

Emerging treatments for pemphigoid diseases

Ralf J. Ludwig¹, Kathrin Kalies², Jörg Köhl^{3,4}, Detlef Zillikens¹, and Enno Schmidt^{1,5}

¹ Department of Dermatology, University of Lübeck, Lübeck, Germany

² Institute of Anatomy, University of Lübeck, Lübeck, Germany

³ Institute for Systemic Inflammation Research, University of Lübeck, Lübeck, Germany

⁴ Division of Cellular and Molecular Immunology, Cincinnati Children's Hospital and University of Cincinnati College of Medicine, Cincinnati, OH, USA

⁵ Comprehensive Center for Inflammation Medicine, University of Lübeck, Lübeck, Germany

Incidence of bullous pemphigoid (BP), the main pemphigoid disease, has increased over the past decades. Despite growing insights into pathogenic mechanisms, treatment is still based on systemic immunosuppression. Furthermore, overall mortality is significantly increased, which can be at least partially attributed to immunosuppressive therapy. Other pemphigoid diseases are also still difficult to treat. Hence, there is a so far unmet need for novel treatment options in patients with pemphigoid disease. Based on recent advances in understanding pathogenesis, several novel potential therapeutic targets have emerged. Interestingly, for some of these targets, lead compounds have been developed. Here, we review emerging therapeutic targets for pemphigoid diseases and discuss which compounds are likely to be used in the future.

Pemphigoid diseases

Pemphigoid diseases are a heterogeneous group of autoimmune blistering diseases (AIBDs) that are clinically characterized by subepidermal (muco)cutaneous blistering (Figure 1, Table 1). Pathogenesis of pemphigoid diseases is complex and can be subdivided into distinct steps: (i) loss of tolerance to the autoantigen; (ii) autoantibody production; (iii) binding of autoantibodies to target antigens; (iv) autoantibody-induced inflammatory response; and (v) tissue injury (Figure 2). Despite increasing insights into the pathogenesis of these diseases, treatment of pemphigoid diseases is challenging and is associated with severe adverse events. Consequently, there is a pronounced medical need to develop novel treatment options for patients with pemphigoid diseases.

At the molecular level, these diseases are characterized by autoantibodies directed to structural proteins of the dermal–epidermal junction [1]. With a few exceptions, autoantibody binding does not directly lead to tissue injury but activates immune mechanisms, which ultimately results in blistering. BP is the most common pemphigoid

disease accounting for approximately 80% of pemphigoid patients. BP is characterized by autoantibodies directed against two hemidesmosomal proteins type XVII collagen (COL17, BP180) and BP230. Other pemphigoid diseases comprise pemphigoid gestations, linear IgA dermatosis, mucous membrane pemphigoid (MMP), anti-p200/laminin γ 1 pemphigoid, lichen planus pemphigoides, and epidermolysis bullosa acquisita (EBA) [1].

Severe medical burden of patients with pemphigoid diseases

Patients with BP suffer from a widespread inflammatory skin disorder and blister formation. Severe itching is almost always present. Topical or systemic corticosteroids effectively control BP [2]. However, after withdrawal, relapse within a few months is observed in 30% to 50% of patients [3]. Following diagnosis of BP, 1-year mortality rate ranges from 20% to 40% [2,4]. Compared with age- and sex-matched controls, mortality of BP patients is increased two to three times [4,5]. Interestingly, BP patients with severe disease treated with (equally effective) topical corticosteroids had a higher survival rate as opposed to those treated systemically with high-dose systemic corticosteroids [2].

Furthermore, incidence of BP has increased over the past decades and ranges between 13 and 62 cases per 1 million inhabitants per year in Central Europe and the UK [4–6]. Owing to the low incidence of other pemphigoid diseases, comparable epidemiological data are not available. Yet, patients with EBA and with esophageal, laryngeal, and conjunctival lesions in MMP are notoriously difficult to treat [7,8].

Pathogenesis of pemphigoid diseases

Identification of autoantigens in pemphigoid diseases and loss of tolerance

Autoantibodies in BP are directed against two hemidesmosomal proteins, BP230 and BP180. The majority of BP sera react with epitopes clustered within the 16th noncollagenous (NC16A) domain of BP180. Pathogenic relevance of anti-BP180 antibodies has been clearly documented by: (i) blister induction in skin sections incubated with either BP sera or anti-BP180 IgG; (ii) BP induction in neonatal mice after transfer of anti-BP180

Corresponding author: Ludwig, R.J. (ralf.ludwig@uksh.de).

