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Immunoglobulin E and bullous pemphigoid

Clinical features and histological findings in bullous pemphigoid (BP) suggest a Th2-oriented inflammatory reaction, especially in the early stages of the disease. Elevated total serum IgE levels, blood eosinophilia, and elevated serum levels of different soluble inflammatory Th2 response mediators have been described in large cohorts of patients with classic clear-cut BP manifestations. Direct immunofluorescence, indirect immunofluorescence, and anti-BP230 and anti-BP180 IgE ELISA testing show self-reactive IgE autoantibodies in a consistent number of BP patients. Both IgE autoantibodies and a Th2-oriented immune response may play a role in the initial phases of BP and atypical cases of BP, such as severe erythematous and urticarial forms of BP, as well as blister formation. Two recently reported experimental murine models employing IgE autoantibodies against BP180 have been reported and the successful treatment of bullous pemphigoid with the anti-IgE antibody, omalizumab, supports the roles played by IgE autoantibodies in BP pathogenesis.

Key words: bullous pemphigoid, Th2, anti-BP230 and anti-BP180 IgE, omalizumab

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Bullous pemphigoid (BP) is a rare autoimmune subepidermal blistering disease that mainly affects the elderly, and is classically characterized by large, tense, serous or haemorrhagic bullae arising on erythematous, urticarial, eczematous or apparently normal skin [1, 2]. However, non-bullous presentations can account for 20% of BP [1].

BP is associated with tissue-bound and circulating autoantibodies directed against two hemidesmosomal proteins: transmembrane BP180 (also termed type XVII collagen or BPAG2) and intracytoplasmic plakin family protein BP230 (also termed BPAG1) [3]. Hemidesmosomes promote the integrity of the dermal-epidermal junction (DEJ) by bonding cytoplasmic keratin intermediate filaments of basal keratinocytes to laminins in the lamina densa [3]. The non-collagenous region 16A (NC16A) of BP180 extracellular domain contains the major epitopes for anti-BP180 autoantibodies [4], while anti-BP230 antibodies in BP sera react with various domains of the entire molecule [5].

The clinical significance [6-9] and pathogenic role [10-15] of IgG autoantibodies in BP have been clearly established. The detection of autoreactive IgG autoantibodies is a cornerstone for diagnosis of BP [1, 2]. Various *in vitro* and *in vivo* studies have provided strong evidence for the pathogenic role of IgG autoantibodies in BP (mainly for anti-BP180 IgG) [12]. More recently, considerable emphasis has been given to the clinical relevance and pathogenic role of circulating and tissue-bound IgE autoantibodies. Patients with BP have been known to have high levels of IgE since 1974 [16], and in 1984, Asbrink *et al.* correlated high IgE serum levels with disease activity [17]. Furthermore, it has been known for two decades that serum IgE antibodies bind to the epidermal side of the basement membrane

zone (BMZ) of split skin in BP [18]. Initially, the target of IgE antibodies in sera of BP patients was identified as BP230 [19]. Later on, anti-BP180 IgE antibodies were also discovered in sera of BP patients [20].

In BP patients, a T-helper cell type 2 (Th2) response is clearly evident. BP patients often present urticarial lesions, peripheral eosinophilia, and high IgE serum levels [21, 22]. These clinical observations are supported by numerous studies demonstrating a Th2 response in BP patients [23]. In this context, the role of IgE in BP pathogenesis is still not fully elucidated. Here, we report the latest developments on the role of IgE in BP and the potential therapeutic implications that this might have.

BP skin lesions: clinical aspects, histological findings, and direct immunofluorescence

Simply by analysing the clinical features of BP patients, a Th2 response is clearly evident. Tense blisters arise on pruritic erythematous and/or urticarial or eczematous plaques. The latter may be the only manifestations during an initial persistent non-bullous phase, lasting several weeks or months, that are seen in up to 20% of BP patients [1, 2]. Bullae may even be absent throughout the disease duration. In elderly patients with intense itching, with or without inflammatory skin lesions, especially when symmetrically distributed, BP should be ruled out [24]. Moreover, initial lesions may be atypical, resembling polycyclic, targetoid, nodular or lichenoid lesions. Therefore, BP is, by definition,

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a polymorphic disease in which the presence of bullae is not always mandatory. However, most of our knowledge on BP pathogenesis regards blister formation, and it is still not fully understood how other BP lesions form.

