



Bullous pemphigoid: From the clinic to the bench

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Abstract Bullous pemphigoid (BP) constitutes the most frequent autoimmune subepidermal blistering disease. It is associated with autoantibodies directed against the BP antigens 180 (BP180, BPAG2) and BP230 (BPAG1-e). The pathogenicity of anti-BP180 antibodies has been convincingly demonstrated in animal models. The clinical features of BP are extremely polymorphous. The diagnosis of BP critically relies on immunopathologic findings. The recent development of novel enzyme-linked immunosorbent assays has allowed the detection of circulating autoantibodies with relatively high sensitivity and specificity. Although potent topical steroids have emerged in the past decade as first-line treatment of BP, management of the disease may be challenging.

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Introduction

The name “bullous pemphigoid” itself is a pleonasm: *pemphigoid* is derived from the Greek and means “form of a blister” (*pemphix*, blister; *eidōs*, form); hence etymologically, the adjective “bullous” should reasonably not be added to designate the blistering in pemphigoid.¹ Despite this redundancy, “bullous” pemphigoid (BP) has emerged as a paradigm for an organ-specific autoimmune disease.

BP belongs to the group of pemphigoids that further include gestational pemphigoid (also called *pemphigoid gestationis* or *herpes gestationis*), mucous membrane pemphigoid, and linear immunoglobulin (Ig) A bullous disease. It represents the most frequent autoimmune blistering disease of the skin in many geographic areas, such as in Europe, North America, Japan, and Australia. The

disease typically affects the elderly and is associated with a significant morbidity and mortality. The cutaneous manifestations of BP are protean. BP is associated with an autoantibody response to BP180, also called BPAG2, or type XVII collagen, and BP230 (BPAG1-e), two components of hemidesmosomes. Although various *in vitro* and *in vivo* animal models have shown IgG autoantibodies to BP180 are pathogenic, the exact role of anti-BP230 antibodies remains to be clarified.

This contribution encompasses the clinical features of BP, the clinical context in which BP occurs and the associated conditions, a diagnostic algorithm, and a discussion of its practical management. Finally, recent advances of our insights into the physiopathology of BP are presented.

Epidemiology and clinical context

The annual incidence of BP was originally estimated in the first prospective studies to be at least 6 to 7 new cases/

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year per 1 million population.²⁻⁴ In our prospective study encompassing the entire Swiss population during a 2-year period, we found an incidence of 12 new cases/year per 1 million population,⁴ whereas another German prospective study from Lower Franconia reported a similar incidence of 13.4.⁵ As seen in some reports describing figures of up to 42.8 new cases/year per 1 million population,⁶ the incidence of BP may be even higher, with a 2-fold to a 4.8-fold increase of incidence in the last decade.^{5,6} Although recruitment biases may have occurred in the latter studies, aging of the general population and a better knowledge of the disease with an improved diagnostic performance most likely account for these increasing figures.

BP typically affects people aged 70 years and older. The incidence in the population older than 80 years is 150 to 180 new patients/year per 1 million, whereas the risk to develop the disease in patients older than 90 years appears to be approximately 300-fold higher than for those aged 60 years or in younger women.³⁻⁵ BP can also occur in infancy and

childhood, however,⁷ and a recent study from Israel suggests that BP in infancy is not so rare as is usually assumed, with an estimated incidence of 23.6 new cases/year per 1 million.⁷

Clinical features

The possibility of BP should be promptly considered in all elderly patients who present with a generalized pruritic bullous eruption.⁸⁻¹⁰

In the bullous stage, dozens to hundreds of vesicles and bullae can arise on apparently normal or erythematous skin together with urticarial and infiltrated papules and plaques or eczematous lesions. The blisters are tense, 1 to 3 cm in diameter or even larger, have a clear exudate, and may persist for several days, leaving eroded and crusted areas. In some cases, blisters rupture rapidly, resulting in large eroded areas. The lesions are frequently distributed symmetrically and predominate on the trunk, abdomen, and proximal



Fig. 1 Bullous pemphigoid. A and B, Erythematous urticarial and infiltrated plaques with tense vesicles, blisters, erosions, and crusts on the trunk. C, Close view of tense serous blisters arising on infiltrated plaques of the thigh. D, Localized bullous pemphigoid on the left foot.

extremities (Figure 1A-C). Lesions may exhibit an annular or figurate pattern and sometimes resemble the lesions of erythema multiforme. Vegetating plaques may be observed in the intertriginous spaces. Healing may leave postinflammatory changes such as hyperpigmentation and hypopigmentation, and more rarely, milia.

Involvement of the oral cavity is observed in 10% to 30% of patients. In our prospective European cohort of BP patients,¹¹ we observed mucosal involvement, almost invariably of the oral mucosa, in 4 of 49 patients (8%) with newly diagnosed BP. The mucosae of eyes, nose, pharynx, esophagus, and anogenital areas may be rarely affected.

A number of patients with BP are first evaluated in the nonbullous phase when BP represents a pitfall even for an experienced dermatologist. Manifestations in this stage are nonspecific: there is frequently an intense pruritus accompanied by a combination of excoriated, eczematous, papular, or urticarial lesions (Figure 2A-C). The features can thus

mimic a wide spectrum of conditions, including those of chronic prurigo, eczema, or a toxic drug reaction that may persist for several months or even remain the only manifestation of BP. In our own prospective Swiss cohort of 164 patients with BP during a 2-year period, the clinical presentation at time of diagnosis in 80% consisted of localized or generalized blistering together with other inflammatory skin lesions. The remaining 20% presented with no obvious blistering, an observation implying the importance of considering BP in all elderly patients with a chronic, relapsing pruritic eruption, even without frank blistering (personal observations).