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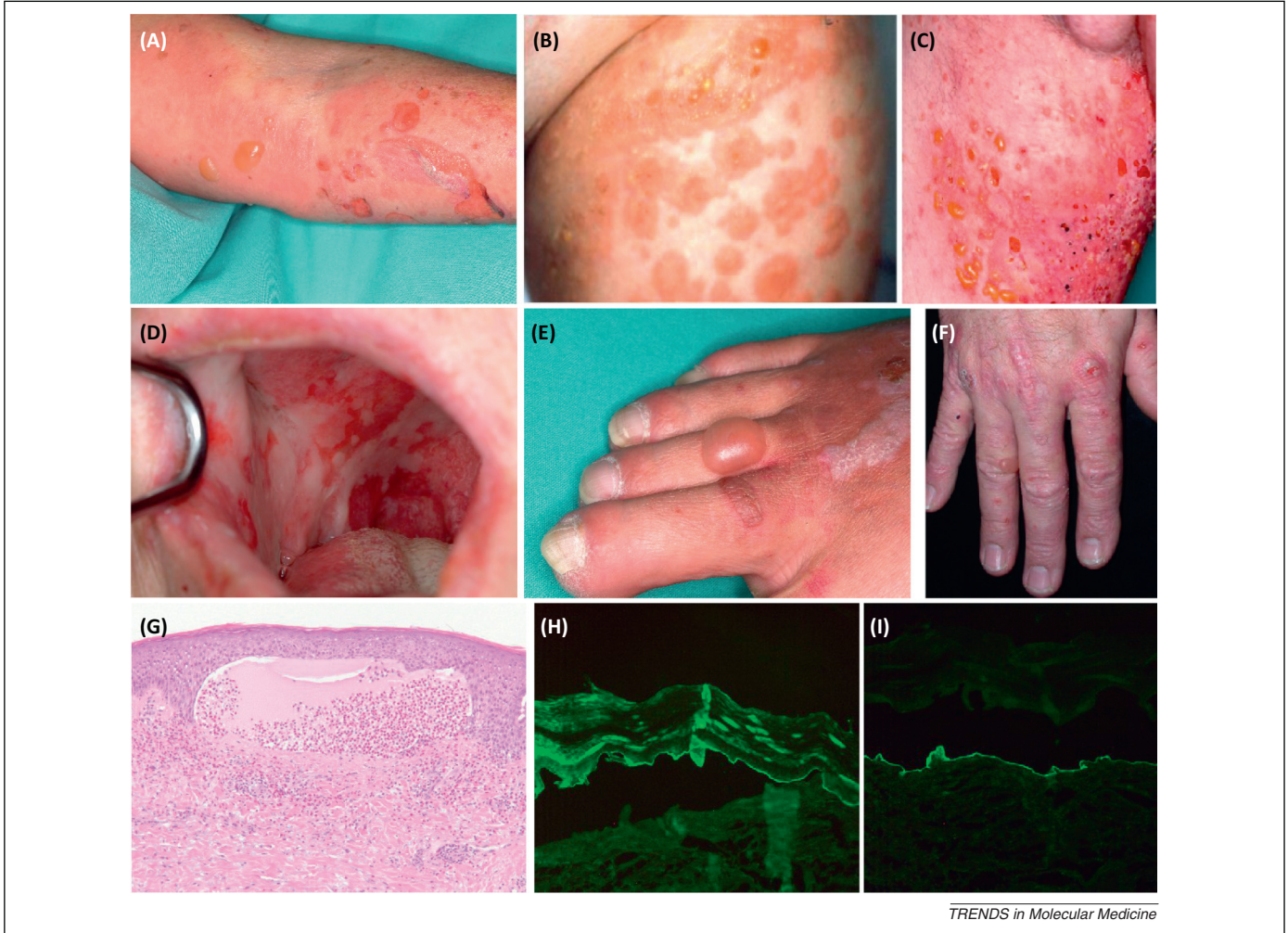


Figure 1. High medical burden of patients with pemphigoid disease. Clinical presentations of patients with (A) bullous pemphigoid (BP) with large blisters and extensive erosions; (B) pemphigoid gestations with erythema multiforme-like vesicles and blisters; (C) anti-p200 pemphigoid with blisters, erosions, and hemorrhagic crusts; (D) mucous membrane pemphigoid with extensive oral erosions; (E) lichen planus pemphigoides with co-occurrence of tense blister with confluent plaque-like papules; and (F) epidermolysis bullosa acquisita with blistering, erosions, and milia on trauma-prone dorsum of the hand. (G) H&E stained lesional skin biopsy from a BP patient with subepidermal blister and a dermal, eosinophil-rich leukocyte infiltrate. (H) Epidermal and (I) dermal binding of serum autoantibodies targeting structural proteins located within the dermal–epidermal junction by indirect immunofluorescence microscopy on human salt-split skin.

Table 1. Pemphigoid diseases

Disease	Pathogenic autoantibody	Additionally targeted antigen(s)	<i>In vitro</i> model	Animal model	Remarks
Bullous pemphigoid	BP180 (NC16A)	BP230	Yes	Yes	–
Pemphigoid gestations	BP180 (NC16A)	BP230 (rarely)	Yes	–	In pregnancy
Linear IgA dermatosis	BP180 ectodomain (LAD-1)	–	–	–	–
Mucous membrane pemphigoid	BP180 α6β4 integrin Laminin 332	BP230 (rarely)	– Yes –	– – Yes	–
Lichen planus pemphigoides	BP180 (NC16A)	BP230	–	–	Co-occurrence with lichen planus
Anti-p200 laminin γ1 pemphigoid	Most likely laminin γ1	–	Yes	–	–
Epidermolysis bullosa acquisita	COL7 (NC1)	COL7 (NC1 and NC2)	Yes	Yes	–

IgG; (iii) transfer of autoreactive lymphocytes; and (iv) BP induction in adult mice after immunization with the murine homolog of human NC16A. By contrast, pathogenic relevance of autoantibodies against BP230 has not yet been univocally demonstrated [1].

In 1984, a protein located in the basement membrane of human skin was described as the autoantigen in EBA [9]. Subsequently, sera from EBA patients were shown to recognize collagen type VII (COL7), the major component of anchoring fibrils. Pathogenicity of antibodies directed

