ectodomain of BP180, these results suggest that BP is a T-cell-dependent autoimmune disease.[20,29,33]

MECHANISMS OF TISSUE INJURY AND BLISTER FORMATION

The release of proteolytic enzymes, complement activation, inflammatory cell recruitment, and direct disruption of the autoantigens' ability to adhere are some of the ways whereby BP autoantibodies are believed to be harmful.

ROLE OF COMPLEMENT SYSTEM

Incubating serum samples from patients with BP with cryosections of normal human skin drew leukocytes from healthy volunteers to the BMZ and led to their separation in a dermal-epidermal junction under a microscope. Leukocytes seemed to be necessary for split formation, which appeared to be facilitated by the addition of fresh human serum as a complement supply. [34]Many preliminary studies have already established that C3 is deposited directly by immunofluorescence at the BMZ of perilesional skin in virtually all patients with BP and that BP autoantibodies fix complement in vitro. Immunoelectron microscopy demonstrates both BP autoantibodies and C3 at the level of immunologically mediated damage: the lamina lucida of the skin. [34]Lesional skin from BP patients has been found to include elements of both the classical and alternative routes of complement, such as C1q, C4, C5, C5-9, factor B, B1H globulin, and properdin.[35-39] The fact that the quantities of individual complement components and of total hemolytic complement in blister fluid from BP patients are less than those found in control blister fluids and in these patients' sera supports further the role of complement activation.[40] Liu et al. used molecular and immunological techniques to further determine whether the complement system is involved in experimental BP. [41,42]They demonstrated the following: (1) rabbit antimurine-BP180 IgG could induce cutaneous blisters in a strain of C5-sufficient mice, but failed to cause disease in the syngeneic C5-deficient strain; (2) Balb/c mice, pretreated with cobra verum factor to deplete complement, were resistant to experimental BP; (3) F(ab′)2 fragments from the pathogenic anti-mBP180 were unable to cause subepidermal blisters in C5-sufficient mice; and (4) C5-deficient mice, reconstituted with C5a, were susceptible to experimental BP. [43]These research' findings provide strong evidence in support of the theory that complement activation is required for the induction of subepidermal blisters caused by anti-BP180 antibodies.

ROLE OF INFLAMMATORY CELLS

Immunohistological data has long supported the theory that inflammatory cells promote the subepidermal blister development in BP caused by anti-BMZ autoantibodies. [41] More than anything else, mast cells, neutrophils, and eosinophils seem to be crucial in mediating tissue damage.

1. MAST CELLS

Wintroub et al. were the first authors to describe and report the existence of mast cells that degranulated in the lesional sites of BP in 1978; subsequent authors reported similar findings. [44-47] Mice administered with pathogenic anti-mBP180 antibodies exhibit mast cell degranulation within the lesional skin, and this is just like in the human BP.[44,48] IgE anti-BP180 autoantibodies have recently been demonstrated to enhance the degranulation of these cells, which could help in the formation of lesions. Antigen-specific histamine release is only observed in patients whose circulating IgE can be detected to react with the noncollagenous stretch of the BP180 ectodomain, NC16A, which is considered to be the major target of IgE class autoantibodies.[49,50] While pathogenic anti-mBP180 antibodies are able to provoke BP skin disease in wild-type mice and mice deficient in T and/or B cells, mice devoid of mast cells, macrophages, or neutrophils are resistant to experimental BP.[51] The pathogenic activity of anti-mBP180 IgG can be restored through reconstitution with MCs. Complement activation-derived fragments C3a and C5a are shown to elicit MC degranulation but the absence of C5 completely excludes MC degranulation. [48, 52]Other important findings included the fact that MC activation actually precedes the infiltration of neutrophils and the fact that degranulation-inhibited MC effectively eliminated blistering. As evidenced by the high levels of histamine, leukotriene B4, IL-1, IL-2, IL-5, and IL-6, as well as TNF-α in BP blister fluids, MCs can produce a range of mediators, including leukotriens, platelet-activating factor, TNF-α, MC tryptase, and other cytokines that have been connected either directly or indirectly to neutrophil influx.[53-65]Furthermore, in MC-deficient

mice reconstituted with neutrophils, IL-8, or TNFα, pathogenic anti-mBP180 antibodies also cause BP skin lesions. [48]Taken together, these studies prove that MCs are involved in subepidermal blistering secondary to pathogenic antimBP180 antibodies. As MCs degranulate, they play a role in attracting neutrophils into the target tissue; hence, after the activation of complement, they are the primary effector cells responsible for the inflammatory cascade that leads to blister formation in BP.

