Pemphigoid diseases: Pathogenesis, diagnosis, and treatment

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Abstract
Pemphigoid diseases (including bullous pemphigoid, mucous membrane pemphigoid, pemphigoid gestationis, linear IgA dermatosis, lichen planus pemphigoides, and anti-p200 pemphigoid) are a subgroup of autoimmune bullous skin diseases characterized by an autoantibody response toward structural components of the hemidesmosome resulting in subepidermal blistering. By the use of different in vitro systems and experimental animal models, the pathogenic relevance of these autoantibodies has been demonstrated. Recent advances in the understanding of autoantibody responses have led to novel diagnostic tools and a more differentiated therapeutic approach for these disorders. This review covers the most recent understanding of the pathophysiology, diagnosis, and treatment of this group of autoimmune diseases.

Keywords: Pemphigoid, skin, antibody, antigen

Introduction
The pemphigoid group of diseases is characterized by subepidermal blisters due to autoantibody-induced disruption of the components of the dermal–epidermal anchoring complex. Pemphigoid diseases include bullous pemphigoid (BP), mucous membrane pemphigoid (MMP), pemphigoid gestationis (PG), linear IgA dermatosis (LAD), lichen planus pemphigoides (LPP), and anti-p200 pemphigoid. In particular, disorders with anti-BP180 (type XVII collagen) reactivity, including BP, PG, LAD, LPP, and a subgroup of MMP may form a continuous spectrum of subepidermal autoimmune blistering dermatoses.

BP is not only the most common disorder within the pemphigoid group, but also represents the most frequent autoimmune blistering disease in general [1,2]. The incidence of BP has been estimated between 4.5 and 14 new cases/million/year in central Europe [1–5]. A higher incidence of 42.8/million/year has recently been reported in Great Britain based on a data registry established on the general practitioner level [6]. Interestingly, in Germany and Great Britain, it has been shown that the incidence of BP has considerably increased within the last 10 years (2-fold and 4.8-fold, respectively) [1,6]. This development may be due to the increasing age of the general population and/or an increased awareness leading to further diagnostic steps. MMP and PG have been identified as the second most frequent pemphigoid diseases in central Europe with an incidence of two new patients/million/year [1].

For the correct diagnosis of pemphigoid diseases, the detection of tissue-bound and circulating autoantibodies is pivotal [7]. Although direct immunofluorescence (IF) microscopy differentiates pemphigus from pemphigoid disorders, serological analyses are necessary to separate pemphigoid diseases from each other and from epidermolysis bullosa acquisita (EBA). In fact, while direct IF microscopy is still the gold standard for the diagnosis of pemphigoid diseases, in the great majority of patients, diagnosis can be made serologically today [7].

By indirect IF microscopy on 1M NaCl-split human skin, anti-laminin 332 MMP and anti-p200 pemphigoid can be distinguished from BP, LAD, PG, and anti-BP180 MMP, but final diagnosis can only be made by more sophisticated methods, i.e. use of cell-derived or recombinant forms of the target antigens (Figure 1). In recent years, various novel detection systems for serum antibodies have been...
developed, some of which are also commercially available. These now allow for a more accurate diagnosis, which is becoming increasingly important to guide treatment decisions, while our knowledge about the therapeutic arsenal of these disorders has also expanded. In this review, we focus on recent progress in our understanding of the pathogenesis, diagnosis, and treatment of the different pemphigoid diseases.

Bullous pemphigoid

Pathogenesis

Two hemidesmosomal proteins, the 230 kDa protein (BP230 or BPAG1), and 180 kDa antigen (BP180, BPAG2, or type XVII collagen) have been identified as the major antigenic targets of BP autoantibodies [8–10]. BP230 is an intracellular plakin protein member showing homology with plectin and desmoplakins I and II and promotes the association of hemidesmosomes with keratin intermediate filaments [11–13]. In contrast, BP180 is a transmembrane protein with a type II orientation that spans the lamina lucida and projects into the lamina densa of the epidermal basal membrane zone (BMZ). The extracellular region consists of 15 collagen domains separated from one another by noncollagen sequences [14,15].

The noncollagenous 16A domain (NC16A), located at the membrane-proximal region of BP180, is considered to harbor major pathogenically relevant epitopes in BP and is recognized by autoantibodies in 80–90% of BP patients [16–19]. The importance of anti-BP180 NC16A reactivity is further highlighted by the observation that serum levels of BP180 NC16A-specific antibodies correlate with the disease activity in BP patients [20].

