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Review

Discovering potential drug-targets for personalized treatment of autoimmune disorders - what we learn from epidermolysis bullosa acquisita

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Abstract

Introduction: Epidermolysis bullosa acquisita (EBA) is a chronic autoimmune bullous dermatosis (AIBD). Treatment of EBA is challenging and mostly relies on systemic immunosuppression. During the last decade, intensive research led to the identification of new potential therapeutic targets that interfere in different phases of disease progression. Therapeutic interventions acting upon these candidate drug targets in animal models of EBA, such as cytokine-modulating biologics and small molecules, have validated them as potential new therapeutic strategies for EBA patients.

Areas covered: In this paper, we review the current treatments for EBA, describe the pathogenesis of the disease, and finally specify new drug candidates for the development of a more specific therapy with minimized side-effects for EBA and potentially other autoimmune diseases.

Expert opinion: We currently understand EBA as a disease that evolves from the interplay of many different signaling pathways. These signaling pathways, which are described in this review, provide new targets for EBA treatment. The ultimate goal of this research field is the development of specific, pathogenesis-based therapeutic strategies. Through identification of up- or downregulated pathways that dominate disease progression in individual patients, we expect therapy in EBA to become more and more precise and move towards a patient-based therapy.

Keywords: drug targets, signaling, pathogenesis, Epidermolysis bullosa acquisita, autoimmune bullous disease

1. EBA - clinical phenotype, diagnosis and treatment

1.1. Clinical phenotype

Epidermolysis bullosa acquisita (EBA) is a rare autoimmune bullous dermatosis (AIBD) that characterized by subepidermal blisters. EBA is caused by autoantibodies directed against type VII collagen (COL7), a major component of anchoring fibrils. The incidence of EBA ranges from 0.2-0.5 new cases per million and per year [1-3]. EBA clinical features resemble those of hereditary dystrophic epidermolysis bullosa. Elliott first proposed the term 'EBA' as a descriptive clinical diagnosis for cases showing similar features of hereditary dystrophic epidermolysis bullosa with adult onset [4]. Later, Roenigk and colleagues distinguished EBA from other bullous diseases because of its distinctive clinical and histopathological features, and suggested the first diagnostic criteria for EBA [5]. Currently, the diagnosis is based on the detection of either circulating anti-COL7 antibodies, the specific binding of the antibodies in direct immune-electron microscopy and/or the pattern of autoantibody binding in direct IF microscopy [6].

The clinical phenotype of EBA is heterogeneous (Figure 1). EBA phenotypes are divided into two groups: a non-inflammatory type and an inflammatory type. The non-inflammatory type, which is also referred to as mechano-bullous or classic EBA, is characterized by the appearance of skin fragility, vesicles, bullae or erosions localized to the extensor skin surface and scarring with milia. Skin involvement appears on trauma-prone sites. The inflammatory EBA variant is observed in approximately 2/3 of patients and characterized by cutaneous inflammation, i.e., erythema, which resembles the clinical presentation of bullous pemphigoid (BP), linear IgA bullous disease (LABD), mucous membrane pemphigoid (MMP) or the Brunsting-Perry type pemphigoid [7-9]. The clinical manifestation of EBA in individual

patients can change to that of the other EBA types during the course of the disease. Furthermore, several different clinical phenotypes may present simultaneously [10].

Besides in the skin, COL7 is expressed in at the basement membrane zone of oral, anal, and vaginal mucosae, and esophagus [11]. This might explain an occurrence of extracutaneous EBA manifestations that may affect the eyes, mouth, larynx, vagina, anus, trachea and esophagus [12-14]. Esophageal strictures represent one of the severe complications in EBA patients. Patients may not be able to swallow foods and thus require endoscopic esophageal dilations [15, 16]. Rarely, laryngeal involvement may cause hoarseness, impaired phonation, and loss of voice, and may lead to irreversible respiratory distress [17, 18]. Mouse models of EBA have further shown blister formation in esophagus, stomach, small intestine, and colon [19, 20]. This anti-COL7-induced gastrointestinal tissue injury is of functional relevance, as a reduced body weight in diseased mice was observed [21]. This might explain a pathogenic link between EBA and inflammatory bowel diseases (IBDs), such as Crohn´s disease (CD) and ulcerative colitis (UC) [22]. CD has been reported in approximately 30% of EBA patients [23-27]. Interestingly, circulating antibodies to COL7 appear in patients with active IBD at frequencies ranging from 6-60% [27-29]. Speculations why not all EBA patients suffer from gastrointestinal symptoms include the distribution patterns of anti-COL7 IgG isotypes in EBA and IBD: In EBA, the autoantibodies mainly belong to the IgG1 and IG4 subclasses, whereas autoantibodies against COL7 in IBD were mainly found to be IgG3 [30]. This finding suggests that progression towards skin blistering diseases is associated with generation of IgG1 and IgG4 autoantibodies against COL7. Alternatively, the epitopes on COL7 targeted by autoantibodies in EBA and IBD may differ, which could further explain the absence of skin-blistering in the majority of patients with IBD.