2. NEUTROPHILS

Many studies have demonstrated the significance of neutrophils in the formation of blisters in human BP and experimental mice. Neutrophils are involved in the separation of the dermal–epidermal junction in the in vitro cryosection model of BP. [34,66,67]In this model system, incubation of human skin with BP serum, complement, and peripheral blood leukocytes results in neutrophil alignment along the BMZ, leading to a loss of dermal–epidermal cohesion. Early neutrophil infiltration in experimental murine BP depends on the activation of the complement system with subsequent mast cell degranulation.[43,48] Eliminating neutrophil infiltration ablates subepidermal blistering and mice with fewer neutrophils are resistant to experimental BP.[68] Increased inflammation of the skin occurs after increased neutrophil recruitment due to further tissue injury caused by additional neutrophil infiltration.[69] This later, or amplification, stage of neutrophil infiltration is mediated by the release of several proteases found in blister fluid and lesional skin, which cause local tissue damage to the basement membrane.[70-72] It has recently been demonstrated that the β2 integrins have important but distinct roles in subepidermal blistering in experimental BP, with Mac-1 mainly in charge of amplifying neutrophil accumulation and apoptosis of tissue-accumulated neutrophils and LFA-1 being absolutely necessary for neutrophil recruitment.[73] Clinical blistering requires a threshold of neutrophil accumulation, and subepidermal blisters are inhibited with a mere 30% decrease in neutrophil influx. [68] In any case, a direct correlation has been established between the number of infiltrating neutrophils and disease severity. Studies published recently question the absolute dependency on granulocytes for the appearance of BP blisters: the passive transfer into newborn hamsters of rabbit polyclonal antibodies produced against hamster BP180 resulted in the formation of subepidermal blisters. While complement activation occurred, leukocytes were not needed for blistering to occur.[74]

3. EOSINOPHILS

Eosinophils appear to play an important role in the pathogenesis and/or progression of BP in humans. In contrast, neutrophils predominate at the lesional site in the animal model, whereas eosinophils dominate the inflammatory infiltrate that characterizes the human disease.[75] Eosinophils are often found at the margin of the dermal–epidermal junction or dispersed throughout the upper dermis.[76] In addition, the peripheral blood contains a greater number of eosinophils in many patients. [77] The blister fluid of BP patients was found to contain high amounts of eotaxin, eosinophilic cationic protein (ECP), and IL-5. Notably, IL-5 enhances both the growth and activation of eosinophils.[78,79] One of the chemoattractants specific for eosinophils, eotaxin, is expressed by ibroblasts, but it is produced likely by keratinocytes also, and the movements of eosinophils are regulated by eotaxin.[80] In BP, it was reported that in patients, basophils and Th2 cells exhibit high levels of the particular receptor for this chemoattractant, namely CCR3. At the level of the BMZ's lamina lucida, IL-5 and eotaxin are thought to enhance the inflammatory response and assist in the influx of granulocytes, which in turn cause the separation of the epidermis and dermis through the release of proteinases or cytotoxic agents like eosinophil major basic protein (MBP) and ECP [45,81]

ROLE OF PROTEOLYTIC ENZYMES

High levels of proteolytic enzymes like neutrophil elastase (NE), cathepsin G, collagenase, plasminogen activators, plasmin, MMP-2 or gelatinase A, MMP-9 or gelatinase B, and MMP-13 have been identified in blister fluid and lesional/perilesional biopsies from patients with BP.[82-90] These enzymes are released into the extracellular space when a cell is activated. Neutrophils and eosinophils specifically express MMP-9 and NE, which are thought to proteolytically degrade a wide range of extracellular matrix proteins in addition to the extracellular domain of BP180. [89,91] Experimental BP does not impact mice with NE or MMP-9 genetic deficiencies. A functional link between MMP-9, NE, and the plasminogen/plasmin system is revealed by dissecting proteolytic processes in experimental BP. [70,71] MMP-9 is mainly initiated by plasmin in early stages of blisters. A plasminogen activators tPA/uPA generate a plasmin from plasminogen, and auto-antibody to BP 180 has now been shown also to directly to modify the activity of tPA expression by human keratinocyte in culture, apart from being activated by a plasmin, MMP 9 can further be activated with the help of the MCspecific serine proteinase MCP4, chymase. [92-94]Activated MMP-9 allows NE to freely act by enzymatically inactivating the physiological inhibitor of NE, α1-proteinase inhibitor. [72]These results indicate that the dermoepidermal junction separates because of the direct action of proteolytic enzymes generated from inflammatory cells against the BMZ.