Several atypical forms or variants of BP have been described; in fact, in a significant subset of patients with BP, skin manifestations are sometimes peculiar and striking due to the extent, localization, or type of lesion.¹² BP can remain occasionally localized^{13,14} (Figure 1D) around stomas on irradiated areas or confined to a paralyzed extremity.¹⁵⁻¹⁷

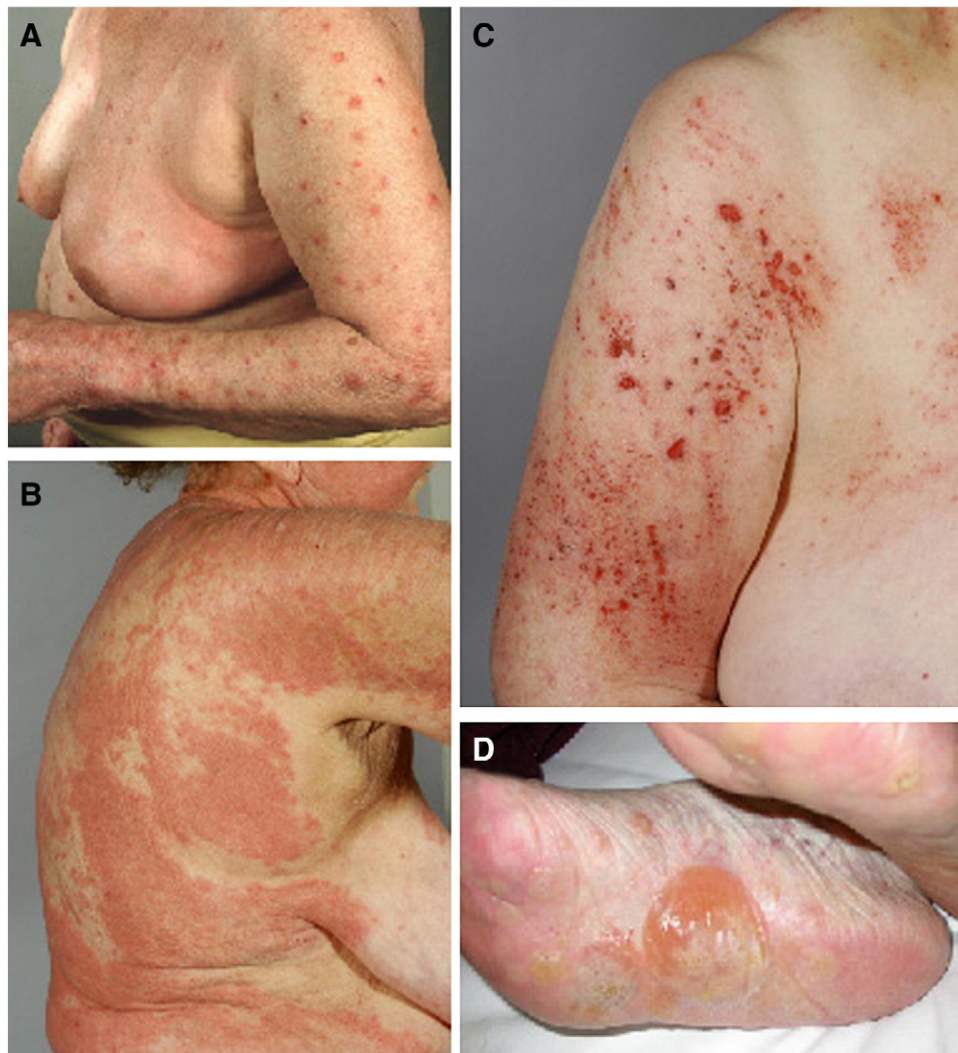


Fig. 2 Bullous pemphigoid: the early stage forms without frank blistering and variants. A, Prurigo-like lesions on the left arm and trunk, with infiltrated urticarial plaques on the trunk. B, Extensive infiltrated urticarial lesions on the trunk and right arm. C, Excoriated eczematous lesions on the right arm and shoulder. D, Localized dyshidrosiform variant on the right foot.

Involvement can be limited to the pretibial area (“pretibial pemphigoid”),¹⁸ to the umbilical area,¹⁹ to the palmoplantar region with features mimicking dyshidrosiform dermatitis^{20,21} (“dyshidrosiform pemphigoid”; Figure 2D), or finally, particularly in young girls, to the vulvar region²² (“vulvar pemphigoid”). In the latter, recurrent genital blisters and erosions that may ultimately result in scarring, and atrophic changes are typically observed.

Several generalized variants of BP have also been reported. Certain patients present with multiple grouped, small, tense vesicles with a symmetric distribution that mimic dermatitis herpetiformis^{23–25} (“vesicular pemphigoid”). Patients have sometimes intertriginous vegetating plaques²⁶ (“pemphigoid vegetans”). In other instances, the inflammatory lesions are constituted by markedly pigmented macules and only later on do bullous lesions develop²⁷ (“pigmented BP”). In our experience, “pemphigoid nodularis” is another quite common and a misleading variant^{18,28–30} in which excoriated nodules and papules, predominantly distributed on arms, legs, and shoulders, are observed in elderly patients. Blisters may precede or follow the development of nodular lesions and arise at sites of nodular lesions or on uninvolved skin. “Erythrodermic BP” is an unusual variant characterized by erythroderma with or without accompanying blistering.³¹

In some of these patients, the diagnosis relies only on the results of the immunopathologic findings. Single cases of extensive “erosive BP” or toxic epidermal necrolysis-like BP have also been described.³² Patients have large eroded areas of skin without pruritus, blisters, or urticarial inflammatory lesions. Large ulcerated ecthyma-like lesions in BP have been described.³³ Another rare variant of BP is “lichen planus pemphigoides,” which is associated with typical features of both lichen planus and BP.^{34,35} Lichen planus pemphigoides usually occurs in the fifth decade, affects the distal extremities, and shows a relatively benign course.