Further associations with other systemic diseases have been reported in EBA patients. In an experimental animal model of EBA, injection of an anti-COL7 antibody caused intestinal

inflammation [21]. Furthermore, neutrophil-predominant infiltration in the tissue has been observed in the histopathological findings of both IBD and EBA [31]. Rheumatoid arthritis and diabetes mellitus, cryoglobulinemia and psoriasis have also been reported [5, 32-35].

Diagnosis

There is no consensus about diagnostic criteria of EBA yet. However, important contributions have been made in the last years that considerably facilitate the serological diagnosis of EBA. The diagnosis of EBA is based on the clinical presentation, histopathological and immunological findings.

Regarding the histopathology, skin samples of EBA patients show subepidermal blistering with various degrees of inflammatory infiltration within the dermis [14]. In inflammatory EBA, the infiltration is dominated by neutrophils, along with various numbers of eosinophils and mononuclear cells [36]. Using electron microscopy, one can observe split formation beneath the *lamina densa* at the dermal-epidermal junction (DEJ) [37].

Antibody deposition at the DEJ is tested by direct immunofluorescence (DIF) or direct immunoelectron microscopy (DIEM). The anti-COL7 autoantibodies are lineally deposited at the DEJ, with IgG as the dominant Ig-subclass, along with IgA and IgM [38]. DIEM provides a more detailed location of the deposition site, i.e., the *lamina densa* and/or *sublamina densa* at the DEJ [39]. This location is distinct from that in other AIBDs [39-41]. DIEM is useful to detect the exact deposition site; however, it is often not available. Therefore, a u-serrated pattern analysis on DIF is used more frequently [42]. Other options for detection of tissue-bound anti-COL7 IgG include immunofluorescence (IIF), western blotting and indirect immunoelectron microscopy (IIEM). Using salt-split skin as a substrate, IIF shows IgG binding on the dermal side of the split. It can thereby easily be distinguished from BP and some MMP cases in which the immunoglobulins bind on the epidermal side of the split.

For the serological diagnosis of EBA, 3 different assays are available: (i) an ELISA that uses the non-collagenous (NC)1 and NC2 domains of COL7 [43, 44], (ii) an ELISA that is based on the NC1 domain alone [45] and (iii) an indirect IF test employing the NC1 domain [45]. Serum levels of IgG against COL7 correlate with disease activity of EBA patients [46]. However, no correlation was detected between antibody specificity and clinical phenotype [47].

In some cases, the diagnosis of EBA is challenging. As an example, EBA is often confused with Linear IgA bullous dermatosis (LABD), with IgA as predominant immunoglobulin, due to the diversity of the clinical phenotype. Some dermatologists therefore consider LABD as a subset of EBA [14].

1.2. 'Standard' treatment of EBA

Systemic corticosteroids are widely used as a first choice for EBA as well as for the other AIBDs [12]. Treatment with corticosteroids leads to significant improvement of symptoms that include skin fragility, vesicles, bullae or erosions and scarring with milia in the most cases. However, specific strategies to reverse scarring have not yet been developed. Colchicine is used as a first line treatment for mild cases of EBA because of its relatively mild side-effects compared to other therapeutic choices [48, 49]. Dapsone has been used in some EBA patients [50]. Monotherapy with any of those treatments is often unsuccessful, and the doses are limited because of their side-effects, particularly in the case of corticosteroids. Therefore, a combined therapy using corticosteroids and other immunosuppressants is often required. More recently, high-dose intravenous immunoglobulin (IVIG) and anti-CD20 have been used in EBA [51]. Rituximab has been used with varying success in EBA. The first report of the successful use of rituximab in EBA was published in 2006 [52]. So far, both

IVIG and anti-CD20 tend to be used in treatment-resistant cases only [53, 54]. As a result, the management of AIBD and especially EBA remains challenging. Adverse effects from standard treatments arise both from their long-term use and abrupt discontinuation. Immunosuppression increases patients' risks of developing infections [55].