Histological findings from lesional skin biopsy specimens confirm the Th2-oriented inflammatory reaction, especially in the early stages of the disease. Histology of early lesions shows subepidermal clefts, eosinophilic spongiosis, and/or an infiltrate of eosinophils in the upper dermis lining the dermal-epidermal junction. Whereas histological changes in established lesions instead are characterized by subepidermal bullae, an absence of acantholysis, and a superficial dermal infiltrate mainly with eosinophils and a few neutrophils or lympho-monocytes [25]. The inflammatory infiltrate, rich in eosinophils is, apart from subepidermal splitting, the key histological feature of BP [26, 27] and seems to play a crucial role in BP pathogenesis [28]. Th2 cytokines activate eosinophils that preferentially infiltrate the skin of BP patients in the presence of IL-5 [29]. In this context, eosinophils express MMP-9 that is capable of cleaving BP180 antigen [30], leading to tissue damage and separation at the dermal-epidermal junction [31]. This process seems to depend on eosinophil adhesion and Fc γ R binding, suggesting that BP autoantibodies directly activate eosinophils and/or attract them to the dermal-epidermal junction [31]. However, conflicting results have been reported on the possible relation between a specific autoantibody isotype and eosinophil activation. Although IgE autoantibody levels showed a strong association with local eosinophil accumulation [22], it seems unlikely that eosinophils directly interact with IgE, because they lack functionally active high-affinity IgE receptors (Fc ϵ RI) [21, 32]. In fact, BP antigen-specific degranulation of basophils and/or mast cells, rather than eosinophils, has been suggested as a mechanism by which IgE may contribute to lesion development [21]. Initial mast cell degranulation and subsequent eosinophil and, to a lesser extent, basophil degranulation have been demonstrated as the sequence of histopathological events in the pathogenesis of BP based on electron and light microscopy [33], and further confirmed in murine models [34]. The role of mast cells in BP pathogenesis is also underlined by reported interaction of C5a-C5aR on mast cells, consequent activation of the p38 MAPK pathway, and subsequent MC degranulation, which is indispensable for development of experimental BP [35]. Indeed, in the skin of BP patients, the presence of IgE on mast cells, but also eosinophils, has been recently reported [36]. Of note, a portion of IgE antibodies were BP-specific. In addition, BP180 and Fc ϵ RI-bound IgE co-localized on mast cells and/or eosinophils. Importantly, the authors demonstrated that BP180-IgE complexes from two BP patients were indeed capable of cross-linking Fc ϵ RI, leading to degranulation of rat basophils. In parallel, eosinophil and mast cell activation in BP patients based on detection of high serum concentrations of major basic protein, eosinophil-derived neurotoxin, eosinophil cationic protein (ECP), and mast cell tryptase have been demonstrated in other studies [37, 38]. Interestingly, ECP serum concentration decreased in patients with ongoing remission, but not in patients who later relapsed [37]. These data once more emphasize the importance of mast cells and eosinophils in disease pathogenesis and the possible relation between BP-specific IgE and granulocyte activity [37].

Direct immunofluorescence (DIF) of perilesional skin shows *in vivo* linear deposition of IgE at the DEJ in 18-41% of BP patients [18, 26, 39-43]. In contrast, other authors could not detect anti-BMZ IgE in the skin of BP patients [23, 44]. However, IgG and IgE in BP patients recognize the same dominant BMZ epitopes and this could be a good explanation for the lack of detectable IgE staining along the BMZ in patients with high IgG levels.