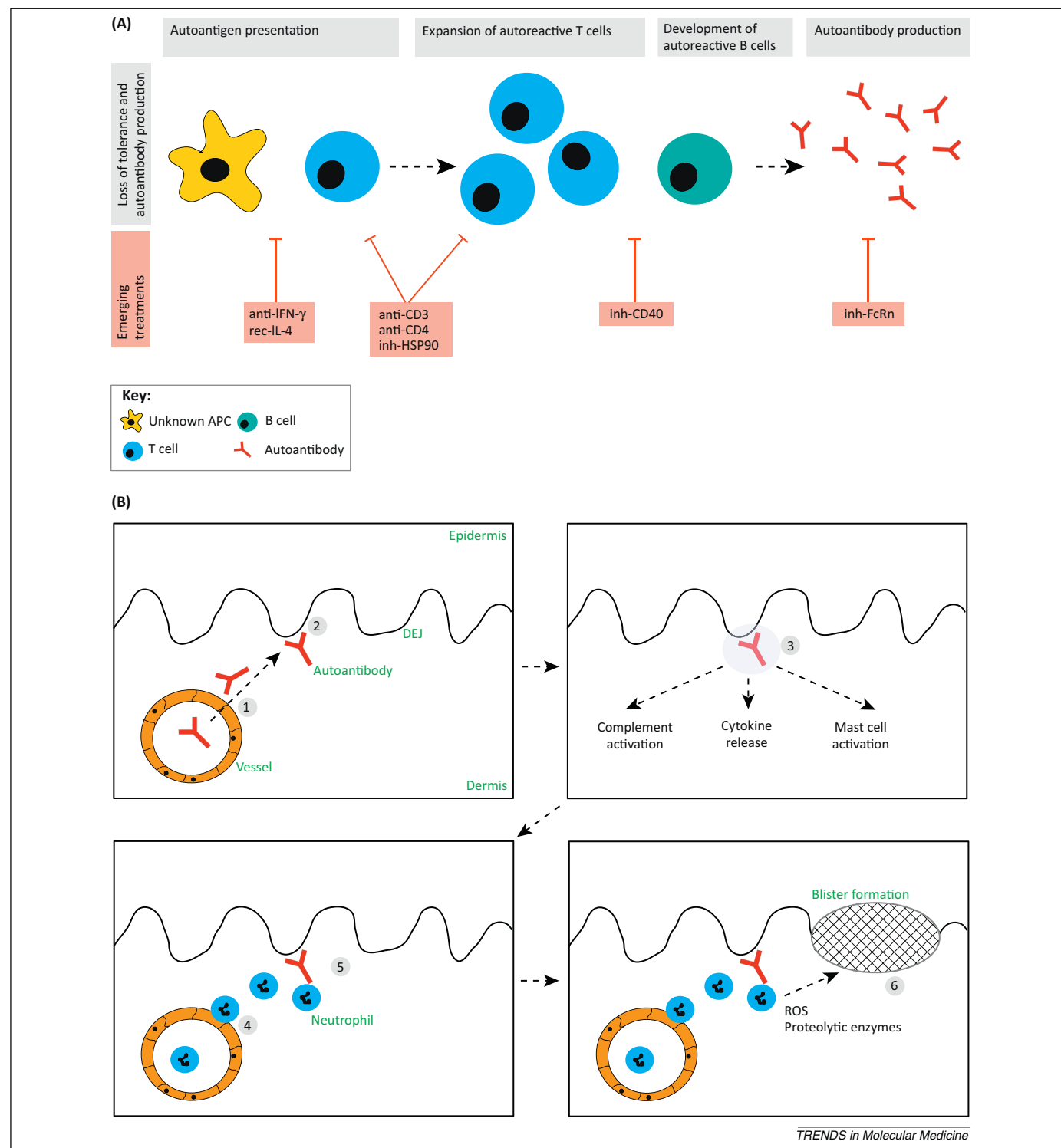


Figure 2. Pathogenesis of pemphigoid disease. **(A)** As a first step, loss of tolerance to structural proteins of the skin occurs. In this process, so far unknown antigen-presenting cells (APCs) interact with T cells. T cells are then polarized towards a T helper cell, type 1 (Th1) phenotype, which is required for the generation of antigen-specific plasma cells. Ultimately, autoantibodies (mainly IgG) are produced, and the half-life of the autoantibodies is controlled by the neonatal Fc receptor (FcRn). Modulation of the cytokine milieu at the site of antigen presentation is one possibility to shift the immune response towards a non/less pathogenic Th2 phenotype. Depletion of CD3/CD4 cells is another potential emerging therapeutic option in pemphigoid disease, as T cells, specifically CD4 T cells, have been shown to mediate the loss of tolerance as well as autoantibody production. In line with this, depleting antibodies or treatment with heat shock protein (HSP) 90 inhibitors have been successfully used to modulate T cell function in pemphigoid disease. **(B)** Schematic diagram of autoantibody-induced tissue injury in pemphigoid disease: (1) autoantibodies locate from the circulation into the skin, (2) where they bind to their respective autoantigen. (3) Autoantibody binding induces complement activation, cytokine release, and activation of immune cells. This collectively leads to the formation of a proinflammatory milieu, which is the prerequisite for (4) neutrophil extravasation into the skin. In the skin, neutrophils bind to the immune complexes located at the dermal-epidermal junction in an Fc receptor-dependent manner. (6) This ultimately leads to neutrophil activation, including the release of reactive oxygen species (ROS) and proteolytic enzymes, which results in blister formation.

against COL7 has been documented by: (i) induction of dermal–epidermal separation in skin sections incubated with anti-COL7 IgG and neutrophils; (ii) disease initiation by transfer of antibodies into mice; and (iii) disease manifestation by immunization of mice with COL7 [7,10].

In MMP, different target antigens have been described at the molecular level, including BP180, BP230, laminin 332, and $\alpha 6\beta 4$ integrin. Autoantibodies in anti-p200 pemphigoid are directed against the C terminus of laminin $\gamma 1$ [11]. In linear IgA disease, autoantibodies belong to the IgA isotype and target the soluble ectodomain of BP180 [12]. Although antibodies to laminin 332 directly cause blister formation without the need of complement activation and infiltration of inflammatory cells into the skin [13], no *in vivo* model reflecting the human disease has yet been described for anti-laminin $\gamma 1$ [14] and IgA anti-BP180 antibodies. As the majority of experimental data has been obtained for BP and EBA, the present review will focus on these two disorders [15,16].

Loss of tolerance to structural proteins of the skin occurs in genetically predisposed individuals and depends on T cells. An in-depth analysis of a possible association of BP or EBA susceptibility with the MHC locus has documented an MHC association for both diseases [17–19]. This finding was also recapitulated in mice with immunization-induced EBA, where only mice with H2s and (to a minor extent) H2k were susceptible to skin blistering [20]. Immunization of genetically heterogeneous outbred mice with COL7 also documented a contribution of genes outside the MHC locus to EBA susceptibility [21]. In addition to the genetic background, T cells are required for induction of EBA, as T cell deficient nude mice were resistant to immunization-induced EBA [22].

Autoantibody production and control of autoantibody half-life

T and B cells are also required for continuous production of autoantibodies; transfer of splenocytes from wild type mice immunized by grafting skin from human COL17-humanized mice into Rag2-deficient/COL17-humanized recipients only led to autoantibody production and subsequent clinical disease if both CD4 T and B cells were transferred. Interestingly, depletion of CD8 T cells from donor cells had no effect [23]. Furthermore, CD40–CD40L interactions are crucial for autoantibody production in this model, as treatment of recipient mice with a function blocking CD40L antibody almost completely abrogated autoantibody production and completely protected from BP induction [24]. In addition to T and B cells, the neonatal Fc receptor (FcRn) significantly contributes to maintain autoantibody concentrations in experimental pemphigoid diseases. The major isotype in pemphigoid diseases is IgG. Compared with other isotypes, IgG has a higher half-life due to rescue from proteolysis by the FcRn [25]. Furthermore, FcRn-deficient mice are protected from induction of experimental BP or EBA by autoantibody transfer [26,27].