ROLE OF DIRECT MECHANISMS

BP usually develops blisters through humoral and cellular processes, unlike pemphigus and anti-laminin 5 mucous membrane pemphigoid. However, it has been proven that autoantibodies may not require the Fc parts of antibodies but the variable sections to self-aggregate and interfere with cell-matrix
adhesion. The simple attachment of the antibody to the BP180 ectodomain might cause blister formation by interfering with the functionality of these molecules through competing with the natural ligand and inhibiting the important binding sites along the BP180 antigen.[26] Actually, autoantibodies from BP patients are mainly of the noncomplement fixing IgG4 subclass; IgG1 subclass autoantibodies are also present, although in smaller quantities.[95,96] It is interesting that IgG4 from BP patients, like IgG1, was recently shown to induce dermal-epidermal separation in human skin cryosections and to recruit and activate leukocytes to the basement membrane.[97] Autoantibodies may also directly cause blisters by activating intracellular signaling pathways that cause hemidesmosomal disintegration or the production of pro-inflammatory cytokines. Autoantibodies to BP180 have recently been shown to directly alter the production of IL-6 and IL-8 in cultured human keratinocytes.[98] However, more work is needed to determine the pathogenic significance of these distinct functions in BP

CONCLUSION:

In conclusion, humoral and cellular immune reactions both play a role in the pathogenesis of Bullous Pemphigoid (BP), a multifactorial autoimmune disease. One of the central elements in the disease process is autoantibodies against the BP180 protein, especially its NC16A domain. Autoantibodies induce tissue damage and blister formation by activating the complement system and attracting inflammatory cells like neutrophils, eosinophils, and mast cells. In addition, these cells release proteolytic enzymes that degrade the extracellular matrix, which further increases the loss of dermal-epidermal adhesion. The intensity of BP is also determined by T-cell responses, i.e., Th1 and Th2 cytokine patterns. While complement activation plays a crucial role, blistering can also occur due to direct antibody-mediated disruption of cell-matrix adhesion. Overall, BP is an immune-mediated process and an intricate one, and to make customized therapies means having a deeper understanding of its pathophysiology.