Yayli *et al.* were the first to investigate IgE deposition along the BMZ and the relationship between this and clinical phenotype. They detected a linear deposition of IgE along the BMZ in 41% of 44 BP patients, including two patients without IgG or C3 reactivity [42]. They found that cases with *in vivo* IgE deposition had more severe urticarial lesions than IgE-negative cases [42].

In line with this, Moriuchi *et al.* also found IgE deposition at the BMZ in 18 of 100 patients based on direct immunofluorescence. Interestingly, four of these had atypical clinical features: a case of BP associated with psoriasis, a case of erythrodermic BP, and two cases of pemphigoid nodularis. Notably, in three patients with these unusual manifestations, no *in vivo* IgG deposition at the BMZ was detectable, even though ELISA and salt-split skin indirect IF revealed circulating IgG autoantibodies in the patients' sera [41]. In all three cases, patients did not develop frank bullous lesions. IgE might perhaps be associated with very rare atypical variants of BP, involving the pre-bullous phase of urticarial lesions, such as erythrodermic BP or pemphigoid nodularis. In contrast to Yayli *et al.*, Moriuchi *et al.* reported no difference in disease severity or clinical course or outcome between *in vivo* IgE-positive and IgE-negative cases of BP [41].

IgE deposition at the BMZ in BP patients is found in a minority of patients. However, in some cases it is the only reactivity apparent on DIF in patients without IgG or C3 reactivity. It is not yet clear whether *in vivo* deposition of IgE is useful for predicting disease course and severity, although predominant IgE deposition seems to be associated with non-bullous lesions.

Total serum IgE levels, blood eosinophilia, and soluble inflammatory Th2 response mediators

Elevated total serum IgE levels have been described in multiple reports in 70-85% of BP sera [17, 43, 45, 46]. In most studies of BP patients, a positive correlation between total IgE serum levels, anti-BMZ antibody levels, and disease activity is reported [16, 17, 47-49]. Recently, Van Beek *et al.* identified a correlation between total serum IgE levels and the extent of urticarial/erythematous lesions, but not blisters/erosions [50]. These data are in contrast with a previous report that showed total IgE serum levels to be rather unrelated to disease severity, with higher total mean serum IgE values in patients with bullae compared to those with urticarial lesions [51].

Elevated serum levels of different soluble inflammatory Th2 response mediators have been found in BP patients compared to healthy controls. In BP patients, serum levels of sCD23, the soluble form of a low-affinity Fc γ RII involved in B-cell growth and differentiation, was found to be

correlated with both disease activity and total serum IgE levels [52, 53]. CD23 expression on peripheral blood B cells was shown to be higher in BP patients than in controls, even though this did not correlate with sCD23 levels. CD23 expression paralleled total serum IgE levels, which were significantly higher in severe compared to moderate BP [54]. BP patients also have significantly higher serum levels of IL-4 and sCD30 [55]. Serum levels of the thymus and activation regulated chemokine (TARC) levels correlated with total serum IgE levels [56, 57]. IL-5 and eosinophil cationic protein levels in blister fluid of BP patients were reported to correlate with total serum IgE levels [58].

In line with this, blood eosinophilia has been reported in 50-60% of BP patients [49, 59, 60]. Blood eosinophilia was described in a large cohort of patients with classic clear-cut BP manifestations [50]. However, no study has, to date, investigated the correlation between the number of blood eosinophils and urticarial lesions. Only one case report described a correlation between the extent of blood eosinophilia and disease severity [49].

The pathogenic mechanisms that link elevated total serum levels of IgEs, blood eosinophilia, and soluble Th2 response mediators with actual formation of BP skin lesions are still unclear. For example, levels of eosinophil chemotactic factor of anaphylaxis, which is responsible for eosinophil chemotactic activity as well as the accumulation of eosinophils in BP lesions, do not appear to correlate with total serum IgE levels or serum eosinophilia in BP [26, 60, 61]. However, Czech *et al.* observed elevated levels of total serum IgE, eosinophil cationic protein, and neutrophil-derived myeloperoxidase both in serum and blister fluid in BP, but not in pemphigus vulgaris patients [62]. Recently, Messingham *et al.* reported Fc ϵ RI expression both on peripheral blood and skin eosinophils in BP patients [63].