Autoantibody-induced tissue injury

Experimental evidence from BP and EBA mouse models suggests that after autoantibodies are present in the circulation, they rapidly bind to antigens located at the

dermal–epidermal junction [28,29]. After binding to the antigen(s), immune complexes are formed. This leads to the formation of a proinflammatory milieu. Mast cells, complement activation, and (possibly) cytokines were shown to be involved in this process [15,30]. This results in integrin-dependent migration of effector leukocytes into the skin [31,32]. In skin, these leukocytes (predominantly neutrophils) bind to the immune complexes in an Fc γ receptor (Fc γ R)-dependent manner [33,34]. Ultimately, Fc γ R ligation drives neutrophil activation causing blister formation as a result of reactive oxygen species (ROS) and proteolytic enzyme release [32,35,36].

Modulation of the generation and maintenance of the loss of tolerance to structural proteins of the skin

T cell depleting strategies

As outlined above, T cells are required for induction of loss of tolerance [22] as well as for autoantibody production in pemphigoid diseases [23]. In other animal models of autoimmune diseases, especially type 1 diabetes, depletion of T cells has been demonstrated to reverse disease and induce long-term remission [37].

Two anti-human CD3 antibodies (teplizumab and oteplizumab) are currently evaluated in Phase I–III clinical studies for type 1 diabetes, rheumatoid arthritis, psoriasis, and/or Graves' ophthalmopathy (www.clinicaltrials.gov). Completed trials demonstrated that patients with new-onset type 1 diabetes treated with oteplizumab (ChA-glyCD3) for 6 days preserved residual β cell function for at least 18 months [38]. The CD4-depleting antibodies, for example, zanolimumab and M-T41, have been successfully evaluated in patients with (cutaneous) T cell lymphoma [39] and trials in multiple sclerosis are ongoing (NCT00004816). However, in patients with chronic inflammatory diseases, CD4-depleting strategies have not shown sufficient therapeutic efficacy [40]. This is in contrast to observations in mice where depletion of CD4 led to decreased disease activity [41]. Therefore, depletion of either CD3 (or CD4) T cells may become an alternative treatment option for patients with severe and treatment refractory pemphigoid diseases, after validation in respective animal models (Table 2).

T cell modulating strategies

As different T cell subsets have been demonstrated to distinctively contribute to disease susceptibility in immunization-induced EBA [42], targeting of disease-promoting T helper, type 1 (Th1) cells may offer the possibility for a more selective treatment. Comparison of the autoantibody response in EBA-resistant and -susceptible mice showed an IgG2-dominated response in susceptible mouse strains. In line with this observation, the interferon- γ /interleukin-4 (IFN- γ /IL-4) ratio in draining lymph nodes of EBA-susceptible mice was significantly increased compared with EBA-resistant strains, pointing towards a Th1 polarization [42]. Future experiments will have to address if the Th1-dominated immune response, which leads to the loss of tolerance to COL7, is also required for the continued production of pathogenic autoantibodies. Given that in experimental EBA, blockade of IFN- γ and/or administration of IL-4 are able to shift the immune response towards a Th2 phenotype and, subsequently, improve disease

Table 2. Possible therapeutic targets for the loss (maintenance) of tolerance to structural proteins of the skin

Targeted mechanism	Molecule or pathway	Possible drug candidates	Evidence
T cells	CD3	Teplizumab, otelixizumab	- Improvement of experimental type 1 diabetes mellitus - Preserved β cell function in type 1 diabetes mellitus patients
	CD4	Zanolimumab, M-T41	- Effective CD4 depletion in patients - Successful in lymphoma - Trials including patients with chronic inflammatory diseases have so far not met the primary endpoint
	IL-4	Recombinant IL-4	IL-4 treatment has therapeutic effects in a preclinical psoriasis model [107]
	IL-17	Anti-IL-17	- Increased IL-17 serum concentrations in BP patients [45] - Anti-IL-17 therapeutic antibodies effective in psoriasis
	IFN- γ	Ni-0501, anti-IFN- γ monoclonal antibody Recombinant IFN- γ	- Blockade of IFN-γ has therapeutic effects in several preclinical models of autoimmunity [108] - Phase I/II trials completed (NCT01459562, NCT00072943) - Administration of IFN-γ improved treatment-refractory BP in an open-label pilot study [43]
	HSP90	Several HSP90 inhibitors [48]	- Clinical trials for cancer patients underway - HSP90 inhibition prevented induction of immunization-induced EBA and had therapeutic effects in the same model [46]
T/B cell interaction	CD40–CD40L (CD154) interaction	CD40: SGN-40	- SGN-40 has been shown to be safe and effective in patients with lymphoma - No clinical data on patients with autoimmunity
		CD154: ruplizumab, toralizumab	Blockade of CD154 is effective in patients with systemic lupus but induces thromboembolic events

activity, use of the anti-IFN- γ antibody and/or recombinant IL-4 in treatment-refractory patients could be considered. In BP the opposite, for example, shifting towards a Th1-dominated immune response, may be of potential therapeutic benefit. This principle has already been tested in ten treatment-refractory BP patients, where administration of IFN- γ led to significant clinical improvement and a drop of anti-BP180 antibodies [43]. Moreover, elevated IL-17 serum levels and high numbers of IL-17-positive cells in skin lesions in BP patients point towards a contribution of Th17 cells in BP [44]. Various drugs target the IL-17–Th17 pathway, for example, the IL-17A inhibitors secukinumab and ixekizumab are already in clinical trials [45] and may be of therapeutic value in BP.

Interestingly, we recently observed a critical contribution of heat shock protein (HSP) 90 in initiation of the autoimmune response towards COL7. In preventive and therapeutic treatment regimes, two different HSP90 inhibitors ameliorated skin blistering in immunization-induced EBA, which was associated with reduced autoantibody serum levels. Of note, total plasma cell numbers, COL7-specific plasma cells, and germinal center B cells were unaffected by HSP90 blockade. However, T cell proliferation was potently inhibited, as evidenced by a reduced response of lymph node cells from immunized mice to *in vitro* restimulation with anti-CD3/CD28 antibody or autoantigen in the presence of HSP90 inhibitors [46]. These findings underscore the importance of T cells for autoantibody production in pemphigoid diseases [47]. Several HSP90 inhibitors are currently being evaluated in patients with malignancies [48].