REFERENCES

1) Giudice GJ, Emery DJ, Zelickson BD, Anhalt GJ, Liu Z, Diaz LA (1993) Bullous pemphigoid and herpes gestationis autoantibodies recognize a common non-collenous site on the BP180 ectodomain. J Immunol 151:5742–575
2) Zillikens D, Rose PR, Balding SD, Liu Z, Olague-Marchan M, Diaz LA, Giudice GJ (1997a) Tight clustering of extracellular BP180 epitopes recognized by bullous pemphigoid autoantibodies. J Invest Dermatol 109:573–579
3) Zillikens D, Mascara JM, Rose PA, Liu Z, Ewing SM, Caux F, Hoffmann RG, Diaz LA, Giudice GJ (1997b) A highly sensitive enzyme-linked immunosorbent assay for the detection of circulating anti-BP180 autoantibodies in patients with bullous pemphigoid. J Invest Dermatol 109:679–683
4) Schumann H, Baetge J, Tasanen K, Wojnarowska F, Schacke H, Zillikens D, Bruckner-Tuderman L (2000) The shed ectodomain of collagen XVII/BP180 is targeted by autoantibodies in different blistering skin diseases. Am J Pathol 156:685–695
5) Kobayashi M, Amagai M, Kuroda-Kinoshita K, Hashimoto T, Shirakata Y, Hashimoto K, Nishikawa T (2002) BP180 ELISA using bacterial recombinant NC16a protein as a diagnostic and monitoring tool for bullous pemphigoid. J Dermatol Sci 30:224– 232
6) Leyendeckers H, Tasanen K, Bruckner-Tudermann L, Zillikens D, Sitaru C, Schmitz J, Hunzelmann N (2003) Memory B cells specific for the NC16A domain of the 180 kDa bullous pemphigoid autoantigen can be detected in peripheral blood of bullous pemphigoid patients and induced in vitro to synthesize autoantibodies. J Invest Dermatol 120:372–378
7) Dopp R, Schmidt E, Shimanovitch I, Leverkus M, Brocker EB, Zillikens D (2000) 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 42:577–583
8) Bernard P, Aucouturier P, Denis F, Bonnetblanc JM (1990) Immunoblot analysis of IgG subclasses of circulating antibodies in bullous pemphigoid. Clin Immunol Immunopathol 54:484– 494
9) Laffitte E, Skaria M, Jaunin F, Tamm K, Saurat JH, Favre B, Borradori L (2001) 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. Bri J Dermatol 144:760–768
10) Liu Z, Diaz LA, Troy JL, Taylor AF, Emery DE, Fairley JA, Giudice GJ (1993) A passive transfer model of the organ-specific autoimmune disease, bullous pemphigoid, using antibodies generated against the hemidesmosomal antigen, BP180. J Clin Invest 92:2480–2488
11) Sitaru C, Schmidt E, Petermann S, Munteanu LS, Brocker EB, Zillikens D (2002) Autoantibodies to bullous pemphigoid antigen 180 induce dermal-epidermal separation in cryosections of human skin. J Invest Dermatol 118:664–671
12) Chorzelski TP, Jablonska S, Beutner EH, Maciejowska E, Jarzabek-Chorzelska M (1976) Herpes gestationis with identical lesions in the newborn. Passive transfer of the disease? Arch Dermatol 112:1129–1131
13) Katz A, Minto JO, Toole JW, Medwidsky W (1977) Immunopathologic study of herpes gestationis in mother and infant. ArchDermatol 113:1069–1072
14) Moncada B, Kettelsen S, Hernandez-Moctezuma JL, Ramirez F (1982) Neonatal pemphigus vulgaris: role of passively transferred pemphigus antibodies. Br J Dermatol 106:465–467
15) Wasserstrum N, Laros RK (1983) Transplacental transmission of pemphigus. JAMA 249:1480–1482
16) Hup JM, Bruinsma RA, Boersma ER, de Jong MC (1986) Neonatal pemphigus vulgaris: transplacental transmission of antibodies. Pediatr Dermatol 3:468–472
17) Ruach M, Ohel G, Rahav D, Samueloff A (1995) Pemphigus vulgaris and pregnancy. Obstet Gynecol Surv 50:755–760
18) Chowdhury MM, Natarajan S (1998) Neonatal pemphigus vulgaris associated with mild oral pemphigus vulgaris in the mother during pregnancy. Br J Dermatol 139:500–503
19) Chen SH, Chopra K, Evans TY, Raimer SS, Levy ML, Tyring SK (1999) Herpes gestationis in a mother and child. J Am Acad Dermatol 40:847–849