These data together point towards a strong systemic and local Th2-oriented inflammatory response in BP [23].

Disease-specific circulating IgE

Already in the 70s, the possible role of other mediators in addition to anti-BMZ IgGs was hypothesized. In fact, anti-BMZ IgG titres did not always correlate with clinical severity of BP, whereas this was always evident in patients with pemphigus [64]. In 1974, Abersman [16] and Provost hypothesized the importance of anti-BMZ IgE antibodies in BP pathogenesis [39]. Serum anti-BMZ IgE reactivity, investigated by indirect IF microscopy on 1-M NaCl-split skin, was described in 0-53% of BP patients according to different case series [18, 19, 26, 45, 65-67], with a mean of 20%. In some, but not all studies, serum anti-BMZ IgE reactivity was associated with elevated total serum IgE levels [19, 65, 67].

Antigen-specific circulating antibodies, anti-BP230 and anti-BP180 IgE can be detected in sera from BP patients by ELISA in 50-67% and 22-77% of cases, respectively [20, 22, 41, 68-75]. Therefore, anti-BP180 IgEs seem to be more frequently represented in BP patients compared to anti-BP230 IgEs. Interestingly, however, our unpublished data show that the mean levels of IgE reactivity against BP230 are higher than those measured for BP180 (unpublished data).

In the late 90s, all attention was focused on anti-BP230 IgEs [19, 76]. Based on the observation that most BP patients had increased serum levels of IgEs, which seemed to correlate with disease severity, Delaporte *et al.* studied the presence of IgE directed towards BP230 in sera obtained from BP patients [68]. The authors identified anti-BP230 IgE autoantibodies only in sera from patients with severe disease. Several studies confirmed these results [19, 76-78]. More recently, anti-BP230 IgE has been found in up to 67% of patients with BP [22]. In a report of two BP patients, IgE, but not IgG, reactivity against a recombinant form of the C-terminal region of BP230 was related to disease activity [76]. In 32 other BP patients, IgE antibodies against BP230 preferentially recognized the C-terminus and paralleled total IgE serum levels [19, 79]. Furthermore, Ishiura *et al.* identified a correlation between anti-BP230 IgE titres and local eosinophil accumulation [22]. In one study, circulating anti-BP230 IgE was found in all BP patients' sera while no BP180-specific IgE autoantibodies were detected in sera and blister fluid of these BP patients [27]. However, most studies reveal both IgE anti-BP230 and anti-BP180 antibodies in the sera of BP patients [22, 79]. Generally speaking, most recent studies focus on anti-BP180 autoimmune reactivity in BP patients rather than anti-BP230 autoimmune reactivity. This is also true for studies on anti-BMZ antibodies. Besides IgG autoantibodies, IgE anti-BP180 autoantibodies of IgA and IgE isotypes were also described in BP patients [21, 71, 80]. All autoantibody isotypes target mainly the NC16A domain [8, 21, 71, 81]. Epitopes outside the NC16A domain have also been described to be recognized by IgE [81-83]. The percentage of BP patients with IgE anti-BP180 NC16A autoantibodies varied substantially in the different reports, ranging from 22% to 100% of BP patients, with a mean of 60% [20, 22, 42, 68-72, 75, 82, 84-86]. The large variation in anti-BP180 IgE reactivity in these studies may be, in part, explained by the different test systems, *i.e.* ELISA or immunoblotting and the use of diluted or undiluted patient sera. For instance, circulating anti-NC16A IgEs can be detected by standard ELISA in 22-30% of BP patients [22, 87], while NC16A-specific IgE class autoantibodies were detected in up to 77% of BP sera using a novel highly specific and sensitive ELISA [72].