In addition, it was reported that autoantibodies preferentially recognize the phosphorylated BP180 ectodomain [21,22]. Recent studies have identified the presence of memory B cells specific for the NC16A domain, which can be induced in vitro to synthesize autoantibodies [23]. These cells belong to the short-lived plasma blast population [24]. The BP180 NC16A-specific autoantibodies are not only of the IgG isotype (mainly IgG1 and IgG4 subclasses), but also of the IgE class [25–27]. In fact, patients with BP frequently have IgE autoantibodies binding to the NC16A domain of BP180 [28].

The presence of IgE autoantibodies has recently been correlated with a severe form of BP, and BP patients, positive for IgE anti-BP180 antibodies, required longer duration for remission, higher dosage of prednisolone, and more intensive therapies for remission [29]. Interestingly, one study showed that a recombinant NC16A fusion protein degranulated basophils opsonized with IgE from patients with BP [30], further supporting the pathophysiological role of IgE antibodies in BP. In addition, other antigenic sites exist on both the extracellular and intracellular

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Figure 1. Indirect IF microscopy on 1 M NaCl split human skin and main target autoantigens of pemphigoid diseases. Differentiation of the diseases and target molecules is based on the binding pattern with either (a) epidermal or (b) dermal binding of circulating autoantibodies on the artificial split.
domain of BP180, which are recognized by IgE and IgG autoantibodies in BP patients [16–18,31–34].

BP patients also exhibit significant reactivity with BP230, which is thought not to be involved in the initiation of the inflammatory response in BP due to its intracellular localization [8,35–40]. Currently, it is unclear whether anti-BP230 autoantibodies in BP patients directly contribute to blister formation or whether they are just a result of keratinocyte injury and determinant spreading of the autoimmune response. Very recently, two retrospective studies with a large series of 138 and 190 patients with active BP, respectively, have determined the outcome of BP230 autoantibody detection using commercial enzyme-linked immunosorbent assays (ELISAs) [39,40].

In the first-mentioned study, 59% of patients had a positive BP230 ELISA result and 86% had a positive BP180 ELISA result, while only 5% had anti-BP230 autoantibodies without anti-BP180 autoantibodies [39]. In the second study, anti-BP230 and anti-BP180 antibodies were detected in 61 and 79% of BP patients serum samples, respectively, while 8% had anti-BP230 autoantibodies without anti-BP180 autoantibodies [40]. Although the study by Charneau et al. found no relationship between a positive BP230 ELISA result and disease extent or presence of mucosal involvement [39], with regard to this specific finding, the study by Roussel et al. [40] showed the opposite finding. Two other studies suggested that anti-BP230 antibodies may be preferentially associated with localized types of BP [37,38].

In contrast to the humoral immune response, the cellular immune response has been less widely studied in human BP. Autoreactive T and B cells are almost constantly found in BP patients. These cells react with the same regions of BP180 and BP230 that are recognized by IgG autoantibodies. The differential epitope recognition of BP180 seems to be associated with distinct clinical severity: T- and B-cell reactivity against the NH2-terminal portion of the BP180 ectodomain is associated with severe BP, while the central portion is more frequently recognized in patients with limited disease.

In contrast, combined T- and B-cell response against the COOH- and NH2-terminal globular domains of BP230 was found in less than 50% [41]. The response to the BP180 ectodomain is restricted by certain HLA class II alleles, such as the DQ8*0301 allele [42,43]. Autoreactive T cells in BP patients produce a Th1/Th2 mixed cytokine profile. We and others have detected elevated levels of IL-1β, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-15, IL-16, eotaxin, MCP-4, tumor necrosis factor α (TNFα), and CCL18 in the sera and/or blister fluids of BP patients [44–55].

Serum levels of TNFα, IL-6, IL-8, IL-15, and CCL18 correlated with patients’ disease activity [47–49,54], pointing to a pathological relevance of these mediators. The observation that Th2-type cytokines are important in human BP is supported by the increased frequency of cutaneous lymphocyte-associated antigen-positive IL-4- and IL-13-producing cells in the peripheral blood [56]. Furthermore, analysis of cell subsets with immunoregulatory functions in BP patients has shown that while the number of circulating CD4+CD25+FOXP3+ regulatory T cells, natural killer T cells, and natural killer cells are normal, γδ T cells are reduced in BP patients [57,58].

Our knowledge about the functionally relevant pathogenic mechanisms in BP is based on in vitro studies using cultured human keratinocytes, ex vivo studies using cryosections of human skin as well as various mouse models [59,60]. When cultured human keratinocytes were treated with anti-BP180 IgG, dose- and time-dependent increases of both mRNA and protein levels of IL-6 and IL-8 were observed, pointing to a signal-transducing event via BP180 [61]. In the same model, decreased expression of BP180 and weakening of keratinocyte attachment in response to anti-BP180 IgG were seen [62].

