Available online at www.sciencedirect.com
SCIENCE DIRECT
ELSEVIER
JNMU
Journal of Nanjing Medical University, 2007, 21(3): 129-133
www.elsevier.com/locate/jnmu

Review

Recent advancement on autoantigens, autoantibodies and inflammatory cells in subepidermal autoimmune bullous diseases

Jianguo Huang[a,*], Qiying Gong[a], Guiming Li[b]

[a]*Department of Dermatology, the Affiliated Huadu Hospital of Southern Medical University, Guangzhou 510800, China;*

[b]*Department of Dermatology, the First Affiliated Hospital of Chongqing University of Medical Science, Chongqing 410006, China*

Received 5 November 2006

Abstract

Subepidermal autoimmune bullous diseases (SABD) are some autoimmune skin diseases that can present in a variety of forms and can be a challenging disease to treat. An overview of the different forms of SABD are discussed including bullous pemphigoid (BP), epidermolysis bullosa acquisita (EBA), cicatricial pemphigoid (CP), bullous systemic lupus erythematosus (BSLE), and Anti-p200 pemphigoid. Emphasis on recent advancement is presented. In recent years, improved knowledge of the mechanisms of intercellular and cell-matrix adhesion has led to better understanding of the blistering process in some SABD. Defects of such structures cause the subepidermal bullous diseases and have also led to the discovery of new diseases (e.g. anti-p200-pemphigoid). Recent studies have outlined the important role of autoantibodies, mast cell lymphocytes and their cytokines in pathogenesis of SABD.

Keywords: subepidermal autoimmune bullous diseases; autoantigens; autoantibodies; inflammatory cells

BACKGROUND

Subepidermal autoimmune bullous diseases (SABD) encompass some disorders, such as bullous pemphigoid (BP) and its variants, epidermolysis bullosa acquisita (EBA), cicatricial pemphigoid (CP), pemphigoid gestationis(PG; or herpes gestationis, HG), linear IgA(bullous) dermatosis (LAD, or LABD), dermatitis herpetiformis (DH), bullous systemic lupus erythematosus (BSLE)[1,2]. They are characterized clinically by the presence of cutaneous and on immunofluorescence (IMF) by the deposition of immunoglobulins deposits in the dermal papillae. Indirect immunofluorescence(IIF) on serum is a routine test for the detection of basement membrane zone antibodies[1,2].

AUTOANTIBODIES AND AUTOANTIGENS

Mulyowa *et al*[1] suggested that the age of patients with autoimmune subepidermal blistering diseases appeared to influence the immunoglobulin class of autoantibodies. In patients with subepidermal blistering diseases, IgG reactivity correlated significantly with old age, whereas younger patients preferentially developed IgA autoantibodies.The high frequency of IgA autoantibodies in Ugandan patients may be explained by the age distribution of the Ugandan population.

Autoantibodies and autoantigens in BP

Bullous pemphigoid (BP) is an acquired autoimmune skin disease characterized by autoantibodies against two hemidesmosomal antigens,BP230 (BPAG1) and BP180(BPAG2). BP antihemidesmosomal autoantibodies can bind to the dermal-epidermal junction and activate the complement system [3]. The majority of BP sera react with epitopes within the NC16A domain of BP180[3]. The serum levels of autoantibodies to BP180 NC16A are correlated with the severity of BP. In addition, NC16A may be detectable at an early clinical stage [2]. NC16A is a 77-

*Corresponding author.

E-mail address: hngjianguo@yahoo.com

amino-acid membrane-proximal noncollagenous region of BP180 .The autoantibodies are usually IgG and IgA, of which IgG4 is the most common subtype[2]. These NC16A-responding T lymphocytes express alpha/beta T cell receptors and CD4 memory T cell surface markers and exhibit a Th1/Th2 mixed cytokine profile. These data demonstrate that antibodies specific for the BP180NC16A domain are pathogenic. It is clear that the binding of anti-BP180 antibody to its target is the first critical step in subepidermal blister formation in BP[3].

Kiss *et al* [4] suggested that BPAG1 had at least three major forms of BPAG1:BPAG1-e (302 kD), BPAG1-a(615 kD) and BPAG1-b (834 kD), which seemed to play an important role in linking the different types of cytoskeletons. Recent findings demonstrate that antibodies against BP230 can elicit the clinical and immunopathological features of BP in neonatal mice, and the fragment representing the C-terminal portion of BP230 was by far the most frequent target within the molecule [5], suggesting that anti-BP230 antibodies may possibly play a pathogenic role in this disease[5].