Also, the correlation between IgE serum levels and BP severity varies according to different studies [32, 75, 86]. Most studies reported a correlation between IgE anti-BP180 antibodies and BP disease activity [68-73, 88]. In contrast, only one study showed no apparent correlation [74]. For a BP patient with both circulating IgG and IgE anti-NC16A autoantibodies, Kamiya *et al.* identified a correlation between IgE autoantibody titres and urticarial erythema, as well as an association between IgG autoantibody titres and blister formation [73]. Very recently, a correlation between IgE anti-BP230 autoantibodies and a nodular, but not erythematous, phenotype was reported [70]. Thus, not only tissue-bound IgEs, but also disease-specific autoreacting circulating IgEs (anti-BP180 and anti-BP230) seem to correlate with atypical BP lesions. A very recent study on 117 BP patients reported no association between circulating BP180 NC16A IgE antibodies and urticarial or erythematous lesions, while the presence of IgG anti-BP180 NC16A antibodies was associated with the occurrence of erosions and blisters, but not urticarial or erythematous lesions [88].

In 2008, Iwata *et al.* performed a study in which anti-BP180 IgG and IgE titres, as well as both anti-BP230 IgG and IgE titres, were considered for each BP patient [87]. The Authors described a tendency for the presence of IgG and IgE antibodies for each antigen. Nonetheless, they also observed cases with negative circulating autoimmune IgGs and positive IgEs, and hypothesized that IgE antibodies may precede IgG antibody production.

Different results are also reported regarding the variation in IgE levels during effective treatment. Most authors report that IgG and IgE autoantibody serum levels decrease after effective medication [72]. However, recently, Bing *et al.* reported a steady rise of IgE levels in two BP patients studied during the first six weeks of oral and topical steroid treatment, even though the disease was being controlled effectively [74]. Another interesting fact is that circulating eosinophil numbers correlate with levels of both NC16A-specific IgG and IgE in BP sera [63], however, it is unknown whether these autoantibodies directly influence lesional eosinophils [89].

This also implies the possibility of using IgE detection in clinical settings for the diagnosis and follow-up of BP patients. The combined use of IgG BP180 and IgE BP230 ELISAs could slightly improve the diagnostic performance of IgG (BP180-BP230) ELISAs. A system based on ISAC microarray system has been proposed for the detection of both IgG and IgE in BP patients [84].

In vitro and in vivo studies

In vitro studies and mouse models further support the hypothesis which emerges from clinical observations, that IgEs and Th2-oriented immune responses play a role in BP. This type of immune response might be responsible for urticarial and eczematous lesions and partially responsible for blister formation.

A study investigating autoreactive BP180-specific CD4(+) T cells in BP patients and healthy controls demonstrated that autoreactive Th2 responses to BP180 are restricted to BP patients and may thus be critical in the pathogenesis of BP [90]. Furthermore, increased levels of IL-4 [91, 92] and IL-5 [91, 93] were found in blister fluid, and an increased number of IL-4- and IL-13-producing cells in peripheral blood and blister fluid [94] were demonstrated in BP patients. All of these observations suggest an involvement of Th2-mediated autoimmunity in BP pathogenesis, which may promote the production of IgE autoantibodies. In line with a Th2-oriented immune response, patients with BP showed a peculiar profile of IgE recognition towards some groups of allergens, which was absent in a group of allergic individuals. Interestingly, a significant, higher prevalence of hen's egg recognition was observed in patients with BP who had specific IgE to BP180, suggesting a possible link between this reactivity and disease pathogenesis [84].

Various *in vitro* studies on cultured human keratinocytes support the potential role of IgE and anti-BP180 IgE in BP. Incubation of cultured human keratinocytes with IgE isolated from BP sera resulted in the release of IL-6 and IL-8, [95, 96] two cytokines that may mediate disease development, *e.g.* by the attraction of inflammatory cells [92, 97, 98]. The secretion from cultured keratinocytes was also seen in response to incubation with a human mono-