Using cryosections of human skin, BP patients’ sera were shown to generate dermal–epidermal separation when co-incubated with leukocytes and complement from healthy volunteers [63]. Characterizing the specificity of pathogenically relevant autoantibodies, we were later able to demonstrate that IgG antibodies to human BP180, purified from sera of BP patients and from rabbits immunized against recombinant human BP180, induce dermal–epidermal separation in this model. This effect was shown to be mediated by binding of autoantibodies to the NC16A domain of human BP180 and to be dependent on the Fc portion of autoantibodies [64].

Passive transfer of rabbit IgG, raised against the murine homolog of the human BP180 NC16A domain, into neonatal wild-type mice produced clinical, histologic, and immunopathologic alterations like those seen in patients with BP [65]. In this model, blister formation is dependent on the activation of complement, degradation of mast cells, and recruitment of neutrophils [65–68].

Blister fluid and lesional/perilesional biopsies from BP patients have been shown to contain high levels of proteolytic enzymes, including neutrophil elastase (NE), cathepsin G, collagenase, plasminogen activators, plasmin, matrix metalloproteinase-2 (MMP-2, gelatinase A), MMP-9 (gelatinase B), and MMP-13 [69–77]. These enzymes, particularly the neutrophil- and eosinophil-derived MMP-9 and NE, are secreted into the extracellular space upon cell activation and are thought to proteolytically degrade various extracellular matrix proteins, including the extracellular domain of BP180 [76,78,79]. Mouse genetically deficient in NE or MMP-9 have been shown to be resistant to experimental BP [80,81].

An interaction between MMP-9, NE, and the plasminogen/plasmin system was demonstrated to
occur during proteolytic events in experimental BP: in the early stages of blistering, MMP-9 is mainly activated by plasmin, which is formed from plasminogen by tissue plasminogen activator (tPA) and/or urokinase plasminogen activator (uPA) [82]. Furthermore, an elevated expression and release of tPA from normal human keratinocytes upon stimulation with antibodies to human BP180 has been reported [83]. In addition to plasmin, the mast cell-specific serine protease MCP-4 (chymase) is also able to activate MMP-9 [84]. Activated MMP-9 is believed to proteolytically inactivate α1-proteinase inhibitor, the physiological inhibitor of NE, which allows unrestrained activity of NE [85]. These findings demonstrate that loss of cell-matrix adhesion within the dermal-epidermal junction (DEJ) is directly mediated by proteinases released by inflammatory cells.

In a first attempt to explore the functional relevance of human anti-BP180 antibodies reacting with the human target antigen, human skin was transplanted onto Severe Combined Immunodeficiency (SCID) mice. The injection of BP IgG into the transplant did, however, not result in blister formation within the observation period of 48 h [86]. Recently, additional “humanized” mouse models have been established. Olasz et al. generated a novel transgenic mouse expressing human BP180 in murine epidermal BMZ by the use of a keratin 14 promoter construct. They showed that wild-type or MHC I−/−, but not MHC II−/−, mice grafted with transgenic skin elicited IgG that bound human epidermal BMZ and BP180. Transgenic grafts on wild-type mice developed neutrophil-rich leukocyte infiltrates and subepidermal blistering [87]. Nishie et al. [88] used the same BP180 transgenic mice to rescue the blistering phenotype of BP180−/− mice by backcrossing experiments. Clinical, histological, and immunopathologic features of BP were achieved by passive transfer of IgG from patients or IgG1 monoclonal antibodies against humanized BP180 NC16A into these BP180-humanized mice [88,89]. To show that pathogenic anti-BP180 autoantibodies act in combination with key innate immune system players also in the humanized BP mouse model, Liu et al. generated a humanized mouse strain, in which murine BP180 NC14A was replaced by the homologous human BP180 NC16A epitope [90]. These BP180 humanized mice pre-treated with mast cell activation blocker or depleted of complement or neutrophils become resistant to BP after passive transfer of human BP autoantibodies. More recently, an active mouse model for BP has been developed by transferring splenocytes from wild-type mice that had been immunized by grafting of human BP180-transgenic mouse skin onto Rag-2−/−/BP180-humanized mice. The recipient mice produced anti-human BP180 IgG antibodies in vivo and developed blisters and erosions corresponding to clinical, histological, and immunopathological features of BP [91].

In two other mouse models, the pathogenic effect of IgE anti-BP180 antibodies has been elucidated, demonstrating that an IgE hybridoma to LABD97 antigen or purified IgE from BP patients was able to induce BP-characteristic inflammatory skin changes and partly subepidermal blistering in human skin grafts on nude mice [92,93].