Finally, BP also occurs in children.³⁶ The features of childhood BP appear to be similar to those observed in the elderly, although frequent involvement of mucosae and palmoplantar region has been suggested.

BP frequently shows a mixture of various features, and dyshidrosiform, vesicular, vegetating, papular, or nodular lesions may coexist. The predominating type of lesions can change in the course of the disease, under therapy, or during relapse. There is no obvious justification to distinguish so many variants of BP from the identical pathogenesis. The ultimate variable phenotypic features observed in BP patients are likely the results of the interplay of a number of genes regulating the adaptive humoral and cellular immune response, the innate immune system, and the inflammatory response.

Trigger factors and associated conditions

Numerous triggers have been implicated in the disease onset of individual BP patients, including trauma, burns,

radiotherapy, ultraviolet (UV) radiation, and a large variety of drugs. A weak association with aldosterone antagonists and neuroleptics was found in a prospective case-control study³⁷ and was recently confirmed by a larger study that showed that chronic use of spironolactone and, among neuroleptics, of phenothiazines with aliphatic side chains represents a risk factor for BP.³⁸

Various autoimmune disorders, inflammatory dermatoses, malignancies, and neurologic disorders have been described in association with BP. Autoimmune disorders reported in BP include rheumatoid arthritis, Hashimoto thyroiditis, dermatomyositis, lupus erythematosus, and autoimmune thrombocytopenia. Although a case-control study did not find an increased risk for autoimmune disorders in BP,³⁹ a genetically determined susceptibility to develop autoimmune diseases is plausible. A statistically significant link has not been provided, however, for inflammatory dermatoses reported in association with BP, in particular psoriasis and lichen planus.^{40–42} The chronic inflammatory process at the dermal–epidermal junction in these disorders could conceivably raise, on occasion, a secondary immune response leading to autoimmunity against the target antigens of BP.

In two case-control studies that included more than 1700 BP patients and age-matched controls in Sweden and Japan, a low association with cancer, in particular gastric carcinoma, was identified only in the Japanese cohort.^{43,44} The previously described higher incidence of malignancies in BP patients was probably biased by the lack of appropriate age-matched controls and the intensive work-up of affected patients in a hospital setting. Patients who develop BP and are aged younger than 60 years may be at higher risk for an underlying malignancy.⁴⁵ Thus, an age-related cancer check based on patient’s history and clinical examination, without a systematic and extensive cancer screening, appears advisable in BP patients.

Several independent hospital- and population-based case-control studies have recently contributed to underlining the association between BP and neurologic disorders.^{38,46–48} A significant association was shown with Parkinson disease, dementia, psychiatric disorders (unipolar and bipolar disorders), and stroke. A strong association was observed with multiple sclerosis in the population-based study,⁴⁷ in line with data obtained in multiple sclerosis patients.^{49,50} In addition, development of specific neurologic diseases preceded the onset of BP.^{46,47}

These observations are particularly intriguing because some evidence has been provided suggesting that antigens BP180 and BP230 are expressed in the central nervous system,^{51–53} and naturally occurring or genetically modified mice null for the dystonin gene, encoding for various isoforms of BP antigen gene (*DST*), including the epithelial isoform BP230/BPAG1-e, develop severe dystonia and sensory nerve degeneration.⁵⁴ Altogether these findings suggest that neurologic diseases may be a predisposing factor for BP, possibly through the development of a humoral

immune response in the nervous system that then spreads to involve skin.

Diagnosis of typical and atypical forms of BP

Diagnosis of BP is in principle derived from a combination of criteria, including clinical features and histopathologic and immunopathologic findings¹⁰ (Figure 3). In elderly patients with the classic generalized bullous eruption, the diagnosis is easy: clinical features (see below) together with findings of the direct immunofluorescence (IF) microscopy studies are sufficient to make the diagnosis of BP with a sensitivity of 90%, a specificity of 83%, and a high positive-predictive value.^{55,56}

Clinical criteria

The typical BP patient is an elderly individual presenting with an intensively pruritic eczematous, urticarial eruption,

with or without frank blistering. Mucous membranes, the face, and neck region are usually not affected. In a number of patients, there are simply excoriated lesions. The diagnosis of BP can be made with high specificity and sensitivity in patients with linear IgG or C3 deposits, or both, along the dermal-epidermal junction when three of these four clinical criteria are present: (1) age older than 70 years, (2) absence of atrophic scars, (3) absence of mucosal involvement, and (4) absence of predominant bullous lesions on the neck and head.⁵⁵

Light microscopy

Studies of an early bulla with light microscopy show a subepidermal blister with a dermal inflammatory infiltrate composed predominantly of eosinophils and neutrophils. In early nonbullous phases, any or all of subepidermal clefts, eosinophilic spongiosis, or an infiltrate of eosinophils in the upper dermis lining the dermal-epidermal junction are found. Histologic features are not specific in the early phase of the