clonal IgE antibody directed against BP180 NC16A [96]. Furthermore, incubation of keratinocytes with anti-BP180 IgE led to internalization of BP180 [99]. Both effects have previously been shown for IgG anti-BP180 antibody [100]. Anti-BP180 IgE can bind FcεRI expressed on the surface of various cells implicated in the inflammatory process of BP lesions: tissue mast cells [21], eosinophils [63, 98], and basophils [18, 39, 42, 63]. In BP biopsies, both IgE and BP180 antigen localize to the surface of mast cells [101]. Dermal mast cell degranulation is known to be the earliest event in BP lesion formation [33], which has further been confirmed by animal models [34]. Therefore, the released ectodomain of BP180, containing the NC16A domain, at the BMZ in BP patients may bind to IgE autoantibodies on mast cells and promote their degranulation [101]. Activated mast cells release histamine, TNFα, IL-6, platelet activating factor (PAF), and vascular endothelial growth factor (VEGF) which induce vasodilation, increase vascular permeability, and enhance leukocyte recruitment, especially of eosinophils, resulting in the development of itchy urticarial lesions and further amplification of the inflammatory process [89, 102] (*figure 1*).

Incubation of basophils with the monoclonal IgE anti-BP180 antibody led to BP180-specific histamine release. In line with this, incubation with recombinant NC16A induced histamine release from basophils of BP patients by crosslinking NC16A-specific IgE on the surface of these cells [21]. IgE may possibly also contribute to eosinophil degranulation, through a process similar to that reported for mast cell activation [101, 103].

Moreover, it has been shown that eosinophils in peripheral blood and up to 70% of eosinophils in lesional BP skin express FcεRI and surface-bound IgE [63, 104], demonstrating the relevance of eosinophils and their link to IgE reactivity in BP. However, a very recent *in vitro* study revealed, based on a cryosection model of BP, that eosinophil localization to the DEJ is dependent on IgG-mediated complement fixation; in particular eosinophils would migrate from the dermis to the DEJ via expression of complement receptors, CR1 and CR3. Therefore, IgE antibodies may not play a direct role in eosinophil activation or localization at the BMZ, but rather an indirect role through mast cell degranulation. Binding of anti-BP180 NC16A IgE to these eosinophils might result in eosinophil degranulation or may trigger mast cell degranulation, which has been suggested to occur as a "piecemeal degranulation" pattern [105, 106], and therefore may contribute to blister development. In addition, gelatinase derived from eosinophils has been reported to cleave the extracellular domain of BP180 [107].

In BP animal models, IgG-mediated subepidermal blistering is easily achieved [11, 12, 108], however, pruritic urticarial lesions, other rare atypical lesions that can be found in BP, and eosinophilic infiltration are not easily reproducible [103]. Furthermore, most data from animal models of BP are generated by the use/induction of IgG anti-BP180 autoantibodies [10, 14, 108, 109], but a few studies have explored the *in vivo* effect of anti-BP180 IgE. A pathogenic role for IgE in reproducing the early phase of BP lesion development in human skin grafted to nu/nu mice was demonstrated by Fairley *et al.* [110]. IgE autoantibodies were isolated from BP patients' sera and injected into human skin, which was then grafted onto immunodeficient nu/nu mice. Twenty-four hours later, erythematous