**Diagnosis**

Clinically, BP is characterized by tense blisters predominantly on flexural aspects of the limbs and abdomen and associated with severe itching (Figure 2a). BP typically affects the elderly with an average age at diagnosis between 75 and 81 years [1,2,94,95]. Before blisters arise, BP is typically preceded by a prodromal, non-bullous stage, in which excoriated, eczematous, papular, and/or urticarial lesions are found that may persist for several weeks or even months.

Light microscopy of lesional skin from patients with BP typically demonstrates a subepidermal blister with an eosinophil- and/or neutrophil-rich leukocytic infiltrate within the papillary dermis and along the epidermal BMZ (Figure 2b). By direct IF microscopy of perilesional skin linear deposits of IgG and/or C3 can be found at the epidermal BMZ (Figure 2c). In approximately 80% of the cases, patient IgG autoantibodies react with the epidermal side of 1 M NaCl split human skin by indirect IF microscopy (Figure 2d) [96].

Based on the finding that NC16A is the immunodominant region of BP180 [17,18], two highly specific and sensitive BP180 NC16A-specific ELISAs for detection of circulating autoantibodies in BP have been commercialized [19,97]. When these assays are combined with other detection systems, including those using BP180 fragments outside the NC16A domain and BP230, the sensitivity of these tests can be increased to even 100% [38,41,98,99]. In contrast to serum levels of antibodies to BP230, BP180 NC16A-specific autoantibodies correlate closely with disease activity and may thus be not only of diagnostic use, but also allow the monitoring of circulating autoantibody levels during the course of the disease, helping to guide treatment decisions [19,20,36].

BP must be differentiated from pemphigus and other subepidermal blistering autoimmune dermatoses, including EBA and dermatitis herpetiformis. In contrast to BP, blister formation in pemphigus results from intraepithelial cell separation and shows an intercellular pattern of IgG between keratinocytes. Differentiation of BP especially from EBA, anti-p200 pemphigoid, and MMP, however, presents a more difficult task. The mechanobullous, non-inflammatory form of EBA is characterized by the appearance of skin fragility and tense blisters at anatomic
areas subjected to trauma, healing of lesions with scarring and milia formation as well as subepidermal split formation with an only scattered dermal inflammatory infiltrate. In contrast, the inflammatory subtype of EBA may mimic clinical, histological, and routine direct IF microscopy findings of BP.

Using higher magnifications of direct IF microscopy, EBA can be sometimes differentiated from BP by distinctly shaped IgG deposits at the epidermal BMZ, i.e. EBA revealing an u-serrated pattern and BP a n-serrated configuration [100]. In addition, as autoantibodies in EBA are directed against type VII collagen, sera from these patients do not bind to the epidermal, but to the dermal side of 1 M NaCl split human skin. Similarly, patients with anti-p200 pemphigoid show autoantibody reactivity to a dermal antigen. This immunopathological finding may be of major importance since clinically, anti-p200 pemphigoid and BP may not be distinguishable. In contrast to BP, MMP is predominantly confined to mucous membranes.

If blisters appear on the skin of MMP patients, they are commonly smaller, transient, and of limited extent. Indirect IF microscopy of sera from MMP patients may reveal epidermal, dermal, or combined staining patterns of IgG on 1 M NaCl split human skin. Some forms of LAD clinically resemble BP or dermatitis herpetiformis. Continuous linear immunodeposits of IgA, most often in the absence of IgG and C3, together with circulating IgA anti-BMZ autoantibodies, can distinguish LAD from these other immunobullous diseases.

**Treatment**

BP is generally a self-limited disease that may remit within a few months or some years. However, the mortality rate in the first year has been shown to be as high as 11–40% [95,101–103], and was related to old age, poor general condition, and use of high doses of oral corticosteroids rather than to the extent of the disease itself [94,102]. Systemic and topical corticosteroids have been widely used in clinical practice [104]. Initially, daily morning doses of prednisolone in the range of 0.5 mg/kg/day usually control the disease within a few weeks. An initial dose higher than 0.75 mg/kg/day has been shown to be not beneficial in a controlled prospective trial [105].

A large, randomized controlled trial showed that initial disease control and 1-year survival were significantly better when treating severe BP with clobetasol propionate cream 40 mg/day compared with oral prednisolone 1 mg/kg/day, while in moderate BP, outcomes using clobetasol cream and prednisolone

![Figure 2. Clinical, histologic, and immunopathologic findings in BP as representative pemphigoid disease. (a) Tense blisters and erosion on erythematous base on the leg. (b) Histopathology of lesional skin demonstrating a subepidermal blister and a mixed dermal inflammatory infiltrate containing numerous granulocytes. (c) Direct IF microscopy of perilesional skin showing continuous linear deposits of IgG at the epidermal BMZ. (d) Indirect IF microscopy on 1 M NaCl split human skin revealing reactivity of circulating IgG against the epidermal side of the artificial split.](image)
0.5 mg/kg/day were comparable [106]. Furthermore, a mild regimen with clobetasol propionate cream 10–30 g/day tapering over 4 months has been shown to be not inferior to the high-dose topical regimen with clobetasol propionate 40 g/day [107].