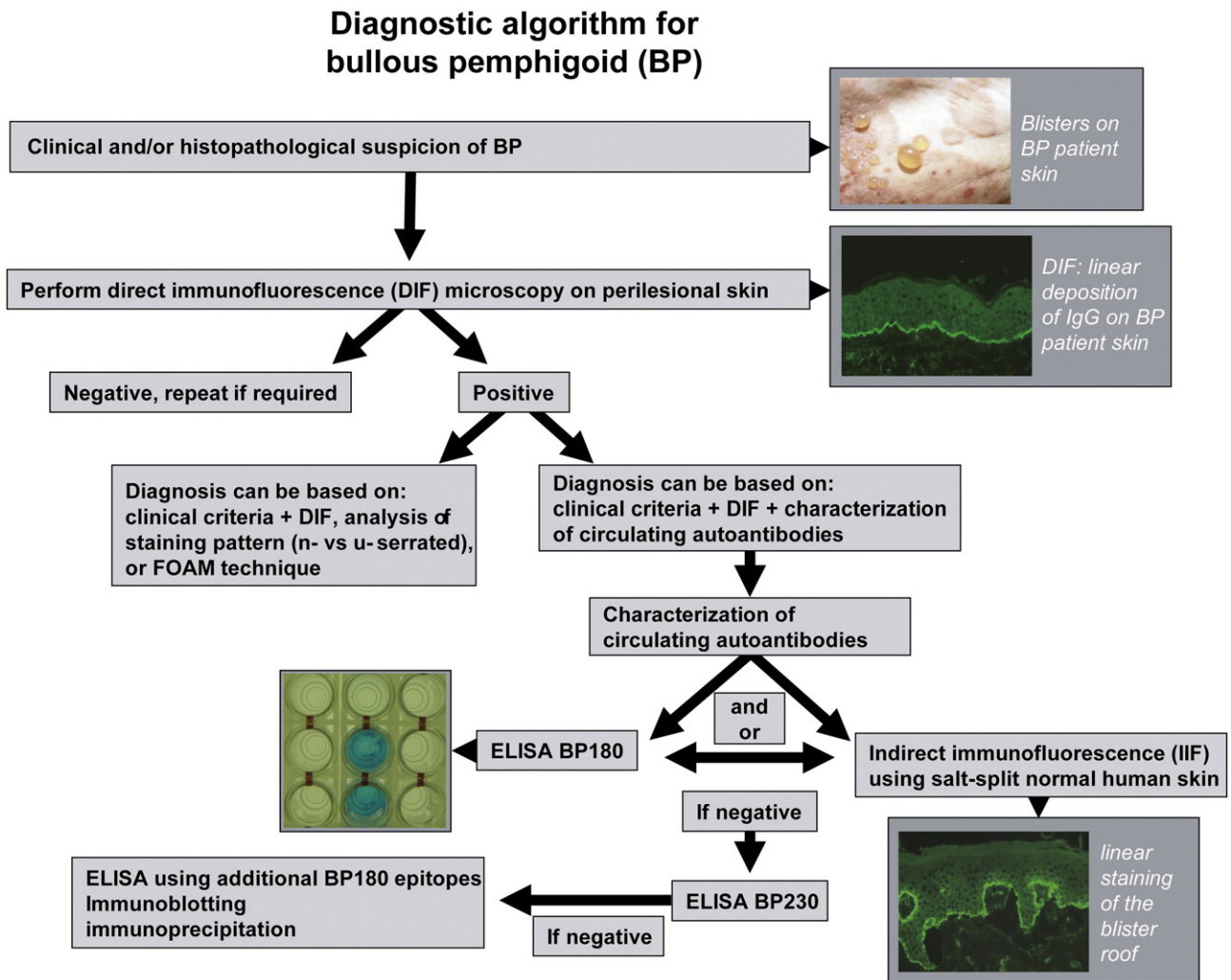


Fig. 3 Diagnostic algorithm for bullous pemphigoid (BP). ELISA, enzyme-linked immunosorbent assay; FOAM, fluorescence overlay antigen mapping; IgG, immunoglobulin G.

disease or in atypical cases of BP. Immunohistochemical studies have recently suggested that the detection of C3d deposits at the dermal-epidermal junction in formalin-fixed tissue is useful for the diagnosis^{57,58}; however, it is not yet known whether this technique is reliable enough to make unnecessary a second biopsy specimen for direct IF microscopy studies.

Direct IF microscopy

Direct IF microscopy studies characteristically show linear deposits of IgG or C3, or both, and more rarely, of other Ig classes, such as IgE and IgA, along the epidermal basement membrane (Figure 3). Testing of autologous patient's skin after treatment with 1 mol/L NaCl can allow the distinction of patients with BP (deposits on the epidermal sides of the split or on both side of the split) from those with epidermolysis bullosa acquisita, anti-epiligrin mucous membrane pemphigoid, and anti-p200 pemphigoid (deposits on the dermal side)⁵⁹ (Figure 3). Finally, close analysis of the linear fluorescence pattern (n-serrated pattern observed in BP vs u-serrated pattern observed in EBA) at the basement membrane zone (BMZ),⁶⁰ or the images obtained by fluorescence overlay antigen mapping (FOAM) technique⁶¹ seems very useful to rapidly distinguish patients with BP from other autoimmune subepidermal blistering diseases (Figure 3).