20) Büdinger L, Borradori L, Yee C, Eming R, Ferencik S, GrosseWilde H, Merk HF, Yancey K, Hertl M (1998) Identification and characterization of autoreactive T cell responses to bullous pemphigoid antigen 2 in patients and healthy controls. J Clin Invest 102:2082–2089
21) Haase C, Büdinger L, Borradori L, Yee C, Merk HF, Yancey K, Hertl M (1998) 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 110:282–286
22) Sams WM Jr., Gleich GJ (1971) Failure to transfer bullous pemphigoid with serum from patients. Proc Soc Exp Biol Med 136:1027–1031
23) Anhalt GJ, Diaz LA (1987) Animal models for bullous pemphigoid. Clin Dermatol 5:117–125 55. Anhalt GJ, Bahn CF, Labib RS, Voorhees JJ, Sugar AA, Diaz LA (1981) Pathogenic effects of bullous pemphigoid autoantibodies on rabbit corneal epithelium. J Clin Invest 68:1097–1101
24) Anhalt GJ, Bahn CF, Labib RS, Voorhees JJ, Sugar A, Diaz LA (1981) Pathogenic effects of bullous pemphigoid autoantibodies on rabbit corneal epithelium. J Clin Invest 68:1097–1101
25) Hall RPI, Murray JC, McCord MM, Rico JM, Streilein RD (1993) Rabbits immunized with a peptide encoded by the 230-kd bullous pemphigoid antigen cDNA develop an enhanced inflammatory response to UVB irradiation: a potential animal model for bullous pemphigoid. J Invest Dermatol 101:9–14
26) Ghohestani R (1998) Pathogenic effects of auto-antibodies in skin diseases. In: Nicolas JF, Thivolet J (ed) Immunodermatology, pp 185–200. John Libbey Eurotext, Paris
27) Korman NJ (1995) In situ-bound antibodies eluted from the skin of patients with bullous pemphigoid are preferentially directed against the 230-kD bullous pemphigoid antigen. J Invest Dermatol 105:824–830
28) Kiss M, Husz S, Janossy T, Marczinovits I, Molnar J, Korom I, Dobozy A (2005) Experimental bullous pemphigoid generated in mice with an antigenic epitope of the human hemidesmosomal protein BP230. J Autoimmun 24:1–10
29) Thoma-Uszynski S, Uter W, Schwietzke S, Schuler G, Borradori L, Hertl M (2006) Autoreactive T and B cells from bullous pemphigoid (BP) patients recognize epitopes clustered in distinct regions of BP180 and BP230. J Immunol 176:2015–2023
30) Lin MS, Fu CL, Giudice GJ, Olague-Marchan M, Lazaro AM, Stastny P, Diaz LA (2000) Epitopes targeted by bullous pemphigoid T lymphocytes and autoantibodies map to the same sites on the bullous pemphigoid 180 ectodomain. J Invest Dermatol 115:955–961
31) Romagnani S (1992) Human Th1 and Th2 subsets: regulation of differentiation and role in protection and immunopathology. Int Arch Allergy Appl Immunol 98:279–285
32) Bernard P, Aucouturier P, Denis F, Bonnetblanc JM (1990) Immunoblot analysis of IgG subclass of circulating antibodies in bullous pemphigoid. Clin Immunol Immunopath 54:484–494
33) D' Auria L, Cordiali Fei P, Ameglio F (1999) Cytokines and bullous pemphigoid. Eur Cytokine Netw 10:123–134
34) Gammon WR, Merritt CC, Lewis DM, Sams WM Jr, Wheeler CE Jr, Carlo J (1981) Leukocyte chemotaxis to the dermal– epidermal junction of human skin mediated by pemphigoid antibody and complement: mechanism of cell attachment in the in vitro leukocyte attachment method. J Invest Dermatol 76:514– 522
35) Provost TT, Tomasi TB Jr (1973) Evidence for complement activation via the alternative pathway in skin diseases. Herpes gestationis, systemic lupus erythematosus and bullous pemphigoid. J Clin Invest 52:1779–1787
36) Jordon RE, Heine KG, Tappeiner G, Bushkell LL, Provost TT (1976) The immunopathology of herpes gestationis. Immunofluorescent studies and characterization of the "HG factor". J Clin Invest 57:1426–1433
37) Katz SI, Hertz KC, Yaoita H (1976) Herpes gestationis. Immunopathology and characterization of the HG factor. J Clin Invest 57:1434–1441
38) Kelly SE, Cerio R, Bhogal BS, Black MM (1989) The distribution of IgG subclasses in pemphigoid gestationis: PG factor is an IgG1 autoantibody. J Invest Dermatol 92:695–698
39) Suzuki M, Harada S, Yaoita Y (1992) Purification of bullous pemphigoid IgG subclasses and their capability for complement fixation. Acta Derm Venerol (Stockh) 72:245–249