Considering other randomized controlled trials in BP, no differences in disease control were seen for azathioprine plus prednisone compared with prednisone alone [108], for prednisolone plus azathioprine compared with prednisolone plus plasma exchange [108], for prednisolone plus mycophenolate mofetil or plus azathioprine [109], and for tetracycline plus nicotinamide compared with prednisolone [110]. Reports have also described successful treatment of BP patients with dapsone [111,112], methotrexate [113], high-dose intravenous immunoglobulins (IVIG) [114], rituximab [115–117], omalizumab [118], and immunoadsorption [119].

**Mucous membrane pemphigoid**

**Pathogenesis**

BP180 is the target antigen in about 70% of MMP patients [120]. However, unlike in BP, only about half of the patients’ sera contain antibodies to BP180 NC16A, and C-terminal epitopes of BP180 are preferentially targeted [120–122]. In addition, patients with MMP may exhibit IgG and/or IgA autoantibodies of other specificity that recognize BP230 [123,124], the 97/120 kDa LAD antigen, laminin 332 (laminin 5), laminin 331 (laminin 6) [125,126], type VII collagen [127], or the α6 and β4 integrin subunit [128]. Considering the latter target antigens, it has been shown that sera from patients with generalized MMP and ocular MMP recognize the β4 integrin subunit, while sera from patients with oral MMP recognize the α6 integrin subunit [129].

The pathogenicity of anti-laminin 332 antibodies has been documented in vivo. Passive transfer of anti-laminin 332 IgG to neonatal or adult mice induced subepidermal blisters of skin and mucous membranes that mimicked clinical, histological, and immunopathologic features seen in MMP patients [130]. The same findings were seen in mice injected with Fab fragments directed against laminin 332 as well as in mice lacking complement, mast cells or T cells, suggesting that such antibodies may elicit epidermal detachment in vivo in a non-inflammatory and direct manner [130,131].

The pathogenicity of patients’ autoantibodies was further confirmed in an experimental human skin graft model, in which human anti-laminin 332 autoantibodies induced subepidermal blisters [132]. Additionally, accumulating evidence indicates that fibrogenic cytokines, matrix metalloproteinases, and collagen type I secreted in high amounts by pemphigoid fibroblasts may actively be involved in ocular MMP-associated scarring processes [133,134].

**Diagnosis**

MMP is a chronic autoimmune blistering disease of mucous membranes and skin. An international consensus conference defined the predominant involvement of mucous membranes as the major clinical diagnosis criterion of MMP [135]. The oral mucosa is the most common site affected by MMP followed by ocular disease (Figure 3). It may also involve nasal, pharyngeal, laryngeal, esophageal, and anogenital mucous membranes. Progressive scarring potentially may lead to serious complications, such as blindness or asphyxiation. Histologically, MMP is characterized by subepidermal blisters with a chronic inflammatory infiltrate in the lamina propria that is composed of lymphocytes, histiocytes, scattered neutrophils, and eosinophils. Anti-BP180 and anti-laminin 332 MMP cannot be differentiated from each other by histology [136].

Direct IF microscopy shows linear IgG and C3 deposits at the BMZ. Indirect IF microscopy using 1 M NaCl split human skin distinguishes between antigens on the epithelial side of the split (BP180, BP230, and αβ4 integrin) and those of the dermal side (laminin 332). However, as only about one-half of sera are reactive in indirect IF microscopy, ELISA and Western blotting using relevant target antigens are important additional diagnostic methods in MMP. Although only half of the MMP patients develop antibodies to the NC16A domain of BP180, the other patients with BP180 reactivity react with LAD-1 or the C-terminal portion of BP180 by Western blotting [130]. Importantly, testing for IgA anti-BP180 antibodies in addition to the IgG isotype will further increase the detection rate [130,137].

Assaying for anti-laminin 332 reactivity is of particular importance as 25% of patients with laminin 332-specific antibodies develop a malignancy. In these
patients, a tumor search is indicated [138,139]. Immunoprecipitation with human keratinocytes has been reported to be the most sensitive technique for the detection of serum autoantibodies against laminin 332, whereas immunoblotting with extracellular matrix of cultured HaCaT cells was found to be the most practical alternative [140]. The term *cicatricial pemphigoid*, previously applied for patients with MMP, is currently only used for the rare clinical variant in which mucous membranes are not predominantly affected and skin lesions heal with scarring [135].