2. Pathogenesis

The development of specific mouse models for the different phases of EBA has greatly improved our current understanding of EBA pathogenesis. However, despite specific pathways that one can analyze by mouse models, we would at this point like to emphasize that not all findings in mice necessarily correspond to humans. Mice can be easily genetically manipulated while human beings are diverse and fiercely outbred. Often therapies have to be withdrawn because they have consequences not predicted earlier. Therefore, findings in mice have to be handled with care. Nevertheless, due to the limited numbers of EBA patients, many of the current findings are based on mouse models. We here discuss all current targets, including studies derived from human as well as murine data.

2.1. Identification of COL7 as the autoantigen in EBA

Dr. Yaoita's observation of linear immunoglobulin and complement deposition along the dermal-epidermal junction in EBA patients in 1981 was a starting point for more extensive studies. In the same year, *in vivo* deposits of IgG were observed below the *lamina densa* associated with the anchoring fibrils in all areas of the skin of an EBA patient. Three years later, an unrecognized protein was identified, which was distinct from other known components of the basement membrane [56]. In 1988, the carboxyl terminus of COL7 was identified as the autoantigen in EBA. Detailed epitope mapping studies with sera from EBA

patients [47, 57-59] demonstrated that most autoantibodies bind to epitopes located within the noncollagenous (NC) 1 domain of COL7. Antibody reactivity to either the collagenous domain [60] or the NC2 domain [61] can be detected in only very few patients.

2.2. Genetic Predisposition to EBA

Extensive studies on the genetic control of EBA point toward a complex interaction of different genes, subsequently leading to EBA development. Basically, genetic involvement consists of (i) genes within the MHC locus and (ii) genes outside the MHC locus. In more detail, an association with the MHC locus (HLA-DR2) has been documented in two independent studies [7, 62]. This finding is paralleled by the observation of a strict association of susceptibility to immunization-induced EBA to the H2s locus [63]. Evidence for the involvement of genes outside the MHC locus arises from the observation that black-skinned patients had a significantly higher risk to develop EBA compared to white-skinned patients [7] in a cohort of EBA patients diagnosed in a French referral center. The contribution of genes outside the MHC locus is further emphasized by mouse studies: After immunization with COL7 in the immunization-induced model of EBA, the disease intensity varied in mice of different strains but with the same MHC haplotype [19]. Another study identified several non-MHC (QTL) associated with susceptibility for immunization-induced EBA [63]. In addition to genetic factors, a recent study further indicated an effect of gene-microbiota interactions on the effector phase of EBA [64].

2.3. The production of autoantibodies directed against COL7

EBA is driven by the production of autoantibodies directed against COL7. Unfortunately, little human data underpins this relevant and complex phase of pathogenesis that includes interaction of various immune cells, such as antigen-presenting cells, autoreactive B cells, T cells and neutrophils that subsequently lead to antibody production. Therefore, all of our understanding of this initial step in EBA pathogenesis has been derived from animal models [65].

COL7-specific T cells can be detected in the circulation of EBA patients [66]. A requirement of T cells for the induction of autoantibody production was further shown in a mouse model: T cell-deficient mice were completely protected from the induction of immunization-induced EBA. Disease susceptibility could be restored by the transfer of T cells from wild-type mice that had been immunized with COL7 [67]. Defining T cell subsets involved in the generation of anti-COL7 antibodies in experimental EBA, CD4+ and CD8+ T cells were depleted in a time-restricted manner at the time of immunization. Depletion of CD4+ T cells delayed both autoantibody production and the onset of clinical disease. In contrast, depletion of CD8+ T cells for the same time period had no effect on autoantibody production or clinical disease manifestation [67]. Therefore, a requirement for CD4+ T cells was demonstrated for the induction of autoantibody production in experimental EBA. To further address the requirement for antigen-presenting cells for the formation of antigen-specific CD4+ T cells, B cell–depleted mice were immunized with COL7, and their antigen-specific CD4+ T cell response was evaluated. The antigen-specific CD4+ T cell response was completely absent in B cell–depleted mice. Depletion of dendritic cells and macrophages had a similar effect, leading to the conclusion that the COL7-dependent development of COL7-specific CD4+ T cells requires the presence of B cells, dendritic cells, and macrophages [67] (Figure 2A).