The CD40–CD40L (CD154) interaction has been shown to be crucial for B cell development [49]. Experimentally, blockade of this interaction completely abolishes production of autoantibodies and subsequent experimental BP [24] in a cell transfer model of BP [23]. The anti-CD40L antibodies

ruplizumab and toralizumab have been evaluated in clinical trials enrolling patients with transplant rejection, idiopathic thrombocytopenia, and systemic lupus erythematosus (SLE). Initially, a good therapeutic response was observed especially in patients with SLE. However, thromboembolic events that occurred during early phase trials and the observation that activated platelets express CD154 hamper use of these compounds. Furthermore, an anti-CD40 antibody (SGN-40) has been developed and was tested as a treatment for lymphoma. Data on autoimmune diseases using SGN-40 have not been published [50]. Owing to adverse events, blockade of CD40L may not be a suitable therapeutic strategy in pemphigoid patients, and before application in patients anti-CD40 treatment requires data from autoimmune disease models.

Modulation of autoantibody concentrations

Owing to the causative contribution of autoantibodies in the pathogenesis of pemphigoid diseases, removal of pathogenic autoantibodies from peripheral blood is a valid therapeutic approach. This can be achieved by either immunoadsorption or plasmapheresis. Alternatively, autoantibody serum levels can also be lowered by blockade of the FcRn. For both BP [26] and EBA [27], the FcRn has been demonstrated to completely or partially protect mice from experimental disease. These findings paralleled similar observations in experimental arthritis, myasthenia gravis, and pemphigus [51]. To the authors' knowledge, no FcRn-targeting compounds have yet been evaluated in humans. Contrary to this, therapeutic antibodies directed against IgE, such as omalizumab, are already in clinical use for asthma. Increased IgE levels are often detected in BP patients. Furthermore, circulating and tissue-bound anti-BP180 IgE are present in up to 50% of BP patients, and case reports suggested a therapeutic benefit of anti-IgE treatment in BP [52].

Emerging targets for autoantibody-induced tissue damage in pemphigoid diseases

Targeting autoantibody localization from blood into skin

Autoantibody-induced tissue injury (Figure 2) may be divided into sequential steps, most of which offer the possibility for drug targeting (Table 3); mechanisms of autoantibody localization from blood into the skin have

not been identified so far. In rheumatoid arthritis, arthritogenic antibodies caused macromolecular vasopermeability localized to sites destined to develop arthritis. This increased vascular permeability enhanced disease severity and depended on mast cells, neutrophils, Fc γ RIII, and vasoactive amides [53]. It is not known whether similar mechanisms are also operative in pemphigoid diseases.

Table 3. Possible therapeutic targets for autoantibody-induced tissue injury in pemphigoid disease

Molecule or pathway	Possible drug candidates	Evidence
Fc γ RIII	Soluble Fc γ RIIB [95]	• Good evidence in experimental arthritis [53], none so far for pemphigoid disease
IgE	Anti-IgE (omalizumab)	• Case reports suggested a beneficial therapeutic effect in BP patients [52]
Vasoactive amides	Ketanserin, H1-antihistamines	• Same as above
Autoantigen	Non-pathogenic or modified autoantibodies	• Anti-NC16A F(ab)s block pathogenicity of polyclonal anti-BP180 IgG <i>in vivo</i> [57] • Enzymatic hydrolysis of sugar moieties at the Fc portion with EndoS protects from/improves many autoantibody mediated diseases, including EBA [59,109]
	Autoantigen-specific immunoadsorption	• Case series provided evidence for efficacy in ABO-incompatible organ transplantation and dilative cardiomyopathy [60,61]
Complement activation	Anti-C5 antibody (eculizumab)	• Effective in several complement, mediated diseases [65,66]
Complement activation	Highly galactosylated IgG1 immune complexes	• Inhibition of C5a- and CXCR2-mediated proinflammatory effects in experimental EBA [70]
C5a receptor	C5a receptor antagonists a) A8 Δ 71–73 b) PMX 53 and PMX 205	• C5a mutein that blocks C5aR1 (CD88) and C5L2-mediated effector functions in several experimental inflammatory disease models including sepsis (e.g., ischemia reperfusion injury) and arthritis [69]. • Cyclic peptides that inhibit C5aR1 (CD88)-mediated effector functions in several inflammatory disease models [67,68].
TNF- α	TNF- α targeting biologics	• Beneficial effects in patients with mucous membrane pemphigoid in case reports
IL-1	Anakinra, canakinumab	• Approved for autoinflammatory syndromes and/or rheumatoid arthritis
IL-6	Tocilizumab	• Approved for rheumatoid arthritis [110] • Anti-inflammatory activity in acute lung injury [111]
CXCR3	NBI-74330 [112]	• Attenuates atherosclerotic plaque formation in LDL receptor-deficient mice [113]
Mast cell activation	Cromoglicic acid	• Prophylactic activity in experimental BP in neonatal mice [79]
CD18	Efalizumab (withdrawn from the market)	• CD18-deficient mice are protected from experimental EBA induction [32] • Anti-CD18, anti-CD11a, or anti-CD11b treatment protects mice from experimental BP induction [31]
PSGL-1	(Modified) heparins	• Modified heparins inhibit PSGL-1 binding to P- and L-selectin [114]
Activating Fc γ R	Soluble Fc γ RIIB [95]	• Evidence in lupus and arthritis models. • Phase II clinical trials in idiopathic thrombocytopenia and lupus
Fc γ R signaling, PI3K β	BKM120, BYL719, SF1126, and others; most blocking also other PI3Ks	• Inhibits experimental arthritis and EBA • Phase I/II clinical trials in cancer patients
Fc γ R signaling, sky	Fostamatinib	• Evidence in preclinical models • The first completed clinical trial failed to improve arthritis
Neutrophil activation, ERK1/2	Several	• Preclinical evidence, including EBA • Several compounds in Phase I/II trials
Neutrophil activation, AKT	Several, e.g., MK2206, GSK690693	• Preclinical evidence, including EBA • Several compounds in Phase I/II trials
Neutrophil activation, p38	Several, e.g., VX-702	• Preclinical evidence, including EBA • Several compounds in Phase I/II trials
Reactive oxygen species	Preclinical	• Experimental evidence in several autoimmune diseases, including EBA
Proteolytic enzymes	Preclinical	• Experimental evidence in several autoimmune diseases, including BP and EBA
Flii	Preclinical	• Experimental evidence in wound healing and EBA

Nevertheless, this process could be relatively easily targeted by blockade of vasoactive amides (e.g., serotonin or histamine). However, so far no clinical studies have addressed the therapeutic benefit of antihistamines in BP patients, despite their frequent use to treat BP-associated itching.