40) Schmidt-Ullrich B, Rule A, Schaumburg-Lever G, Leblanc C (1975) Ultrastructural localization of in vivo-bound complement in bullous pemphigoid. J Invest Dermatol 65:217–219
41) Jordon RE, Kawana S, Fritz KA (1985) Immunopathologic mechanisms in pemphigus and pemphigoid. J Invest Dermatol 85:72–78
42) Jordon RE, Day NK, Sam WM Jr, Good RA (1973) The complement system in bullous pemphigoid. Complement and component levels in sera and blister fluids. J Clin Invest 52:1207–1214
43) Liu Z, Giudice GJ, Swartz SJ, Fairley JA, Till GO, Troy JL, Diaz LA (1995) The role of complement in experimental bullous pemphigoid. J Clin Invest 95:1539–1544
44) Wintroub BU, Mihm MC Jr, Goetzl EJ, Soter NA, Austen KF (1978) Morphologic and functional evidence for release of mastcell products in bullous pemphigoid. N Engl J Med 298:417–421
45) Dvorak AM, Mihm CM, Osage JE, Kwan TH, Austen KF, Wintroub BU (1982) Bullous pemphigoid, an ultrastructural study of the inflammatory response: eosinophil, basophil and mast cell granule changes in multiple biopsies from one patient. J Invest Dermatol 78:91–101
46) Maynard B, Peters MS, Butterfield JH, Leiferman KM (1990) Bullous pemphigoid: eosinophil, neutrophil and mast cell degranulation in lesional tissue. J Invest Dermatol 94:A553
47) Borrego L, Maynard B, Peterson EA, George T, Iglesias L, Peters MS, Newman W, Gleich GJ, Leiferman KM (1996) Deposition of eosinophil granule proteins precedes blister formation in bullous pemphigoid. Comparison with neutrophil and mast cell granule proteins. Am J Pathol 148:897–909
48) Chen R, Ning G, Zhao M-L, Fleming MG, Diaz LA, Werb Z, Liu Z (2001) Mast cells play a key role in neutrophil recruitment in experimental bullous pemphigoid. J Clin Invest 108:1151–1158
49) Dimson OG, Giudice GJ, Fu CL, Van den Bergh F, Warren SJ, Janson MM, Fairley JA (2003) Identification of a potential effector function for IgE autoantibodies in the organ-specific autoimmune disease bullous pemphigoid. J Invest Dermatol 120:784–788
50) Fairley JA, Fu CL, Giudice GJ (2005) Mapping the binding sites of anti-BP180 immunglobulin E autoantibodies in bullous pemphigoid. J Invest Dermatol 125:467–472
51) Chen R, Fairley JA, Zhao M-L, Giudice ML, Zillikens D, Diaz LA, Liu Z (2002) 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 169:3987–3992
52) Muller-Eberhard HJ (1988) Molecular organization of the complement system. Annu Rev Biochem 57:321–347
53) Galli SJ, Gordon JR, Wershil BK (1991) Cytokine production by mast cells and basophils. Curr Opin Immunol 3:865–872
54) Galli SJ (1993) New concepts about the mast cell. N Engl J Med 328:257–265
55) Baba T, Sonozyki H, Seki K, Uchiyama M, Ikesawa Y, Torisu M (1976) An eosinophil chemotactic factor present in blister fluids of bullous pemphigoid patients. J Immunol 116:112–116
56) Katayama I, Doi T, Nishioka K (1984) High histamine level in the blister fluid of bullous pemphigoid. Arch Dermatol Res 276:126–127
57) Kawana S, Ueno A, Nishiyama S (1990) 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 Venerol 70:281–285
58) Grando SA, Glukhenky BT, Drannik GN, Epshtein EV, Kostromin AP, Korostash AT (1989) Mediators of inflammation in blister fluids from patients with pemphigus vulgaris and bullous pemphigoid. Arch Dermatol 125:925–930
59) Takiguch Y, Kamiyama O, Saito E, Nagao S, Kaneko F, Minagawa T (1989) Cell-mediated immune reaction in the mechanism of blister formation in bullous pemphigoid. Dermatologica 179:137
60) Endo H, Iwamoto I, Fujita M, Okamoto S, Yoshida S (1992) Increased immunoreactive interleukin-5 levels in blister fluids of bullous pemphigoid. Arch Dermatol Res 284:312–314
61) Schmidt E, Ambach A, Bastian B, Bröcker E-B, Zillikens D (1996) Elevated levels of interleukin-8 in blister fluid of bullous pemphigoid compared with suction blisters of healthy controls. J Am Acad Dermatol 34:310–312