Indirect IF studies

Indirect IF studies demonstrate in 60% to 80% of patients the presence of circulating IgG autoantibodies that typically bind to the epidermal side of salt-split normal human skin⁶² (Figure 3). The latter substrate has been found to be superior to intact skin and other substrates such as monkey esophagus. Additional circulating autoantibodies of the IgA, IgE, and IgM class can also be found. In gestational pemphigoid, patients have IgG1 and IgG3 complement-fixing antibodies that are best detectable by a complement-binding indirect method.

Enzyme-linked immunosorbent assays

Enzyme-linked immunosorbent assays (ELISAs) that use recombinant proteins encompassing various portions of BP180, such as the NC16A domain, the COOH-terminal portion, or its entire ectodomain, have been found to be specific and sensitive (Figure 3). These ELISAs have several advantages compared with traditional diagnostic approaches, including that they allow multiple sample testing, are rapid and easy to perform, and can be better standardized. Antigens are tested under native conditions, and reactivity against conformational antigens is not missed (Table 1).⁶³⁻⁷⁹

ELISAs using BP230 recombinant proteins have been so far less sensitive and specific compared with BP180 ELISAs

but have confirmatory diagnostic value.^{71,72,74,77} Recent studies in France have suggested that ELISA BP230 testing be performed only in the subset of suspected BP patients in whom the ELISA BP180 NC16A is negative.^{78,79} Under these circumstances, the BP230 ELISA allows the detection of circulating autoantibodies in up to 5% to 10% of anti-BP180-negative patients.

Clinical experience and data from several published ELISA studies indicate that the sensitivity of the commercially available BP180 ELISAs (70% to 98%) is not superior to indirect IF microscopy studies using salt-split healthy skin (Table 1). However, the sensitivity can be increased up to 100% when various ELISAs using the NC16A domain and other extracellular portions of BP180 or of BP230 are used together.^{11,64,68,70,71,74,78,79} ELISAs have nowadays largely replaced immunoblot and immunoprecipitation techniques, which are technically much more demanding. These technical approaches are only performed in particular cases, such as for ELISA BP180-negative or BP230-negative serum samples or in investigative studies.

Current diagnostic challenges

In localized and atypical variants, diagnosis of BP critically relies on direct IF microscopy studies and the detection of circulating autoantibodies directed against BP180 or BP230, or both. Although we usually make the diagnosis of early-stage of BP (pemphigoid incipiens, or prebullous BP) in patients with pruritic skin conditions in whom positive direct IF findings even when blisters are lacking, this approach is personal and questionable. A small number of these patients do not appear to have an autoimmune blistering eruption, even after a follow-up of years, as was recently observed.¹

In fact, we lack well-accepted diagnostic criteria. Furthermore, we still have problems with how to interpret the presence of circulating autoantibodies in patients with eczematous, urticarial, excoriated, or ulcerated lesions without obvious blistering, in whom results of direct IF microscopy studies are negative. In addition, can we realistically continue to talk about BP in a patient without bullae, missing the characteristic elementary lesions of the disease? The naming of a disease involves phenotypic description and knowledge about the natural history and the prognosis. We do have some information in the case of typical BP, but not really in the atypical variants; therefore, a working set of diagnostic criteria for a *definite* and a *probable* diagnosis of BP is urgently needed.¹

Prognosis

Although few data are available on the evolution of the disease, BP appears to have a chronic course with frequent exacerbations or relapse, even after successful treatment. A recent prospective study of 114 BP patients found up to

Table 1 A survey of published enzyme-linked immunosorbent assay studies in bullous pemphigoid

Study	BP sera	Control sera	IIF *	Antigen	Recombinant protein †	Se ‡	Sp
First author			(%)			(%)	(%)
Zillikens, 1997	50	107	84	BP180	P-NC16A (AA 490-562)	94	100
Nakatani, 1998	110	50	89	BP180	P-NC16A (AA 507-548)	96	100
					P-C-terminus (AA 1365-1413)	38	100
Husz, 2000	43	60	NK	BP180 BP230	Combination of 3 peptides (BP180: AA 507-528; BP230: AA 1814-1834, AA 1793-1813)	91	88
Hata, 2000	83	40	100	BP180	E-ectodomain (AA 490-1497)	66	95
					P-NC16A (AA 490-566)	70	93
Kobayashi, 2002	64	538	100	BP180	P-NC16A (AA 490-566)	84	99
Hofmann, 2002	116	100	100	BP180	E-NC16A+ Col 15 (AA 490-811)	80	99
					E-C-terminus (AA 1351-1497)	47	97
Sakuma-Oyama, 2004	102	94	100	BP180	BP180-NC16A (commercial kit) §	89	98
Mariotti, 2004	78	107	100	BP180	P-NC16A (AA 490-562)	82	100
					Combination of 2 P-fragments (AA 1080-1107, AA 1331-1404)	41	99
Thoma-Uszynski, 2004	127	51	NK	BP180 BP230	E-ectodomain (AA 485-1497)	95	94
					Combination of 3 eukaryotic fragments (N: AA 1 1307; C1: AA 1881-2649; C2: AA 2077-2649;)	82	65
Kromminga, 2004	56	76	100	BP230	Combination of 5 E-fragments (N1: AA 1-671; N2: AA 607-1073; N3: AA 1014-1758; C1 AA 1710-2189; C2: AA 2137-2649)	63	93
Tsuji-Abe, 2005	14	447	100	BP180	BP180-NC16A (commercial kit) §	79	99
Yoshida, 2006	239	430	100	BP180	BP180-NC16A (commercial kit) §	70	99
		109		BP230	Combination of 2 P-fragments (N: AA 1-979; C: AA 1870-2650)	72	99
		NK		BP180 + BP230	Combination of both assays	97	NK
Sitaru, 2007	118	494	94	BP180	Tetramers of NC16A	90	98
Di Zenzo, 2008	49	80	96	BP180 + BP230	Combination of 1P and 2 E-fragments BP180-NC16A/ BP180-ECD/BP230-NH2term	100	82
Feng, 2008	42	24	100	BP180	BP180-NC16A (commercial kit) §	98	96
Tampoia, 2009	20	82	100	BP180	BP180-NC16A (commercial kit) §	90	98
				BP230	BP230 (commercial kit) §	60	98
Charneux, 2011	138	0	62 (70/113)	BP180	BP180-NC16A (commercial kit) §	86	NK
				BP230	BP230 (commercial kit) §	59	NK
				BP180 + BP230	Combination of both assays	91	NK
Roussel, 2011	190	78	81	BP180	BP180-NC16A (commercial kit) §	79	90
				BP230	BP230 (commercial kit) §	61	96
				BP180 + BP230	Combination of both assays	87	88