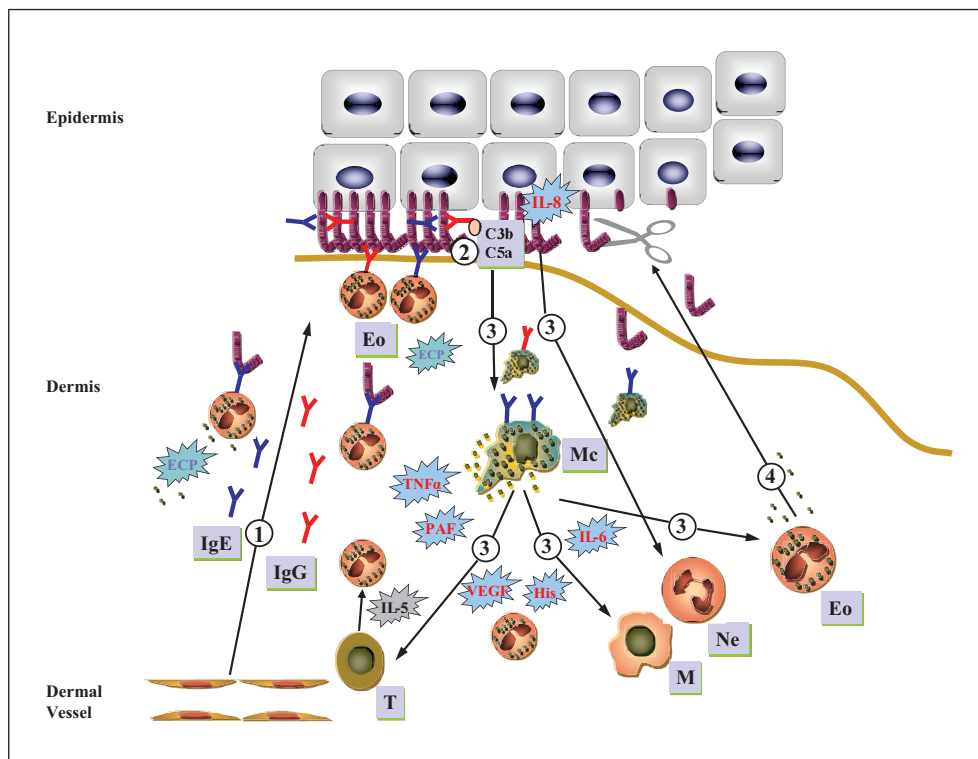


Figure 1. Schematic representation of the pathogenesis of BP demonstrating the mechanisms of blister formation in bullous pemphigoid. Binding of IgG and IgE autoantibodies to the target antigens results in an inflammatory response with complement activation, degranulation of mast cells, accumulation of neutrophils and eosinophils, and liberation of proteolytic enzymes. The following steps are shown: (1) IgG and IgE autoantibodies bind to BP180 and induce the internalization in the basal keratinocytes of BP180; (2) the complement system is activated, while basal keratinocytes produce interleukin-8 (IL-8); (3) macrophages (M), neutrophils (Ne), mast cells (Mc) and eosinophils (Eo) are recruited and activated; mast cells bind IgE and IgG antigen-specific autoantibodies and release their content of histamine (His), TNF α , IL-6, platelet activating factor (PAF), and vascular endothelial growth factor (VEGF) which induce vasodilation, increase vascular permeability, and enhance leukocyte recruitment, including T cells (T) that release IL-5 and especially eosinophils; (4) eosinophils bind IgE and IgG antigen-specific autoantibodies, release eosinophil cationic protein (ECP), and secrete various proteases (MMP-9) that degrade various extracellular matrix proteins, including the extracellular domain of BP180.

and elevated plaques were observed in all human skin grafts, similar to those clinically observed in BP patients. Histological and ultrastructural examination of the lesions showed engorgement of blood vessels and a dermal infiltrate composed of neutrophils, eosinophils, and mast cells, many of which were degranulated [110]. Interestingly, if high doses of BP IgE were injected into athymic nude mice, histological separation of the epidermis from the dermis was observed [110].

Based on a similar model, Zone *et al.* produced an IgE hybridoma to LABD97 antigen, a shed form of the BP180 ectodomain, that was subcutaneously injected in severe combined immunodeficiency (SCID) mice (which lack T and B lymphocytes) with engrafted human skin. After the injection, the mice presented erythema and intense scratching. Three weeks later, the mice developed intense eosinophil infiltration and degranulation of the human mast cells within the grafts, and most of these also presented histological, but not clinically-visible, basement membrane blisters. This model reproduced the histological inflammatory process observed in BP mediated by anti-BP180 IgE antibodies [111]. Both *in vivo* models do not entirely mirror the patient situation. While one model used IgE isolated from BP sera but only included an observation period of 24

hours and, thus, only addressed the very early events [110], the other model included a considerably longer observation period but applied a monoclonal IgE anti-BP180 antibody that may have targeted only one pathogenic epitope [111]. In addition, in both models, the effector cells express the murine Fc ϵ Rs, which have a lower affinity to human IgE [110]. Interestingly, mice with a genetic deletion of the immunodominant NC14A domain of BP180 (corresponding to the NC16A domain in humans) show elevated total IgE serum levels compared to wild-type animals and start scratching at Weeks 10-16 of life without developing blisters or erosions [112].