Brunsting–Perry pemphigoid is a rare variant of cicatricial pemphigoid first described in 1957 [141]. It is characterized clinically by blisters, hemorrhagic crusts and atrophic scars confined to the head and neck without mucosal involvement. It probably represents a heterogeneous disorder with several target antigens. Most patients with this type of pemphigoid disease have been reported to have autoantibodies against type VII collagen, therefore, representing a localized form of EBA [142]. Although the clinical features of Brunsting–Perry pemphigoid are completely different from those of MMP, it has recently been reported that they may share the same target antigens, e.g. the C-terminal domain of BP180 and laminin 332 [143,144].

**Treatment**

Treatment of MMP is largely tempered by its severity and sites of involvement. A consensus conference on this disease differentiated high-risk and low-risk patients. Although in low-risk patients, lesions are limited to the oral cavity and the skin and are more amenable to treatment, patients with conjunctival involvement usually require aggressive management to avoid blindness. Mild lesions of the oral mucosa and skin can sometimes be effectively treated with topical glucocorticoids or topical calcineurin inhibitors combined with good oral hygiene. For more severe lesions in low-risk patients, initial treatment may include dapsone (1.0–1.5 mg/kg/day) and prednisolone (0.5–1.0 mg/kg/day).

An alternative for these patients may also be tetracyclines combined with nicotinamide. Prednisolone (1.0–1.5 mg/kg/day) in combination with oral cyclophosphamide (1.0–2.0 mg/kg/day) may be tried in patients at high risk (ocular, nasopharyngeal, genital, and esophageal involvement) [145,146]. Alternatively, i.v. dexamethasone pulses (100 mg on 3 consecutive days) combined with i.v. cyclophosphamide pulses (500–1000 mg) in 2- to 4-week intervals can be given. In patients who do not tolerate cyclophosphamide, other immunosuppressants such as azathioprine, mycophenolate mofetil, and methotrexate can be applied [145,146].

Good therapeutic efficacy of IVIG has been reported in patients with MMP. IVIG therapy, usually given at 2 g/kg/cycle initially every 4 weeks, avoided progression of the disease and was more effective than conventional immunosuppressive treatment [104]. In one study, IVIG given as monotherapy halted progression of scarring and visual deterioration during the study period, and serological and clinical remission was sustained for several months after cessation of IVIG infusions [147]. Recent case reports also described the beneficial use of rituximab and TNF-α inhibitors for severe and treatment-resistant cases of MMP [105,107,148].

**Pemphigoid gestationis**

**Pathogenesis**

PG, formerly referred to as herpes gestationis, is another disease in which BP180 has been identified as the major target [149]. Occasionally, PG is associated with a trophoblastic tumor, hydatidiform mole or choriocarcinoma. The disease may appear for the first time during any pregnancy, but then rarely skips subsequent gestations [149]. The immunopathologic hallmark of PG is linear deposits of C3 and, to a lesser extent, of IgG along the DEJ in perilesional skin biopsies. Circulating-complement fixing autoantibodies “HG factor” can be detected by indirect IF on intact or NaCl split skin.

The autoantibodies react with BP180 and, less frequently, with BP230 [16,150]. IgG autoantibodies in most PG sera target the NC16A domain of BP180 [16,32,151–153]. However, antigenic sites outside NC16A have also been identified both extra- and intracellullarly [153]. Epitope mapping studies identified two well-defined antigenic sites within a 22 amino acid segment of BP180 (NC16A2 and NC16A2.5) as major antigenic targets for PG sera [152]. In addition, using overlapping synthetic peptides, PG autoantibodies were shown to bind to two defined epitopes within the NC16A domain (aa 500–514 and aa 511–523). Importantly, preadsorption using an affinity matrix containing these epitopes completely abolished dermal–epidermal separation *ex vivo* induced by PG autoantibodies [154].

In contrast to findings in BP [155], reactivity to NC16A is dominated by IgG1 and IgG3 antibodies in PG patients [152]. As IgG1 and IgG3 are the IgG subclasses with the strongest complement fixing properties, these observations may well explain complement deposition at the DEJ, which is the most consistent immunopathological feature in PG. In PG patients tested for T-cell autoreactivity, BP180-specific T cells exclusively reacted with the NC16A2 domain. Proliferative responses of these cells were restricted to HLA-DR and cells expressed a CD4+ Th1-type memory phenotype [156]. These results underline other findings that PG is strongly associated with HLA-DR3 and -DR4 haplotypes [149].
Diagnosis

PG usually presents during the second or third trimester of pregnancy or in the immediate postpartum period with a pruritic urticarial, papulovesicular eruption. Skin lesions often start around the umbilicus and then spread over abdomen and thighs [149]. PG frequently presents as a nonbullous disease with eczematous lesions, erythema multiforme-like changes or erythematous papules and plaques. Diagnosis is based on detection of C3 deposits in perilesional skin biopsies as well as circulating autoantibodies by the complement binding test and ELISA using recombinant BP180 NC16A [19,157].