Targeting formation of pathogenic immune complexes at the dermal–epidermal junction

Epitope mapping studies clearly documented a polyclonal autoantibody response in patients with pemphigoid diseases, which can be attributed, at least partially, to intra- and intermolecular epitope spreading [54]. Therefore, prior identification of pathogenically relevant epitopes is a prerequisite for targeting formation of immune complexes at the dermal–epidermal junction. Provided that such epitopes will be identified, treatment with non-pathogenic autoantibodies or autoantigen-specific immunoabsorption would be elaborate treatment options. In BP, the pathogenic autoimmune response is directed to epitopes located within the relatively small NC16A domain [55]. Interestingly, monoclonal anti-NC16A F(ab) blocked binding of polyclonal anti-NC16A IgG isolated from patients in indirect immunofluorescence (IF) microscopy. Furthermore these F(ab)s blocked complement deposition *in vitro*. Most strikingly, these F(ab)s were also capable of blocking anti-NC16A- or BP-IgG-induced subepidermal blisters in neonatal COL17-humanized mice [56,57]. Similarly, a recombinant anti-NC16A IgG1 autoantibody, which had been mutated at the Fc portion to not activate complement, also failed to induce experimental BP in mice [58]. This and other non-pathogenic antibodies could also be used to competitively block the binding of pathogenic autoantibodies.

In line with these findings, treatment of anti-COL7 IgG with EndoS (a novel secreted endoglycosidase in *Streptococcus pyogenes*; Box 1) completely abolished their *in vitro* and *in vivo* pathogenicity. Furthermore, administration of EndoS into mice with already established experimental EBA showed significant therapeutic effects, indicating that in addition to hydrolysis of circulating immune complexes, EndoS is also capable of hydrolyzing IgG immune complexes deposited in the skin [59]. Importantly, we have recently shown that EndoS differentially modulates the expression of FcγR on inflammatory cells not only in blood but also in skin lesions of experimental murine EBA, that

is, it upregulates the inhibitory FcγRIIB and downregulates the activating FcγRI, -III, and -IV. Targeting the glycosylation status of autoantibodies is a promising novel therapeutic avenue for autoantibody-mediated diseases [59].

Removal of autoantibodies is another potential therapeutic approach for pemphigoid diseases. Antigen-specific immunoabsorption has become available for ABO-incompatible organ transplantation and dilative cardiomyopathy [60,61]. In addition, from case reports, there is evidence for a therapeutic efficacy of IgG immunoabsorption in patients with pemphigoid disease [62]. Furthermore, antigens for selective immunoabsorption, which are currently applied in diagnostic assays for pemphigoid diseases, are available. Thus, selective removal of anti-NC16A antibodies by immunoabsorption seems a promising therapeutic approach for patients with BP and pemphigoid gestations.

Targeting the generation of a proinflammatory milieu in the skin

After the formation of immune complexes at the dermal–epidermal junction, a proinflammatory milieu is generated in the skin, leading to neutrophil recruitment (Figure 3). Different cells and molecules contribute to generation of this proinflammatory milieu. Among these, complement activation has so far been particularly well characterized. Lack of complement activation protects mice from induction of experimental BP/EBA via autoantibody transfer [63,64]. In patients, inhibition of terminal complement pathway activation can be achieved by treatment with anti-C5 antibody eculizumab. Eculizumab has successfully been used in paroxysmal nocturnal hemoglobinuria and antibody-mediated rejection of renal transplants [65,66], and is currently being evaluated in several other autoimmune diseases (NCT00727194, NCT01303952, NCT01275287). Furthermore, several inhibitors have been developed that specifically target cleavage fragments of C5, that is, C5a, or its cognate C5a receptors CD88 and C5L2 [67–69] (Table 3). In addition to a direct inhibition of complement activation, we recently identified that in an antigen-independent manner, highly galactosylated IgG1 immune complexes associate with FcγRIIB and dectin-1, resulting in inhibition of C5a effector functions. In detail, injection of IgG1 immune complexes reduced C5a-mediated neutrophil migration into the peritoneum in mice. Detailed analysis of this observation showed that galactosylated IgG1 immune complexes through binding to the inhibitory FcγRIIB and dectin-1 block C5aR-mediated proinflammatory events in neutrophils. In line with this observation, administration of galactosylated IgG1 immune complexes also blocked experimental EBA manifestation [70]. Thus, galactosylated IgG1 and FcγRIIB exert anti-inflammatory properties by their regulatory impact on activating Fcγ and complement receptors. Importantly, this new inhibitory feedback loop not only suppresses C5aR signaling but also other chemoattractant G-protein-coupled receptors, including CXCR2, suggesting a broad anti-inflammatory effect of highly galactosylated IgG1 immune complexes.

Unfortunately, compared with other chronic inflammatory diseases, little functional data have been published regarding the contribution of cytokines to the pathogenesis

Box 1. Enzymatic removal of terminal sugar residues alters IgG function

Another strategy to render pathogenic autoantibodies non-pathogenic is enzymatic modification of the glycosylation pattern at the Fc portion. In 2001, a novel secreted endoglycosidase in *Streptococcus pyogenes*, termed EndoS, was identified and characterized; EndoS specifically hydrolyzes the β-1,4-di-*N*-acetylchitobiose core of the asparagine-linked glycan of IgG [115]. This enzymatic modification of sugar residues profoundly changes the effector functions of IgG. Binding of EndoS-hydrolyzed IgG to activating FcγRs is decreased, whereas the affinity to the inhibitory FcγRIIB is increased. The preventive and therapeutic effects of EndoS have been well documented in several autoantibody-mediated diseases, including idiopathic thrombocytopenia, autoimmune hemolysis, experimental arthritis, nephritis, and SLE [115].