AA, amino acid; ECD, extracellular domain; IIF, indirect immunofluorescence; NK, not known; Se, sensitivity; Sp, specificity.

* Percentage of positivity by indirect immunofluorescence microscopy.

† Recombinant proteins used as substrate. P- for prokaryotic or E- for eukaryotic expressed protein.

‡ In most studies, sensitivity is largely overestimated because selected serum samples from BP patients with positive IIF studies were used.

§ A commercially available enzyme-linked immunosorbent assay kit (MBL, Naka-ku, Nagoya, Japan).

45% of the evaluable individuals experienced a relapse, which in most cases occurred within 6 months after cessation of therapy.⁸⁰

Besides a significant effect on the quality of life because of the severe itch and potentially widespread inflammatory lesions, BP has a considerable mortality rate, which varies among studies, of between 12% and 40% in the first

year.^{81,82} These different figures likely reflect recruitment biases, different management practices in the use of systemic corticosteroids and immunosuppressive drugs,⁸¹ and inclusion of patients of different ages and variable general conditions.^{83,84} In our prospective study encompassing all BP patients collected in a 2-year period in Switzerland, we found the probability of death 1, 2, and 3 years was 20.9%,

28%, and 38.8%, respectively (Cortes B et al submitted, personal observation). These data should appropriately reflect the true overall prognosis of BP, because the study prospectively encompassed patients managed in both ambulatory and hospital settings, making inclusion biases unlikely. Several European studies found the mortality rate was increased in patients with BP compared with age-adjusted control subjects.^{81,85,86} In our study, we found a standardized mortality ratio ranging from 2 to 15, according to the age range. The increase in the risk of death was particularly high for the people younger than 70 years.

Principles of therapy

The management of BP has undergone significant changes in the past decade.^{87,88} A large multicenter study demonstrated that potent topical corticosteroids, such as clobetasol propionate, are superior than the previous gold therapy standard of oral corticosteroids in the control of the disease, side effect profile, and overall survival.⁸⁹ In addition, a mild regimen with clobetasol propionate cream (10 to 30 g/d, tapered over 4 months) was not inferior to the original treatment protocol (40 g/d initially, with tapering over 12 months). This mild regimen allowed a 70% reduction of the cumulative doses of corticosteroids and further improved the outcomes and side effect profiles of BP patients.⁹⁰

Systemic corticosteroids continue to be widely used in clinical practice, and their efficacy has been confirmed in uncontrolled and controlled studies^{89,91,92}; however, their use can be associated with significant side effects.^{81,89} For patients with extensive disease, oral prednisone, at the dosage of 0.5 to 1 mg/kg/d, usually controls the disease within 3 weeks. This dose is then progressively tapered over a period of 6 to 9 months. Practically, whenever possible (ie, self-application feasible, good general condition of the patient, external help available, and/or presence of contraindications to systemic steroids) we prefer to introduce topical corticosteroid therapy; whereas we prefer systemic therapy in bedridden patients or when regular topical application cannot be assured.

The use of immunosuppressive drugs, such as azathioprine,^{93,94} mycophenolate mofetil,⁹⁵ methotrexate, and, less frequently, chlorambucil and cyclophosphamide, is a matter of debate. Some clinicians introduce them only when corticosteroids alone fail to control the disease or if the latter are contraindicated. The only controlled study available so far failed, however, to prove an advantage of a combination of prednisone and azathioprine vs prednisone alone, because more complications were observed in the azathioprine group. In the latter study, thiopurine methyltransferase levels were not assessed, precluding dose optimization. The choice of an immunosuppressive drug depends on the profile of its side effects, the patient's overall condition, the experience of the physician with the molecule,

and finally, cost issues. Although mycophenolate mofetil, for example, is likely to have less hepatic side effects compared with azathioprine, the latter is much cheaper and may show a more rapid onset of action with a better corticosteroid-sparing effect.⁹⁵

Dapsone, as well as the association of nicotinamide and minocycline or tetracycline, have also been tried alone or as adjuvant therapy with some success^{96,97} and may be helpful in mild disease. The experience with biologicals in BP, such as rituximab, is still anecdotal,⁹⁸ but in certain situations, they likely represent the last therapeutic option available to try.⁸⁰ Finally, in all BP patients, it is important to undertake all measures aimed at preventing the complications of both the cutaneous lesions and of the treatment.

