Diagnosis and clinical severity markers of bullous pemphigoid

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Abstract

The use of a broad spectrum of novel detection systems for autoantibodies to the basement membrane proteins BP180 and BP230 has greatly facilitated the diagnosis of bullous pemphigoid, which most likely explains its increasing incidence in central Europe. Because the pathogenic relevance of antibodies to human BP180 has been convincingly shown both *in vitro* and *in vivo*, repeated testing for these antibodies appears to be helpful in guiding treatment decisions during the course of the disease.

Introduction and context

Bullous pemphigoid (BP) is the most frequent auto-immune bullous disorder and is clinically characterized by tense blisters and crusts on erythematous or apparently uninvolved skin. Direct immunofluorescence (IF) microscopy of a perilesional skin biopsy that typically reveals linear deposits of complement component 3 (C3) and/or immunoglobulin G (IgG) at the basal membrane zone (BMZ) is still the diagnostic gold standard. The most sensitive substrate for screening of serum autoantibodies in subepidermal blistering autoimmune diseases is 1 molar sodium chloride (1 M NaCl) split human skin. In BP, the circulating IgG autoantibodies label the epidermal side of the artificial split. These autoantibodies are directed against two hemidesmosomal proteins, BP180 and BP230 [1,2]. BP180, also known as type XVII collagen or BP antigen 2, is a transmembrane glycoprotein of about 1500 amino acids which ultrastructurally spans the lamina lucida before kinking back from the lamina densa to the lamina lucida. In contrast, BP230 is an intracellular constituent of the hemidesmosomal plaque and belongs to the plakin family of proteins. In recent years, analysis of the fine specificities of various isotypes of autoantibodies against the two target antigens has led to both better diagnostic tools and new insights into the pathogenicity of autoantibodies in BP.

Recent advances

Diagnosis of bullous pemphigoid

For more than a decade, B-cell epitopes have been known to be unequally distributed on BP180 in BP. The extracellular portion of the 16th non-collagenous (NC16A) domain was shown to be recognized by about 80% of BP sera [3]. It then became obvious that the majority of BP patients also raise antibodies against epitopes outside the NC16A domain [4–9]. Interestingly, IgG reactivity with C-terminal epitopes appeared to be associated with the presence of both skin and mucosal lesions [6–8] whereas the intracellular domain was preferentially targeted at an early clinical stage [8]. Testing for anti-BP180 antibodies outside the NC16A domain therefore is required in those patients unreactive with the immunodominant domain and has been included in our routine work-up. Most recently, the extent of phosphorylation of BP180 was shown to be important for binding of antibodies to its ectodomain [10]. Because antibodies raised against the murine homolog to the NC16A domain, expressed in *Escherichia coli*, induce subepidermal blisters when injected into neonatal mice, the relevance of phosphoepitopes on BP180 needs to be determined.

The other major target antigen in BP, BP230, is recognized in between 60 and 70% of patients by enzyme-linked immunosorbent assay (ELISA)

[4,9,11,12]. As for BP180, B-cell epitopes are not equally distributed but preferentially localize to the globular C-terminal domain of BP230 that mediates interaction with keratin filaments and is targeted by about 80% of BP230-reactive BP sera [9,13,14]. Interestingly, reactivity to BP230 was predominantly found in patients with limited BP, and only less than 5% of BP sera exclusively recognized BP230 [4]. By the combined use of detection systems for anti-BP180 and anti-BP230 antibodies, all 49 BP sera were reactive in a recent multicenter prospective study [9].

The novel diagnostic assays have greatly facilitated the diagnosis of BP (Figure 1), which may explain the increased incidence of BP that was recently observed in a prospective study with patients from a well-defined region, Lower Franconia in Germany. Compared with a study of the same region 10 years earlier, the incidence of BP has increased from 6.6 to 13.4 new cases per million per year [15,16]. An even higher incidence (including all subepidermal bullous diseases) of 43 new cases per million per year, which has increased almost fivefold within the last 10 years, was recently reported in the UK [17]. The data, however, were retrieved from a database for general practitioners and have been challenged by the report that in St. John's Institute of Dermatology, London, the diagnosis of BP, based on direct IF microscopy, has not increased within the last decade [18].

Whereas IgG reactivity to BP180 has been studied intensively, the importance of BP180-specific antibodies of the IgA and IgE isotypes has been highlighted only recently. Indeed, the majority of BP sera contain IgA antibodies to BP180 and most sera of patients with linear IgA disease raise IgG anti-BP180 antibodies in addition to IgA reactivity [19]. There appears to be a spectrum of the autoimmune response against BP180, ranging from pure IgG to pure IgA reactivity, with mixtures in between. This hypothesis is supported by the observation in Uganda that younger patients with BP/linear IgA disease were more likely to develop IgA anti-BMZ antibodies whereas older patients tended to raise IgG autoantibodies [20]. IgE reactivity against BP180 has been reported in 30 to 95% of BP patients [21–25]. The majority of sera contain IgE reactivity to the NC16A domain, and IgE antibodies to the intracellular domain of BP180 have also been detected recently [23,25].