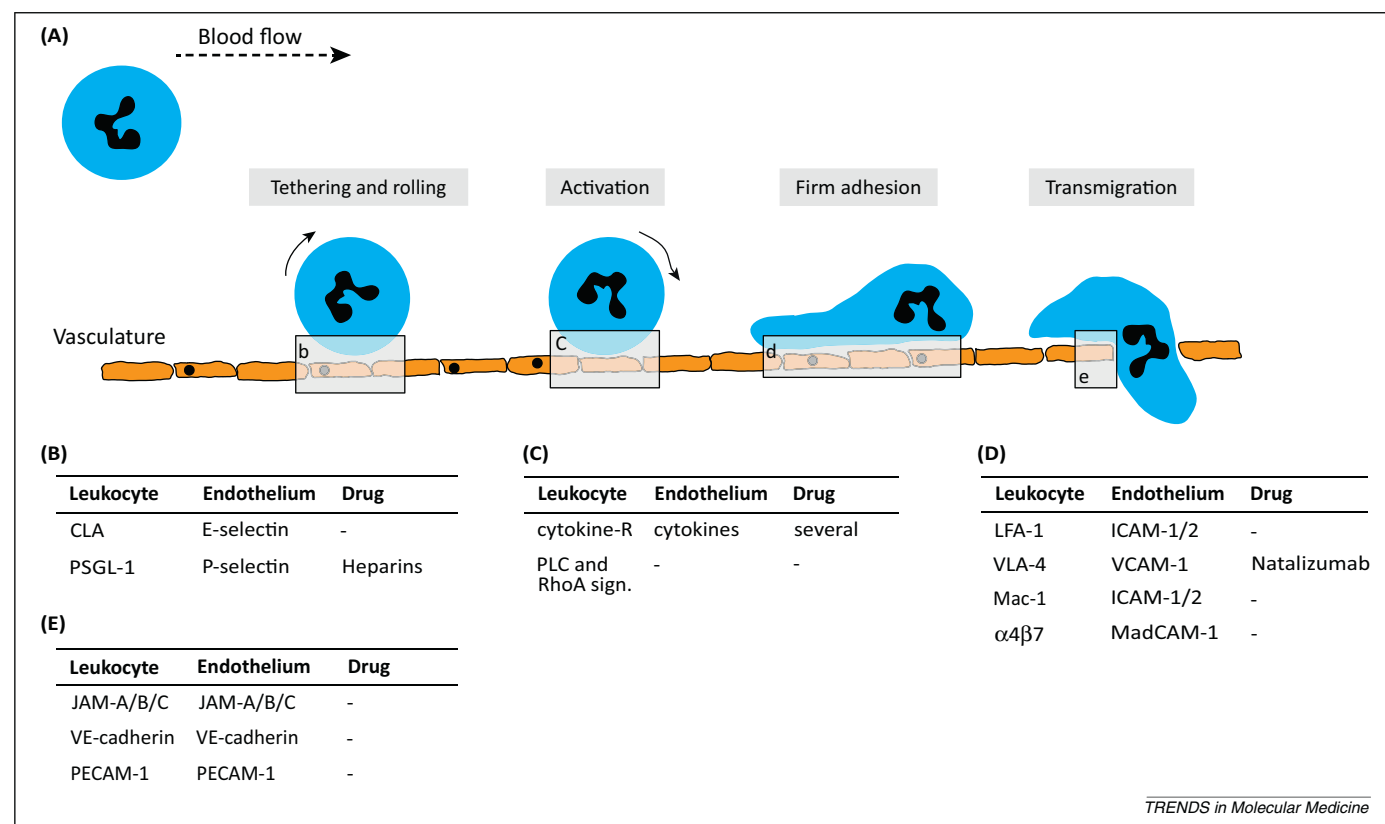


Figure 3. Targeting leukocyte extravasation in pemphigoid disease. **(A)** Schematic overview of leukocyte extravasation. Emerging therapeutic targets in pemphigoid disease in leukocyte **(B)** rolling, **(C)** activation, **(D)** firm adhesion, and **(E)** transmigration.

of pemphigoid diseases (Box 2). However, there is clinical evidence from case reports pointing to a therapeutic effect of tumor necrosis factor- α (TNF- α) blockade in patients with MMP [71–73]. Application of other cytokine-targeting agents in patients with pemphigoid diseases other than MMP needs to be carefully considered, as a possible induction of pemphigoid has been reported by the use of anti-TNF- α [74]. Interestingly, binding of autoantibodies to targeted cells (e.g., keratinocytes) leads to cytokine release, such as IL-6 and IL-8 [75]. Furthermore, certain cytokines, for example, IL-6, may even have anti-inflammatory effects [76]. In line with this notion, we recently showed a potent anti-inflammatory contribution of IL-6 to tissue destruction in experimental EBA. In detail, IL-6 induced

upregulation of IL-1 receptor antagonist (IL-1ra), which controlled the extent of IL-1-driven inflammation in this model. Accordingly, blockade of IL-1 function impaired the induction of experimental EBA [77]. Therefore, cytokine functions may differ in different diseases, and a thorough analysis in appropriate model systems is required before applying cytokine-targeted therapies in patients with pemphigoid diseases.

In addition, mast cells have been shown to contribute to autoantibody-induced tissue injury in neonatal mice injected with anti-NC16A IgG [78]. Prophylactic treatment with the mast cell stabilizer cromoglicic acid (cromolyn) protected mice from experimental BP induction [79].

Targeting leukocyte extravasation

To carry out their effector functions in inflamed tissues, leukocytes have to move from blood stream into tissues. This process of leukocyte extravasation is tightly controlled by expression and/or avidity of adhesion molecules, as well as by different cytokines [80]. In pemphigoid diseases, relatively little is known regarding the contribution of adhesion molecules. However, the clear dependency of disease manifestation on neutrophils [32,81] implies that adhesion molecules significantly contribute to disease manifestation (Figure 3). In accordance with these findings, induction of both experimental BP [31] and EBA [32] were completely dependent on CD18, the $\beta 2$ chain of several leukocyte integrins [82]. Whereas the contribution of CD18 in EBA has not been studied in detail, differential roles for $\beta 2$ integrins in experimental BP have been

Box 2. Cytokines in AIBDs

Based on observational studies of increased cytokine expression in the serum or the targeted tissues in chronic inflammatory diseases, functional studies in preclinical disease models of chronic inflammatory diseases have demonstrated a critical contribution of cytokines, for example, TNF- α , IL-1, or IL-6 to disease pathogenesis. This has subsequently led to the development and approval of cytokine-modulating compounds (biologics), which have greatly improved the therapy for patients with rheumatoid arthritis, psoriasis, and other chronic inflammatory diseases [116]. There is ample evidence for an increased expression of several cytokines, including TNF- α , IL-1, and IL-6 in pemphigoid diseases [117]. Furthermore, recent gene expression profiling studies identified IL-24, CXCL3, CXCL5, and IL-1ra as differentially expressed cytokines in the skin of mice with experimental EBA [34].

demonstrated. LFA-1 (CD11a/CD18) is required for neutrophil recruitment, whereas Mac-1 (CD11b/CD18) mediates late neutrophil accumulation and apoptosis of infiltrating neutrophils [31]. Owing to reports of progressive multifocal leukoencephalopathy in patients treated with the anti-CD11a antibody efalizumab (<http://www.gene.com/gene/news/press-releases/display.do?method=detail&id=11667>), the manufacturer has withdrawn the drug from the market.