In the present study researchers presented five patients with erythematous plaques, subepidermal blister formations of the skin, and the presence of circulating autoantibodies directly against a so far unrecognized 190 kDa antigen in human keratinocytes[6]. Amino acid sequence analysis identified the protein as IQGAP1, and the protein is selectively recognized by a monoclonal anti-IQGAP1 antibody on western blots and immunoprecipitates from keratinocyte extracts [6]. Indirect immunofluorescence located IQGAP1 within individual keratinocytes in a cytoplasmic pattern and along the cell periphery at adhesive sites [6]. These results demonstrate IQGAP1, a newly described multifunctional protein, to be constitutively expressed in human keratinocytes where it may contribute to the integrity of the epidermal layer. Furthermore, researchers found autoantibodies reacting with IQGAP1 in patients with bullous skin eruptions most apparently belonging to the spectrum of BP[6].

Apart from BP1 (230) and BP2 (180), there are more autoantigens in BP. Anti-p200 pemphigoid is an SABD characterized by autoantibodies against a 200-kDa protein (p200) of the dermal-epidermal junction (DEJ) [7]. Most recently p200 has been demonstrated to be distinct from all major DEJ autoantigens and is thought to be important for cell-matrix adhesion[7]. Two-dimensional gel electrophoresis demonstrated that p200 was an acidic protein with an isoelectric point of 5.4 to 5.6. Six different p200-specific sera recognized an identical protein spot of two-dimensionally separated dermal extracts, confirming that patients with this novel autoimmune disease indeed have a single pathobiochemical change[7]. The present research concludes that in anti-p105 pemphigoid, p105 is an unique basement membrane component produced by keratinocytes and fibroblasts. The morbidity of Anti-p450 pemphigoid is very rare[8].

Autoantibodies and autoantigens in CP

Anti-laminin 5 cicatricial pemphigoid (CP) is a mucosal-dominant subepithelial blistering disease characterized by IgG anti-basement membrane zone (anti-BMZ) autoantibodies, which bind to dermal side split skin of 1 mol/L NaCl and immunoprecipitate laminin 5. Laminin 5 is an epidermis-specific extracellular matrix consisting of alpha3, beta3 and gamma2 subunits[9]. Mutations in genes of laminin5, which result in the loss of the protein, cause epidermolysis bullosa Herlitz. Recent studies suggest that autoantibodies of anti-laminin 5 CP recognize the G domains of alpha3 subunit[10].

Bhol *et al's*[11] observations indicate that the ocular cicatricial pemphigoid (OCP) autoantibody recognizes only the intracellular domain of human β4 integrin and not the extracellular domain. In subsequent studies researchers used several clones representing fragments of the intracellular portions of β4 (IC 1.0, IC 2.0, IC 3.0, IC 3.4). Results indicated that, for Mucous membrane pemphigoid (MMP) sera, the dominant antibody binding epitope was IC 3.4 (83 amino acids). Normal human sera, preimmune rabbit sera, and sera from patients with pemphigus vulgaris did not bind to this fragment of β4 integrin.Present observations strongly suggest that anti-BMZ antibodies in the sera of MMP patients target the same peptide in the intracellular portions of β4 (IC 3.4) as do autoantibodies in OCP[11].

Haas *et al* [12] showed that some antiepiligrin cicatricial pemphigoid(AECP) complicated by some internal malignancies (5 of 16 cases, 31.2%). Non-small cell carcinoma of the lung and oral squamous cell carcinomas are found to have a focal loss of laminin-5 in conjunction with a loss of the cellular receptor, and increased synthesis of laminin-5 by budding tumor cells has been observed along with deposition of the protein in the stroma. The patients with AECP have an increased relative risk for solid cancer (lung, colon, mouth, kidney, and urinary

bladder, etc.) rather than lymphomas or leukaemias, especially in the first year after blister onset. The majority of cancers were adenocarcinomas. This circumstance is thought to account for a high incidence of mortality among AECP patients who develop an associated cancer. The researchers calculated the expected numbers of cancers in a cohort of 35 such patients based on respective incidence rates of all cancers in the National Cancer Institute´s Surveillance, Epidemiology, and End Results (NCI SEER) Registry. Ten patients in this cohort had solitary solid cancers; eight patients developed cancer after onset of AECP (seven within 14 months)[13]. The relative risk(RR) for cancer in this cohort was 6.8 (95% confidence intervals [CI]: 3.3-12.5)[13]. AECP seems to be associated with an increased relative risk for cancer [13]. Immunoblotting studies demonstrated the presence of antibodies against the alpha3 and the gamma2 subunit of laminin 5 [14]. Hence, the new concept about Paraneoplastic CP is described[14].