In addition to reactivity against BP180 and BP230, antibodies to the $\alpha 6$ chain of $\alpha 6\beta 4$ integrin and laminin 332, also known as laminin 5, have been reported in 50% and 40% of BP patients, respectively [26,27]. The relevance of these findings is elusive and both findings

Figure 1. Diagnostic pathway in bullous pemphigoid (BP)

Direct immunofluorescence (IF) microscopy of a perilesional biopsy is the gold standard for the diagnosis of BP and differentiates subepidermal blistering autoimmune diseases from pemphigus. By indirect IF microscopy on 1 M NaCl split human skin, BP patients' sera are screened for anti-basal membrane zone (BMZ) autoantibodies. Whereas sera from patients with epidermolysis bullosa acquisita, anti-laminin 332 mucous membrane pemphigoid, and anti-p200 pemphigoid label the dermal side of the artificial split, sera of BP patients bind to the blister roof. Anti-BP180 antibodies can be detected by BP180 NC16A-specific enzyme-linked immunosorbent assay (ELISA), Western blotting with conditioned concentrated medium of cultured HaCaT cells, which detects reactivity against LAD-1 (linear IgA disease antigen 1) that corresponds to the cell-derived ectodomain of BP180, and Western blotting with various other recombinant fragments of BP180. Since four different entities are associated with IgG antibodies to BP180, the clinical phenotype determines the final diagnosis. When no BP180 reactivity is found, sera are assayed for BP230-specific antibodies that, only in conjunction with a positive direct IF microscopy and compatible clinical features support the diagnosis of BP. In case of epidermal binding by indirect IF microscopy and failure to detect IgG reactivity to both BP180 and BP230, testing for antibodies against $\alpha 6\beta 4$ integrin is recommended (for example, by Western blotting of keratinocyte extract) [44]. The shaded boxes represent commercially available test systems. NC16A, the extracellular portion of the 16th noncollagenous (domain).

await confirmation. A more recent report demonstrates that the detection of antibodies to laminin 332 is rather uncommon in BP [28].

Clinical severity markers of bullous pemphigoid

The first evidence for a pathogenic role of human antibodies to human BP180 came from the observation that levels of circulating antibodies to the NC16A domain correlate with disease activity in BP patients [29]. This notion has been corroborated in different patient populations within recent years [30–34]. Thus, the NC16A domain appears to be an important target for monitoring circulating autoantibody levels and for guiding treatment decisions; for example, the reduction of the corticosteroid dose during the course of the disease. By the use of an NC16A tetramer, a second commercially available ELISA that is a highly sensitive and specific tool for the detection of serum antibodies in BP has been established [35]. Autoantibody levels to a C-terminal stretch of BP180 have subsequently been shown to parallel clinical activity in BP [9].

The pathogenic relevance of human antibodies to human BP180 had previously been suggested *in vitro* when the incubation of cultured human keratinocytes and cryosections of human skin with BP IgG resulted in increased mRNA levels followed by the release of proinflammatory cytokines or dermal-epidermal separation, respectively [36,37]. Most recently, a decrease of cell-surface BP180 that led to weakened cell attachment has been observed in response to the incubation of cultured human keratinocytes with anti-BP180 antibodies [38]. In addition, *in vivo* data have been provided by Nishie *et al.* [39], who induced BP-like skin lesions by injection of IgG from BP patients into Col17 knockout mice rescued by the human ortholog.

The importance of IgE autoantibodies to BP180 NC16A has been highlighted by demonstrating a correlation between clinical activity and IgE NC16A-specific circulating antibodies that were detected in 60% of BP patients [21]. Subsequently, IgE antibodies directed against this domain were linked to both a greater extent of skin lesions and a longer time period until remission was induced [23]. These observations have been substantiated in two passive-transfer mouse models. The injection of BP IgE or an IgE-producing hybridoma against the BP180 ectodomain into human skin grafted on to athymic nude mice or SCID (severe combined immunodeficiency disease) mice, respectively, resulted in an erythematous itchy plaque, which showed dermal-epidermal separation by histopathology [40,41]. Whether measurement of IgE anti-BP180 NC16A antibodies will be included in the routine analysis of BP sera needs to be addressed in future studies.

Whereas the pathogenic relevance of antibodies to BP180 is well established, the role of anti-BP230

reactivity is less clear. Serum levels of anti-BP230 antibodies repeatedly failed to parallel disease activity in BP patients [4,9,12]. In addition, experimental evidence for the pathogenicity of anti-BP230 antibodies is inconclusive and needs further exploration.

Implications for clinical practice

Direct IF microscopy of a perilesional biopsy is still regarded as the gold standard in the diagnosis of BP. A large body of evidence, however, has been collected supporting the notion that, in a large majority of BP patients, the disease can be diagnosed serologically by the detection of BP180 NC16A-specific autoantibodies. Rarely, anti-BP180 antibodies have also been found in patients with pruritic disorders, diabetes mellitus, or dementia, even when there have been no associated clinical signs of BP and direct IF microscopy has been negative [42,43]. Usually, however, in patients with clinical lesions typical for BP, the detection of antibodies to BP180 NC16A suffices to establish the diagnosis of BP, whereas serum antibodies to BP230 require a positive direct IF microscopy to diagnose BP. Finally, based on our personal experience, repeated testing for serum anti-BP180 NC16A antibodies during the course of the disease may be a helpful guide for treatment decisions.

Abbreviations

BMZ, basal membrane zone; BP, bullous pemphigoid; C3, complement component 3; ELISA, enzyme-linked immunosorbent assay; IF, immunofluorescence; IgG, immunoglobulin G; NC16A, the extracellular portion of the 16th noncollagenous (domain).

Competing interests

The authors run a non-profit laboratory that is integrated into the Department of Dermatology at the University of Lübeck. This laboratory specializes in the diagnosis of autoimmune bullous diseases and receives sera from all over the world. In this laboratory, the test systems discussed in this report are applied.

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