Disease activity and relapse markers

In retrospective cohorts with a limited number of patients, IgG ELISA BP180 NC16A values appeared to parallel disease severity or activity, or both.^{99,100} We were able to confirm in our recent prospective multicenter study with 49 BP patients that disease severity and activity of BP correlate not only with the levels of IgG against the BP180-NC16A domain but also against a COOH-terminal epitope of BP180.¹¹ Two prospective studies recently showed that disease severity fluctuates in parallel with the levels of IgG against BP230 in at least some patients⁷⁴ (our unpublished observations), whereas two other recent retrospective studies in France indicated that performing an ELISA BP230 is of little help.^{78,79} Although the usefulness of repeated ELISA BP180 NC16A testing in guiding the reduction of therapy is still to be demonstrated, a recent prospective study has indicated that the detection of a moderate titer ELISA BP180 (NC16A) score (3 times higher than the cutoff) at cessation of therapy represents a good indicator for an increased relapse risk in BP patients.⁸⁰ Because of these findings, we now check the ELISA BP180 values before cessation of therapy to identify those patients with increased relapse risk who need closer follow-up in the next 6 months.

Pathogenesis

Targeted antigens and humoral immune response

BP180 and BP230 are components of hemidesmosomes, junctional complexes promoting adhesion of epithelial cells to the underlying BMZ in stratified and other complex epithelia, and are involved in the maintenance of dermoepidermal adhesion¹⁰¹ (Figure 4). BP180 is a transmembrane molecule with a large collagenous extracellular domain^{102,103} (Figure 4). Mutations in the gene encoding BP180 underlie a form of nonlethal junctional epidermolysis bullosa characterized by skin blistering and fragility,

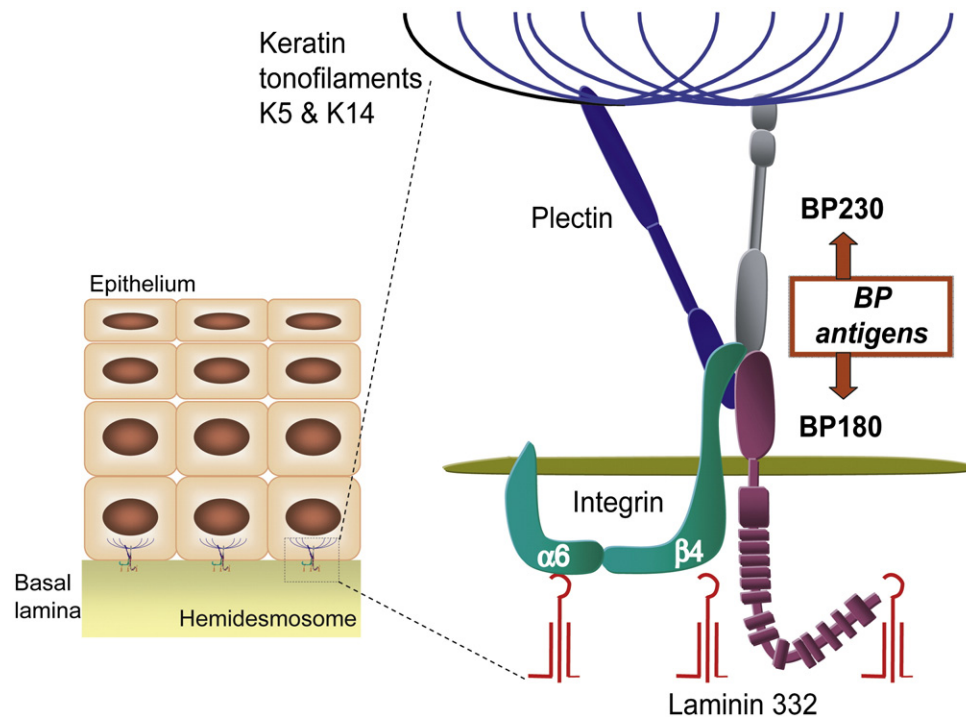


Fig. 4 The epidermal basement membrane zone (BMZ). The diagram depicts the major components of the dermal–epidermal junction. The components include the keratin filaments, the hemidesmosomal proteins, and the extracellular matrix proteins, such as laminin 332. Bullous pemphigoid (BP) BP180 and BP230 are components of hemidesmosomes, junctional complexes promoting adhesion of epithelial cells to the underlying basement membrane zone in stratified and other complex epithelia, such as skin and mucous membranes.

alopecia, and dental and nail abnormalities,^{104,105} an observation attesting to the importance of this molecule for cell adhesion.

In contrast, BP230 is a cytoplasmic component that belongs to the plakin family of proteins that consist of a central coiled-coil region flanked by two globular end domains^{106–108} (Figure 4). The recent description of a mild form of epidermolysis bullosa simplex due to mutations in a part of the dystonin gene encoding for the coiled-coil domain of the epithelial isoform of BP230 (BPAG1-e) confirms the role of BP230 in maintenance of cytoarchitecture and cell resilience.¹⁰⁹

Almost all BP patients have autoantibodies binding to an immunodominant region of BP180, the NC16A domain, which is located extracellularly close to the transmembrane domain. Additional antigenic sites exist on the extracellular and intracellular domain of BP180, which is recognized by up to 96% of the BP sera.^{11,63,64,68,70,110–119} A subgroup of patients react with different BP180 extracellular epitopes, but not with the NC16A domain, suggesting the involvement of non-NC16A epitopes in the pathogenesis of the disease⁷⁰ (own unpublished observations).