In addition to integrins, expression profiling has identified PSGL-1 to be significantly upregulated in mice with experimental EBA [34]. PSGL-1 is expressed on leukocytes and endothelial cells and is capable of binding all selectins [83]. Interactions of selectins with their corresponding ligands mediate leukocyte rolling, which initiates leukocyte extravasation [80]. In experimental models of inflammation, genetic deficiency [84–86] or pharmacological blockade [87–89] of these interactions potentially alleviate pathogenic inflammatory responses. However, with the exception of $\beta 2$ integrins, no functional experimental data are available regarding the contribution of adhesion molecules to the pathogenesis of AIBDs.

Targeting neutrophil activation

In pemphigoid diseases, after extravasation into the skin, neutrophils bind to the immune complexes located at the dermal–epidermal junction. This process is mediated by Fc γ R. The equilibrium of activating inhibitory Fc γ R expression at the cellular surface determines if the neutrophil is activated [90]. Compared with wild type controls, mice deficient for inhibitory Fc γ RIIB develop a significantly enhanced EBA phenotype [34]. Interestingly, in a model of BP in neonatal mice, lack of Fc γ RIIB expression had no impact on the disease phenotype [33]. However, in adult mice with EBA, a difference in disease phenotype was only observed at days that were not investigated in the neonatal mouse model, in which the disease severity is evaluated hours after disease induction. Furthermore, in mice with EBA autoantibody-induced tissue injury is solely mediated by Fc γ RIV, whereas the neonatal BP model is Fc γ RIII-dependent [33,34]. In models reproducing immune complex-induced neutrophil activation in BP patients, Fc γ RIIA and Fc γ RIIIA are required [91]. The importance of Fc γ Rs in pemphigoid diseases is further underscored by different Fc γ R polymorphisms in BP patients [92].

When IgG immune complexes in the skin bind to activating Fc γ R, a series of signaling events is initiated downstream of activation of the γ -chain-associated immunoreceptor tyrosine-based activation motif (ITAM) [90]. These signaling events involve src family tyrosine kinases [93]. In models of pemphigoid, signaling through class I phosphoinositide 3-kinase (PI3K) β has been demonstrated to be essential for neutrophil-dependent blister formation. In an autoantibody transfer model of EBA, PI3K β -deficient mice were almost completely protected from disease induction, whereas a partial inhibitory effect was observed in the K/BxN model of arthritis [94].

Recently, several possible therapeutics to target this Fc–Fc γ R interaction have been identified; binding of activating Fc γ R to immune complexes can be inhibited by soluble Fc γ RIIB (sIIB, SM101) [95]. In lupus-prone mice,

therapeutic application of sIIB attenuated the disease pathology [96]. In collagen-induced arthritis, sIIB treatment improved already established arthritis and decreased levels of circulating anticollagen antibodies [97]. Furthermore, in experimental arthritis and lupus models, blockade of syk signaling impaired disease induction or had therapeutic effects [98,99]. However, in a clinical trial, the syk inhibitor fostamatinib did not improve rheumatoid arthritis in patients who had previously failed biological agents. In addition, recent findings from our laboratory demonstrated a crucial role for p38 mitogen-activated protein kinase (MAPK), extracellular signal-regulated kinase 1/2 (ERK1/2), and AKT phosphorylation in immune complex activation of neutrophils [100]. Several inhibitors of these pathways are currently being evaluated in Phase I/II clinical trials (Table 3).

Targeting of neutrophil effector molecules

As a result of neutrophil activation, several effector molecules are released, including ROS and proteolytic enzymes [101]. Interestingly, mice lacking functional NADPH oxidase activity were completely protected from the induction of experimental EBA by autoantibody transfer. Furthermore, pharmacological inhibition or deficiency of human NADPH oxidase abolished dermal–epidermal separation caused by autoantibodies and granulocytes in cyrosections of human skin [32]. Several ROS inhibitors have been developed and they efficiently inhibit ROS-dependent pathology in different animal models [102,103].

Furthermore, several proteolytic enzymes, such as elastase and matrix metalloproteinase 9, have been demonstrated to be crucial for autoantibody-induced tissue injury in experimental BP and EBA [35,36]. In addition to a mere ‘destructive’ role of these proteases, recent observations in experimental BP suggest a more complex contribution, that is, BP180 is a direct substrate of elastase, which cleaves BP180 at specific positions. One of these elastase-generated BP180 fragments has potent chemotactic properties. Furthermore, injection of elastase into the skin of mice induced neutrophil recruitment [104].

Additional emerging therapeutic targets

The actin remodeling protein, Flightless I (Flii) has recently been demonstrated to have an important role in mediating blister formation in EBA. Previous work in wound healing models showed that reducing Flii expression improves wound healing. Flii overexpression resulted in delayed wound closure. Similar findings were obtained in experimental EBA, where induction of disease led to increased cutaneous Flii expression. In accordance, reduced Flii expression in Flii^{+/-} mice significantly impaired blister formation [105]. Further work also demonstrated that topical treatment with Flii-neutralizing antibodies has therapeutic effects in experimental EBA [106], suggesting that this concept might be translated into patient care.

In addition, gene expression profiling in the skin of mice with experimental EBA has identified several other differently expressed genes [34]. Interestingly, the genes encoding for Fc γ RIV, proteolytic enzymes, and adhesion molecules were among these. From these sets of differently expressed genes, S100a8, CXCL3, CXCL5, and members of

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the Trem family are potential targets. Although awaiting validation using *in vitro* and/or *in vivo* model systems, these pathways provide additional emerging targets for the treatment of EBA and potentially other pemphigoid diseases.

Conventional or emerging treatment for patients with pemphigoid diseases?

The plethora of emerging therapeutic targets for pemphigoid diseases raises the question of which strategy is the most suitable. Ideally, the different possible approaches should first be evaluated in appropriate preclinical model systems, including animal models. Given favorable safety data, the corresponding compounds may then be tested in early Phase II clinical studies and subsequently validated in randomized controlled trials. These efforts will aim to reduce the current burden of both increased morbidity and mortality associated with unspecific conventional immunosuppression.

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