In addition, the present findings demonstrate that autoantibodies in CP target epitopes on both extra and NC16A domains of BP180, and highlight the importance of testing for both IgG and IgA reactivity in these patients´ sera[15].

Autoantibodies and autoantigens in EBA

Jonkman *et al*[16] suggested that induction of complement-fixing autoantibodies against type VII collagen resulted in subepidermal blistering of EBA in mice. In susceptible animals, deposits of IgG1, IgG2 and complement C3,were detected at the dermal-epidermal junction. In contrast, in the nondiseased mice, tissue-bound autoantibodies were predominantly of the IgG1 subclass, and complement activation was weak or absent.

The sera of EBA react with type VII collagen, a major component of anchoring fibrils, in which the major epitopes have been considered to be present in the N-terminal noncollagenous (NC) 1 domain. Recently studies clearly identified the presence of epitopes in the N-terminal noncollagenous (NC) 2 domain, and shows that the epitope in the NC1 domain was in the lamina densa and the pitope in the NC2 domain was in the dermis below the lamina densa[16].

The current studies revealed the simultaneous presence of circulating IgG autoantibodies against type VII collagen and laminin alpha3. This case illustrates that the clinical and immunological overlap between EBA and anti-epiligrin cicatricial pemphigoid, a unique finding that may have developed as a consequence of epitope spreading[17].

CELLS MEDIATING SABD

Neutrophils

Numerous inflammatory cells infiltrate the upper dermis in BP. In the present study demonstrated that the relative contribution of neutrophils, mast cells (MCs), macrophages (Mphi), lymphocytes and their functional relationship in the immunopathogenesis of this disease model by using mice deficient in these cells [18]. Wild-type, T cell-deficient, and T and B cell-deficient mice injected intradermally with pathogenic anti-murine BP180 IgG ,exhibited extensive subepidermal blisters. In contrast, mice deficient in neutrophils, MCs and Mphi were resistant to experimental BP [18]. These findings provide the first direct evidence to our knowledge that MC's play an essential role in neutrophil recruitment during subepidermal blister formation in experimental BP[18]. Furthermore, Mphi-mediated neutrophil infiltration depends on MC activation or degranulation[18]. Antihuman BP180NC16A antibodies and neutrophils are responsible for this tissue injury .

Gelatinase B (MMP-9, 92 kD gelatinase) is present in BP blister fluid and can cleave BP180. However, gelatinase B-deficient mice reconstituted with neutrophils from normal mice, developed blistering in response to anti-mBP180 antibodies [19]. The pre sent study results implicate neutrophil-derived gelatinase B in the pathogenesis of experimental BP and might lead to novel therapeutic strategies for BP[19]. Neutrophil elastase (NE) directly in the dermal-epidermal cleavage was induced by anti-BP180 antibodies in the experimental BP model[20].

Mast cell

At present, the possible involvement of mast cell tryptase and chymase in subepidermal bullous diseases was studied enzyme-histochemically in specimens from erythematous and vesicular skin and from non-involved skin of patients with DH, BP, erythema multiforme, infective bullous eruption and LAD[21]. These research results showed significant alterations in mast cell chymase and protease inhibitors in many different bullous diseases, suggesting mast cell involvement [21]. The apparent inactivation of chymase could be due to the action of chymase inhibitors detected in numerous mast cells. However, these alterations probably reflect general inflammation rather th an a specific reaction in a certain bullous disease[21].

B cell

More research in this field shows that B cell expression of CD22 was 20% lower in BP patients

when compared to healthy control subjects. In addition, B cells from BP patients showed decreased expression of L-selectin, which is an indicator of leukocyte activation, and CD22 expression levels were correlated with L-selectin expression. These results suggested that the decreased CD22 expression might be associated with the activation of B cells in BP and might not be associated with BP-specific antibody production[21].

CYTOKINES

In current investigations, on the basis of the constant presence of TGF-β1 mRNA in the different lesion phases of CP, and its overlapping expression in BP. The dermatologist hypothesize that the involvement of additional factors is responsible for the scarring course typical of CP [22]. Theses results suggest that BP is a unique organ-specific autoimmune disease characterized by an expansion of skin-homing interleukin-13-producing cells. In addition, corticosteroids may control such type 2 biased inflammatory responses in bullous pemphigoid by promoting the expansion of interleukin-10-producing cells[23].

A specific function for each cytokine in bullous pemphigoid induction still cannot be defined. However, on the basis of significant (direct or inverse) correlations found between disease intensity and the blister fluid/serum levels; such as the following cytokines IL-7 and IL-15, regulated upon activation normal T cell, expressed and presumably secreted (RANTES), vascular endothelial growth factor (VEGF) and tumor necrosis factor-alpha (TNF-alpha), may be considered relevant to this disease[24].