Treatment

Treatment is aimed at suppression of new blister formation and relief of the intense pruritus. In milder cases, this can be achieved by the use of topical corticosteroids in combination with oral antihistamines. In more severe cases, the administration of prednisolone at an initial dose of 0.3–0.5 mg/kg/day is generally sufficient. This dose can usually be decreased during the course of the disease. Flares postpartum may require a temporary increase in the corticosteroid dose [149].

Linear IgA dermatosis

Pathogenesis

LAD is an autoimmune subepidermal blistering disease characterized by circulating IgA anti-BMZ autoantibodies. Autoantibodies in LAD were shown to target antigens with various molecular weights, including 97-, 120-, 180-, 200-, 230-, 280-, 285- and 290-kDa proteins [158–160]. The two most characteristic target antigens are the protein of 97 kDa and the 120 kDa protein, termed the LABD antigen 1 (LABD97) and LAD-1, respectively [159,161]. These proteins present a cleaved portion of the extracellular domain of BP180 [160,162–165]. Recently, the sheddases ADAM 9 and 10 have been reported to be involved in generation of LAD-1 from BP180, while formation of LABD97 has been shown to be dependent on plasmin [166,167].

In contrast to BP, only 20% of sera of patients with LAD react with the NC16A domain [168]. In addition, IgA antibodies to BP230, type VII collagen and laminin 332 have been reported [169–171]. Since the majority of LAD sera also contain IgG antibodies against BP180 and in most BP sera, IgA anti-BP180 antibodies can be detected, LAD and BP may be regarded as different ends of a continuous spectrum [172]. In fact, the isotype of anti-BMZ reactivity was associated with the age of the patients: in younger patients, IgA autoantibodies predominated, whereas in older patients, predominantly IgG anti-BMZ antibodies were found [173].

LAD may be either idiopathic or drug-induced (e.g. vancomycin). The mechanism for blister formation is not fully understood, but is likely to involve IgA- and complement-mediated neutrophil chemotaxis. The pathogenic relevance of LAD-associated autoantibodies has been shown by the passive transfer of IgA murine monoclonal antibodies against LAD autoantigen to SCID mice bearing human skin grafts. In some of these challenged mice, the transferred autoantibodies produced neutrophil-rich infiltrates and subepidermal vesicles [174].

Diagnosis

LAD mainly shows vesicobullous lesions affecting the skin and mucosal surfaces, sometimes forming characteristic collarettes of vesicles or blisters as new lesions arise in the periphery of old lesions (Figure 4). On histopathology, the bullae are subepidermal, with collections of neutrophils along the epidermal BMZ and occasionally in the dermal papillary tips. Direct IF microscopy of perilesional skin shows linear deposition of IgA at the epidermal BMZ, sometimes in combination with linear IgG deposits. Patients have

Figure 4. Clinical presentation of LAD. Erythema multiforme-like lesions with partly clustered annular tense blisters on proximal lower extremity.
circulating IgA autoantibodies that mostly bind the epidermal side of 1M NaCl split human skin on indirect IF microscopy (lamina lucida type), but combined epidermal and dermal staining as well as dermal labeling alone (sublamina densa type) has also been observed. Autoantibodies to LAD-1 in the lamina lucida type of LAD are detected by immunoblotting with conditioned concentrated medium of cultured human keratinocytes [159].

Treatment

Skin lesions in LAD commonly respond when treated with dapsone. As dapsone may cause hemolytic anemia, decreased hemoglobin values or even methemoglobinemia, glucose-6-phosphate dehydrogenase deficiency should be excluded before the drug is initiated. An alternative treatment is Sulfapyridine, usually given at a dose of 15–60 mg/kg/day [175]. Some patients may require low-dose prednisone initially to suppress blister formation. In unresponsive patients, erythromycin, colchicine, flucloxacillin, IVIG, and immunoadsorption have been administered successfully [176].

Lichen planus pemphigoides

Pathogenesis

Regarding the pathogenesis of LPP, it is most likely that the lymphocytic inflammatory process directed against basal keratinocytes in lichen planus leads to a release of hidden antigenic determinants within the DEJ, resulting in an autoimmune response against hemidesmosomal structures [177–180]. Immunoblotting studies have identified BP180 and BP230 as target molecules but also a new antigen of 200 kDa of keratinocyte derivation. The latter finding reinforces the hypothesis that LPP might have a unique antigen and thus represent a distinct entity from BP [178,179]. In line with this assumption, it has been shown that the epitope within the C-terminal NC16A domain of BP180 antigen, targeted in LPP, differs from BP, localizing to NC16A4 as opposed to NC17A1 through NC16A3 [180].