62) Schmidt E, Bastian B, Dummer R, Tony HP, Bröcker E-B, Zillikens D (1996) Detection of elevated levels of IL-4, IL-6, and IL-10 in blister fluid of bullous pemphigoid. Arch Dermatol Res 288:353–357
63) He S, Peng Q, Walls AF (1997) Potent induction of neutrophil and eosinophil-rich inflitrate in vivo by human mast cell tryptase: selective enhancement of eosinophil recruitment by histamine. J Immunol 159:6216–6225
64) Huang C, Friend DS, Qiu WT, Wong GW, Morales G, Hunt J, Stevens RL (1998) Induction of a selective and persistent extravasation of neutrophils into the peritoneal cavity by tryptase mouse mast cell protease 6. J Immunol 160:1910–1919
65) Compton SJ, Cairns JA, Holgate SH, Walls AF (1998) The role of mast cell tryptase in regulating endothelial cell proliferation, cytokine release, and adhesion molecule expression: tryptase induces expression of mRNA for IL-1β and IL-8 and stimulates the selective release of IL-8 from human umbilical vein endothelial cells. J Immunol 16:1939–1946
66) Sitaru C, Schmidt E, Petermann S, Munteanu LS, Brocker EB, Zillikens D (2002) Autoantibodies to bullous pemphigoid antigen 180 induce dermal-epidermal separation in cryosections of human skin. J Invest Dermatol 118:664–671
67) Shimanovich I, Mihai S, Oostingh GJ, Ilenchuk TT, Brocker EB, Opdenakker G, Zillikens D, Sitaru C (2004) Granulocyte-derived elastase and gelatinase B are required for dermal-epidermal separation induced by autoantibodies from patients with epidermolysis bullosa aquisita and bullous pemphigoid. J Pathol 204:519–527
68) Liu Z, Giudice GJ, Zhou X, Swartz SJ, Troy JL, Fairley JA, Till GO, Diaz LA (1997) A major role for neutrophils in experimental bullous pemphigoid. J Clin Invest 100:1256–1263
69) Springer TA (1994) Traffic signals fo lymphocyte recirculation and leukocyte emigration: the multistep paradigm. Cell 76:301– 314
70) Liu Z, Shipley JM, Vu TH, Zhou X, Diaz LA, Werb Z, Senior RM (1998) Gelatinase B-deficient mice are resistant to experimental BP. J Exp Med 188:475–482
71) Liu Z, Shapiro SD, Zhou X, Twining SS, Senior RM, Giudice GJ, Fairley JA, Diaz LA (2000) A critical role for neutrophil elastase in experimental bullous pemphigoid. J Clin Invest 105:113–123
72) Liu Z, Zhou X, Shapiro SD, Shipley JM, Twining SS, Diaz LA, Senior RM, Werb Z (2000) The serpin α1-proteinase inhibitor is a critical substrate for gelatinase B/MMP-9 in vivo. Cell 102:647–655
73) Liu Z, Zhao M, Li N, Diaz LA, Mayadas TN (2006) Differential roles for β2 integrins in experimental autoimmune bullous pemphigoid. Blood 107:1063–1069
74) Yamatomo K, Inoue N, Masuda R, Fujimori A, Saito T, ImajohOhmi S, Shinkai H, Sakiyama H (2002) Cloning of hamster type XVII collagen cDNA, and pathogenesis of anti-type XVII collagen antibody and complement in hamster bullous pemphigoid. J Invset Dermatol 118:485–492
75) Dubertret L, Bertaux B, Fosse M, Touraine R (1980) Cellular events leading to blister formation in bullous pemphigoid. Br J Dermatol 103:615–624
76) Iryo K, Tsuda S, Sasai Y (1992) Ultrastructural aspects of infiltrated eosinophils in bullous pemphigoid. J Dermatol 19:393–399
77) Bushkell LL, Jordon RE (1983) Bullous pemphigoid: a cause of peripheral blood eosinophilia. J Am Acad Dermatol 8:648–651
78) Wakugawa M, Nakamura K, Hino H, Toyama K, Hattori N, Okochi H, Yamada H, Hira K, Tamaki K, Furue M (2000) Elevated levels of eotaxin and interleukin-5 in blister fluid of bullous pemphigoid: correlation with tissue eosinophilia. Br J Dermatol 143:112–116
79) D'Auria L, Pietravalle M, Mastroianni A, Ferraro C, Mussi A, Bonifati C, Giacalone B, Ameglio F (1998) IL-5 levels in the serum and blister fluid of patients with bullous pemphigoid: correlations with eosinophil cationic protein, RANTES, IgE and disease severity. Arch Dermatol Res 290:25–27
80) Frezzolini A, Teofoli P, Cianchini G, Barduagni S, Ruffelli M, Ferranti G, Puddu P, Pita OD (2002) Increased expression of eotaxin and its specific receptor CCR3 in bullous pemphigoid. Eur J Dermatol 12:27–31
81) Czech W, Schaller J, Schopf E, Kapp A (1993) Granulocyte activation in bullous diseases: release of granular proteins in bullous pemphigoid and pemphigus vulgaris. J Am Acad Dermatol 29:210–215