A study showed that BP-associated autoantibodies to the human BP180 ectodomain triggered a signal transducing event that lead to expression and secretion of interleukin-6 and interleukin-8 from human keratinocytes [25]. The N-terminal sequence of the 120-kDa fragment of BP180 will help to further dissect the physiologic and pathologic relevance of the cleavage process[26].

ANTI-CD20 (RITUXIMAB) AND ANTI-CD25 (DACLIZUMAB) THERAPY

Szabolcs *et al*[27] reported: in one case(a 10-year-old boy) monitoring the BP-specific circulating antibodies and CD25-expressing activated T lymphocyte subset, which led researchers to combine anti-CD20 (Rituximab) mediated B cell ablation with anti-CD25 (Daclizumab) therapy to block $CD4^+$ T cell help. Complete clinical and serologic response was achieved within 4 weeks of initiation of therapy allowing global immunosuppression to be dramatically reduced[27]. Because Anti-CD20 antibody (rituximab) is a chimeric monoclonal antibodies (MoAb), IgG1 kappa antibody that mediates complement-dependent cell lysis and antibody-dependent cellular cytotoxicity [27]. Anti-CD25 antibody interrupted the helper function of CD4+ T cells facilitating the secretion of antibodies against BPAG2 by CD20-plasma cells. This therapy was necessary to achieve a complete clinical response and a decrease in the disease-specific antibody titers. This is the first reported combination treatment of monoclonal antibodies targeting T cell-dependent B cell diseases, such as BP[27].

CONCLUSION

Recent findings should help to elucidate the immunopathologic mechanisms responsible for SABD and may have significant implications in the diagnosis and treatment of these autoimmune diseases [1,28]. In addition, with the elucidation of genetic defects in the different variants of Epidermolysis bullosa (EB), genotype-phenotype correlations research now begin to arise and genetic counseling has been improved. Explaining this phenomenon, which although probably linked to different expressions of MHC, is one in the challenges for the future.

References

[1] Mulyowa GK, Jaeger G, Kabakyenga J, Brocker EB, Zillikens D, Schmidt E. Autoimmune subepidermal blistering diseases in Uganda: correlation of autoantibody class with age of patients. *Int J Dermatol* 2006;45:1047.

[2] Wong SN, Chua SH. Spectrum of subepidermal immunobullous disorders seen at the National Skin Centre, Singapore: a 2-year review. *Br J Dermatol* 2002;147:476-80.

[3] Di Zenzo G, Grosso F, Terracina M, Mariotti F, De Pita O, Owaribe K, *et al*. Characterization of the anti-BP180 autoantibody reactivity profile and epitope mapping in bullous pemphigoid patients. *J Invest Dermatol* 2004;122:103-10.

[4] Kiss M, Husz S, Janossy T, Marczinovits I, Molnar J, Korom I, *et al*. Experimental bullous pemphigoid generated in mice with an antigenic epitope of the human hemidesmosomal protein BP230. *J Autoimmun* 2005;24:1-10.

[5] Kromminga A,Sitaru C,Hagel C,Herzog S,Zillikens D. Development of an ELISA for the detection of autoantibodies to BP230. *Clin Immunol* 2004;111:146-52.

[6] Presslauer S, Hinterhuber G, Cauza K, Horvat R, Rappersberger K, Wolff K, *et al*. RasGAP-like protein IQGAP1 is expressed by human keratinocytes and recognized by autoantibodies in association with bullous skin disease. *J Invest Dermatol* 2003;120:365-71.

[7] Cho SB, Kim SC. A Korean case of anti-p200 pemphigoid. *Yonsei Med J* 2003 30;44:931-4.

[8] Chan LS, Woodley DT. The 105-kDa basement membrane autoantigen p105 is N-terminally homologous to a tumor-associated antigen. *J Invest Dermatol* 1996;107:209-14.