Diagnosis

Most patients with LPP have typical lichenified plaques or papules of lichen planus initially, followed after weeks to months by tense vesicles and blisters which arise, in contrast to bullous lichen planus, independent of the lichenoid lesions (Figure 5). Histologically, blisters resemble those in BP [177]. Direct IF microscopy of a papule shows Civatte bodies, but when a blister is evaluated, IgG and C3 are present as in BP. Indirect IF microscopy on salt split skin shows binding of IgG to the epidermal side.

By immunoblotting, reactivity is primarily directed against BP180 with NC16A being the immunodominant domain [180]. Less often, antibodies against BP230 or other yet uncharacterized antigens are found [177–179]. The following observations suggest that LPP is not a coincidental association of BP and lichen planus: (i) the average age of BP patients is 77 years, whereas in LPP it is 44 years; (ii) lichenoid papules are not seen in BP; (iii) autoantibodies in LPP react with C-terminal epitopes of NC16A, which is very uncommon in BP; and (iv) LPP is usually a milder disease and more therapy-responsive than BP.

Therapy

It is important to treat existing lichen planus to avoid further immunostimulation. In this context, acitretin has proven valuable. Otherwise, treatment is the same as in BP.

Anti-p200 pemphigoid

Anti-p200 pemphigoid is a subepidermal blistering skin disease characterized by circulating autoantibodies against a 200-kDa molecule (p200 protein) of the lower lamina lucida of the BMZ [181]. Recently, it has been shown that 90% of the anti-p200 pemphigoid sera react with the C-terminus of laminin γ1 [182]. Subsequently, the term anti-laminin γ1
pemphigoid was proposed for this disease. Because not all anti-p200 pemphigoid sera react with laminin γ1 [182,183], we suggest not applying the two terms synonymously and only diagnosing an anti-laminin γ1 pemphigoid when reactivity with laminin γ1 has actually been identified.

Pathogenesis

Anti-p200 pemphigoid shows organ-specific pathogenicity, although laminin γ1 is expressed not only at the DEJ but also in the BMZ of dermal blood vessels [184]. This has been explained by the assumption that laminin γ1 in the epidermal BMZ has different posttranslational modifications, such as glycosylation, compared with laminin γ1 expressed in blood vessels [185]. However, the pathogenicity of anti-p200/laminin γ1 antibodies has hardly been studied yet, with the exception of a single patient whose IgG, in contrast to BP IgG, did not induce IL-8 secretion following incubation with human cultured keratinocytes [186].

Diagnosis

Patients with anti-p200 pemphigoid typically develop tense blisters and urticarial eruptions, sometimes with itching, closely resembling those of BP (Figure 6). Usually, patients with anti-p200 pemphigoid can only be clinically differentiated from BP by their considerably younger age with an average of 61 years at diagnosis [187]. Histopathologically, skin biopsy specimens demonstrate subepidermal blistering and a linear infiltrate of neutrophils, and sometimes eosinophils, along the epidermal BMZ.

With this method, anti-p200 pemphigoid cannot be differentiated from other pemphigoid disorders [188]. Direct IF microscopy of perilesional skin biopsies from patients with p200 pemphigoid demonstrate linear deposits of IgG and C3 along the epidermal BMZ. Typically, autoantibodies bind to the floor of the artificial blister by indirect IF microscopy on 1 M NaCl split human skin [181]. Classically, autoantibodies in anti-p200 pemphigoid react with a 200-kDa protein in extracts of human dermis by Western blotting. Recently, laminin γ1-specific antibodies could be detected by Western blotting or ELISA using the recombinant C-terminus of laminin γ1 [182,183].

Treatment

Anti-p200 pemphigoid can be treated in a similar way as BP and typically shows a prompt response to topical class IV corticosteroids and dapsone (1.0–1.5 mg/kg/day). In more severe cases, prednisolone (0.5 mg/kg/day) may be added [187]. Recently, adjuvant immunoabsorption has been successfully applied in a severe case of anti-p200 pemphigoid with concomitant active gastric ulceration [189].

Conclusion

Considerable progress has been made in the last years regarding our understanding of pemphigoid diseases. Experimental animal models of these diseases and advances in translational research provided essential insight into the pathophysiology of these disorders. Intense scientific research on the autoantibody response has led to new diagnostic techniques, allowing the serological diagnosis in the great majority of patients. Novel, more specific therapeutic avenues are, however, needed to further reduce the long-term adverse effects of the currently available immunosuppressants.

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