BP patients also exhibit significant reactivity with BP230. Approximately 60% of BP sera have IgG autoantibodies that recognize the COOH-terminal domain of BP230.^{120,121} IgG autoantibodies binding to the N-terminal and the central α -helical coiled-coil domain are also found.^{71,120,122–125} In addition to the IgG isotype, IgE and IgA autoantibodies

bound to both BP180 and BP230 are frequently found in BP patients.^{11,126–130}

We recently found that epitope-spreading phenomena occur in BP patients involving not only BP180 but also BP230. Noteworthy, IgG reactivity against the intracytoplasmic BP230 never preceded that against the ectodomain of the BP180 protein (our unpublished observations).

Cellular immune response

The involvement of autoreactive T cells in the pathogenesis of BP and related disorders has been somewhat neglected so far. Autoreactive T-cell responses to the ectodomain of BP180 in BP, gestational pemphigoid, and in healthy individuals have been identified.^{131,132} Autoreactive T cells react with the same regions of BP180 and BP230 that are recognized by IgG autoantibodies.¹¹⁹ Strikingly, epitope recognition appeared to be restricted by certain human leukocyte antigen (HLA) major histocompatibility class II alleles, such as the *HLA-DQB1*0301* allele, that are prevalent in BP.¹³³ CD4 T-cell lines and clones derived from BP patients were shown to produce both Th1 and Th2 cytokines.¹³¹

Because Th1 cytokines, such as interferon- λ (INF- λ) are able to induce the secretion of IgG1 and IgG2, whereas Th2 cytokines, such as interleukin (IL)-4, IL-5, and IL-13, have regulate the secretion of IgG4 and IgE,¹³⁴ the detection of anti-BP180 and anti-BP230 antibodies of the

IgG1, IgG4 and IgE isotype^{128,135} in BP patients suggests that autoreactive Th1 and Th2 cells are both involved in the regulation of the immune response. Lesional tissue and serum samples of BP patients show an increased expression of various cytokines, the level of which grossly correlates with the skin lesion number. The relative low concentrations of IFN- λ and IL-2 compared with those of IL-4, IL-5, and IL-10 suggest a predominance of a Th2 response.¹³⁶

Animal models

The relative contribution of humoral and cellular immune response in BP has been investigated in detail in animal models. Transferred antibodies were raised against the murine homologue of the human BP180 (hBP180)-NC16A domain or were obtained and purified from BP patients and subsequently injected into hamster, neonatal wild-type, and/or “humanized” mice expressing hBP180. Most of the data obtained in these models are in agreement with findings in BP patients.¹³⁷⁻¹⁴⁷ Overall, the results demonstrate that IgG autoantibodies are pathogenic by triggering complement activation, recruitment of inflammatory cells (neutrophils, eosinophils, basophils, mast cells), and release of proteases, such as matrix metalloproteinase (MMP)-9 and neutrophil elastase.¹⁴⁸⁻¹⁵² The latter, which are strongly expressed by neutrophils and eosinophils and are found in lesional skin, are thought to proteolytically degrade various extracellular matrix proteins, including the extracellular domain of BP180.^{149,150,153-156} Besides IgG, IgE anti-BP180 autoantibodies also contribute to lesion development by stimulating degranulation of basophils or mast cells, or both.¹⁵⁷ IgE purified from patients with BP injected into human skin grafted onto nude mice was able to induce histologic changes reminiscent of those observed in BP.¹⁵⁸ Eosinophils appear to be important in mediating the inflammatory process and tissue injury.¹⁵⁹

The pathogenic relevance of autoantibodies to BP230 has received less attention so far. Although reactivity against this cytoplasmic antigen has been often considered an epiphenomenon, some observations suggest its involvement in tissue damage. Specifically, immunization of rabbits with BP230-derived peptides and passive transfer of anti-BP230 antibodies in neonatal mice was able to induce a local inflammatory response and subepidermal microdetachments, respectively, in the animals.^{160,161}

We recently assessed the dynamics of the humoral response to hBP180 in mice grafted with skin obtained from transgenic mice expressing hBP180. The results showed that antibodies develop first against epitopes on the extracellular domain of BP180 and later against intracellular ones.¹⁶² Altogether, findings in human (see above) and in animals are in keeping with a model indicating the initial involvement of an extracellular immunodominant epitope of BP180 that triggers an inflammatory cascade with tissue damage leading subsequently to exposure and

recognition of antigenic reactive sites on the intracellular domain of BP180 and BP230.

With regard to the cellular immune response in BP, a recent animal model has demonstrated that the IgG response to BP180 is critically dependent on major histocompatibility complex class II-CD4⁺ T-cell interactions.¹⁶³ In addition, an active BP animal model, established by the transfer of splenocytes from wild-type mice immunized with hBP180 to hBP180 humanized mice, produced anti-hBP180 IgG antibodies and developed BP-like features¹⁶⁴; hence, T cells and other innate immune system players critically contribute to the development and progression of tissue damage in BP.

Conclusions

The autoimmune etiology of BP is now established:

1. patients have autoantibodies and autoreactive T cells to well-characterized self-antigens
2. tissue injury occurs where antibody-antigen complexes are found
3. *in vivo* animal models of the disease have provided unequivocal evidence for the pathogenic role of autoantibodies and cellular immune response
4. in gestational pemphigoid, the transplacental transfer of anti-BP180 autoantibodies from the mother into the neonate can cause a transient bullous eruption
5. the levels of IgG and IgE autoantibodies directed against the immunodominant region of BP180 autoantibodies parallel disease activity
6. the disease occurs in association with distinct human leukocyte antigen genotypes and responds to immunosuppressive therapy

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