[9] Nousari HC, Rencic A, Hsu R, Yancey KB, Anhalt GJ. Anti-epiligrin cicatricial pemphigoid with antibo dies against the

gamma2 subunit of laminin 5. *Arch Dermatol* 1999 ;135:173-6.
[10] Hisamatsu Y, Nishiyama T, Amano S, Matsui C, Ghohestani R, Hashimoto T. Usefulness of immunoblotting using purified laminin 5 in the diagnosis of anti-laminin 5 cicatricial pemphigoid. *J Dermatol Sci* 2003;33:113-9.
[11] Bhol KC, Colon JE, Ahmed AR. Autoantibody in Mucous Membrane Pemphigoid Binds to an Intracellular Epitope on Human β4Integrin and Causes Basement Membrane Zone Separation in Oral Mucosa in an Organ Culture Model. *Journal of Investigative Dermatology* 2003;120:701.
[12] Haas M, Berndt A, Hyckel P, Stiller KJ, Kosmehl H. Laminin-5 in diseases of the oral cavity. *Mund Kiefer Gesichtschir* 2000; 4:25-9.
[13] Egan CA, Lazarova Z, Darling TN, Yee C, Cote T, Yancey KB. Anti-epiligrin cicatricial pemphigoid and relative risk for cancer. *Lancet* 2001;357:1850-1.
[14] Setterfield J, Shirlaw PJ, Lazarova Z, Bryant BM, Bhogal BS, Harman K, *et al*. Paraneoplastic cicatricial pemphigoid. *Br J Dermatol* 1999;141:127-31.
[15] Schmidt E, Skrobek C, Kromminga A, Hashimoto T, Messer G, Brocker EB,*et al.* Cicatricial pemphigoid: IgA and IgG autoantibodies target epitopes on both intra- and extracellular domains of bullous pemphigoid antigen 180. *Br J Dermatol* 2001; 145:778-83.
[16] Jonkman MF, Schuur J, Dijk F, Heeres K, de Jong MC, van der Meer JB, *et al.* Inflammatory variant of epidermolysis bullosa acquisita with IgG autoantibodies against type VII collagen and laminin alpha3. *Arch Dermatol* 2000;136:227-31.
[17] Leverkus M, Schmidt E, Lazarova Z, Brocker EB, Yancey KB, Zillikens D. Antiepiligrin cicatricial pemphigoid: an underdiagnosed entity within the spectrum of scarring autoimmune subepidermal bullous diseases? *Arch Dermatol* 1999;135:1091-8.
[18] Chen R, Fairley JA, Zhao ML, Giudice GJ, Zillikens D, Diaz LA, *et al.* 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;169:3987-92.
[19] Liu Z, Shipley JM, Vu TH, Zhou X, Diaz LA, Werb Z,*et al*. *Gelatinase B-deficient mice are resistant to experimental bullous pemphigoid. J Exp Med* 1998;188:475-82.
[20] Liu Z, Shapiro SD, Zhou X, Twining SS, Senior RM, Giudice GJ.,*et al*. A critical role for neutrophil elastase in experimental bullous pemphigoid. *J Clin Invest* 2000;105:113-23.
[21] Inaoki M, Echigo T, Hayashi H, Nagaoka T, Hasegawa M, Takehara K,*et al.* Decreased expression levels of CD22 and L-selectin on peripheral blood B lymphocytes from patients with bullous pemphigoid. *J Autoimmuno* 2006;27:196-202.
[22] Caproni M, Calzolari A, Giomi B, Santucci M, Ficarra G, Fabbri P.. IL-4, IL-5, TGF-beta1 and IFN-gamma mRNAs detected by a new in situ amplification system in cicatricial pemphigoid. *Exp Dermatol* 2002;11:421-7.
[23] Yamamoto K, Inoue N, Masuda R, Fujimori A, Saito T, Imajoh-Ohmi S., *et al.* 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;118:485-92.
[24] D´Auria L, Cordiali Fei P, Ameglio F. Cytokines and bullous pemphigoid. *Eur Cytokine Netw* 1999;10:123-34.
[25] Sitaru C, Chiriac MT, Mihai S, Buning J, Gebert A, Ishiko A,.*et al*. Induction of complement-fixing autoantibodies against type VII collagen results in subepidermal blistering in mice. *J Immumol* 2006;177:3461-8.
[26] Hirako Y, Nishizawa Y, Sitaru C, Opitz A, Marcus K, Meyer HE, *et al*. The 97-kDa (LABD97) and 120-kDa (LAD-1) Fragments of Bullous Pemphigoid Antigen 180/Type XVII Collagen Have Different N-Termini. *Journal of Investigative Dermatology* 2003;121:1554.
[27] Szabolcs P, Reese M, Yancey KB, Hall RP, Kurtzberg J. Combination treatment of bullous pemphigoid with anti-CD20 and anti-CD25 antibodies in a patient with chronic graft-versus-host disease. *Bone Marrow Transplant* 2002;30:327-9.
[28] Verdolini R, Cerio R. Autoimmune subepidermal bullous skin diseases: the impact of recent findings for the dermatopathologist. *Virchows Arch* 2003;443:184-93.