82) Oikarinen AI, Zone JJ, Ahmed AR, Kiistala U, Uitto J (1983) Demonstration of collagenase and elastase activities in blister fluids from bullous skin diseases. Comparison between dermatitis herpetiformis and bullous pemphigoid. J Invest Dermatol 81:261–266
83) Welgus HG, Bauer EA, Stricklin GP (1986) Elevated levels of human collagenase inhibitor in blister fluids of diverse etiology. J Invest Dermatol 87:592–596
84) Grando SA, Glukhenky BT, Drannik GN, Kostromin AP, Chernyavsky AL (1989) Cytotoxic proteinases in blister fluid of pemphigus an pemphigoid patients. Int J Tissue React 11:195–201
85) Grando SA, Glukhenky BT, Drannik GN, Epshtein EV, Kostromin AP, Korostash TA (1989) Mediators of inflammation in blister fluids from patients with pemphigus vulgaris and bullous pemphigoid. Arch Dermatol 125:925–930
86) Gissler HM, Simon MM, Kramer MD (1992) Enhanced association of plasminogen/plasmin with lesional epidermis of bullous pemphigoid. Br J Dermatol 12:272–277
87) Jensen PJ, Baird J, Morioka S, Lessin S, Lazarus GS (1988) Epidermal plasminogen activator is abnormal in cutaneous lesions. J Invest Dermatol 90:777–782
88) Kramer MD, Reinartz J (1993) The autoimmune blistering skin disease bullous pemphigoid. The presence of plasmin/α2- antiplasmin complexes in skin blister fluid indicates plasmin generation in lesional skin. J Clin Invest 92:978–983
89) Ståhle-Bäckdahl M, Inoue M, Giudice GJ, Parks WC (1994) 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 93:2022–2030
90) Niimi Y, Pawankar R, Kawana S (2006) Increased expression of matrix metalloproteinase-2, matrix metalloproteinase-9 and matrix metalloproteinase-13 in lesional skin of bullous pemphigoid. Int Arch Allergy Immunol 139:104–113
91) Verraes S, Hornebeck W, Polette M, Borradori L, Bernard P (2001) Respective contribution of neutrophil elastase and matrix metalloproteinase 9 in the degradation of BP180 (type XVII collagen) in human bullous pemphigoid. J Invest Dermatol 117:1091–1096
92) Liu Z, Li N, Diaz LA, Shipley M, Senior RA, Werb Z (2005) Synergy between a plasminogen cascade and MMP-9 in autoimmune disease. J Clin Invest 115:879–887
93) Schmidt E, Wehr B, Tabengwa EM, Reimer S, Brocker EB, Zillikens D (2004) Elevated expression and release of tissuetype, but not urokinase-type, plasminogen activator after binding of autoantibodies to bullous pemphigoid antigen 180 in cultured human keratinocytes. Clin Exp Immunol 135:497–504
94) Coussens LM, Raymond WW, Bergers G, Laig-Webster M, Behrendtsen O, Werb Z, Caughey GH, Hanahan D (1999) Inflammatory mast cells up-regulate angiogenesis during squamous epithelial carcinogenesis. Genes Dev 13:1382–1397
95) Bird P, Friedmann PS, Ling N, Bird AG, Thompson RA (1986) Subclass distribution of IgG autoantibodies in bullous pemphigoid. J Invest Dermatol 86:21–25
96) Bernard P, Aucouturier P, Denis F, Bonnetblanc JM (1990) Immunoblot analysis of IgG subclasses of circulating antibodies in bullous pemphigoid. Clin Immunol Immunopathol 54:489– 494
97) Mihai S, Herrero-Gonzales J, Goodall M, Jefferis R, Savage CO, Zillikens D, Sitaru C (2005) Non-complement fixing IgG4 autoantibodies from bullous pemphigoid patients activate leukocytes and induce blisters in cryosections of human skin. J Invest Dermatol 125:A45
98) Schmidt E, Reimer S, Kruse N, Jainta S, Brocker EB, Marinkovich MP, Giudice GJ, Zillikens D (2000) Autoantibodies to BP180 associated with bullous pemphigoid release interleukin-6 and interleukin-8 from cultured human keratinocytes. J Invest Dermatol 115:842–848