Most animal models are based on passive transfer of autoantibodies or immune cells. Hirose *et al.* created a more realistic mouse model of BP by actively immunizing immunocompetent mice with a recombinant form of the immunodominant 15th non-collagenous domain of murine BP180 (type XVII collagen). Immunization of female SJL/J mice with the murine peptide led to clinical disease within 14 weeks in 56% of mice. The clinical disease manifested for at least eight weeks without further manipulation. This novel immunization-induced model reflects key immunopathological characteristics of human BP, including binding of complement-fixing autoantibodies along the

dermal-epidermal junction, elevated total IgE serum levels, and infiltration of skin lesions with eosinophilic granulocytes [109].

IgE autoantibodies seem to be implicated in the induction of non-bulbous lesions in BP, mainly via mast cell degranulation, however, they probably also directly and indirectly contribute to blister formation, through partially-understood mechanisms, such as the internalization of BP180 in basal keratinocytes and eosinophil gelatinase-mediated cleavage of BP180.

Therapeutic implications

The presence of IgE antibodies reactive to BP180 seems to be associated with severe or atypical cases of BP, which usually exhibit a poor response to conventional therapy [41]. Therefore, from a strictly clinical point of view, a specific therapy directed towards IgE antibodies could be a possible second-line treatment option to manage BP patients.

Omalizumab, a humanized IgG1k monoclonal antibody that selectively binds to human IgE, specifically targets the receptor binding site on IgE; therefore, it binds only to circulating IgE and not to IgE bound via high-affinity IgE receptor on mast cells or basophils [113]. In addition, it also reduces IgE synthesis in B cells [114] and decreases the expression of FcεRI on mast cells [115]. The first therapeutic trials of omalizumab for BP were conducted in 2014, following the results of a major study by Messingham *et al.* which demonstrated both IgE and BP180 antigen localized on the surface of mast cells in BP biopsies, and incubation of circulating basophils from these patients with BP180 protein triggered degranulation resulting in substantial improvement in the patients' disease [63].

To date, 10 cases of BP treated with omalizumab have been reported in the literature, of which nine showed a reduction in circulating IgE level and eosinophil count, with significant improvement [72, 116-119]. Interestingly, of these 10 cases, two were regarded as severe forms of BP not responding to conventional therapy: a woman with pruritic BP and very high levels of IgE and eosinophils, who was refractory to standard aggressive immunosuppressive regimens for BP [117] and a man with BP and very high levels of total IgE (5,000 kU/L), who was refractory to standard aggressive immunosuppressive regimens (systemic steroids and daily cyclophosphamide). Both patients rapidly responded to omalizumab [118].

The successful use of the anti-IgE antibody, omalizumab, in individual patients with BP provides *ex juvantibus* criteria supporting a pathogenic role of IgE autoantibodies in this disease. However, these are small uncontrolled studies with clear limitations, and further structured investigation is needed to prove the efficacy of omalizumab in BP patients.

Conclusions

IgE autoantibodies have been known to be potentially pathogenic in BP since the early 90s. Perhaps self-reactive IgE might play a role in the initial phases of BP and atypical cases of BP, such as severe erythematous and urticarial forms of BP, as well as blister formation. Although

detection of serum anti-BP180 IgE is not of diagnostic importance, it may be relevant for therapeutic decisions (e.g. the use of anti-IgE treatment) [88]. The efficacy of omalizumab is yet to be thoroughly assessed as treatment for BP, however, results are very promising and it could be a key to treat atypical forms of BP, which are frequently non-respondent to standard therapy, as well as classic non-responding BP. ■

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