Further effort has been made in the analysis of Th1 polarization in the peripheral lymph nodes in experimental EBA. Because only few strains develop skin blistering after immunization

with COL7, the autoantibody response of clinically healthy versus diseased mice after COL7 immunization was compared, showing that complement-fixing antibodies were associated with clinical EBA manifestation [68]. This antibody response reflected the Th1 polarization of the immune response. This assumption was further supported by an increased IFN-γ/IL-4 ratio in the draining lymph nodes of EBA-susceptible mice compared with EBA-resistant strains [68]. Regarding the involvement of neutrophils in autoantibody production, GM-CSF(-/-) mice produced lower a serum autoantibody titer, which was associated with lower neutrophil numbers in draining lymph nodes. The same effect was observed in neutrophil-depleted wild-type mice [69].

Differently than T cells, autoreactive B cells almost exclusively reside in peripheral lymph nodes in immunization-induced EBA [70, 71]. A possible explanation of this finding is their lack of homing-associated CXCR3 and CXCR4 chemokine receptor expression. It was further demonstrated that murine COL7-specific plasma cells have a half-life of approximately 7 weeks, resembling an intermediate between short-living (a few days) and long-living (many months or years) plasma [70]. These findings correspond to the frequently observed slow decline of the autoantibody titer in patients with autoimmune bullous diseases within 8-12 weeks of successful B cell-targeting immunosuppressive treatment [72].

Another important factor relevant to the modulation of autoantibody production is heat-shock protein 90 (Hsp90). In an *in vivo*-study, HSP90 inhibition in mice immunized with COL7 led to the suppression of autoantibody production. Furthermore, HSP90 inhibitors prevented the onset of immunization-induced EBA when injected prior to immunization with COL7 and also ameliorated clinical disease in already established EBA in a therapeutic setting. Total plasma cell numbers, COL7-specific plasma cells, and germinal center B cells were unaffected by the HSP90 inhibition. Interestingly, T cell proliferation was significantly inhibited, identifying T cells as targets of HSP90 inhibition in EBA [66].

After the autoantibodies are released to the circulation, their half-life is controlled by the neonatal Fc receptor (FcRn). FcRn is constructed as a heterodimer, consisting of an alpha-chain and a beta-2-microglobulin (B2M) light chain. The alpha chain is a major MHC class I-like molecule, and its official gene name is the "Fc receptor, IgG, alpha chain transporter" (FCGRT). Similar to all MHC class I proteins, FCGRT must complex with the B2M light chain to exit the endoplasmic reticulum and for the efficient pH-dependent binding of IgG [73]. Among other functions, the FcRn protects IgG from catabolism [74]. Inhibition of the FcRn leads to the enhanced clearance of IgG, including autoantibodies. In animal models of AIBD, including pemphigus, bullous pemphigoid and EBA, disease induction in mice is completely blocked [75, 76]. However, this protection can be overridden by the transfer of high amounts of antibodies [75].

2.4. Effector phase

Based on the current understanding of EBA pathogenesis, the effector phase of EBA, i.e., autoantibody-induced blistering, can be divided into five distinct events (Figure 2B): (**i**) autoantibody binding to their target antigen, (**ii**) complement recruiting, (**iii**) Fc-dependent effects on blister formation, (**iv**) neutrophil extravasation and activation, including the release of reactive oxygen species (ROS) and matrix metalloproteinases (MMP), and (**v**) autoantibody-induced tissue injury resolution.

Autoantibody-induced tissue injury in EBA is initiated by **(i) the deposition of autoantibodies at the DEJ**. Apart from the skin, morphological investigations after anti-COL7 IgG antibody transfer into mice showed IgG binding in the esophagus, stomach, small intestine, and colon [11, 21]. However, not all isoclasses of anti-COL7 have the potential to

induce dermal-epidermal separation: IgG1 and IgG3, but not IgG2 and IgG4, induced blister formation in an ex vivo study [77].

Subsequently, **(ii) a pro-inflammatory milieu is generated** in the skin, including complement activation [78]. The complement system consists of circulating proteins that upon activation initiate a highly controlled cascade that is an integral part of the innate humoral immune response [79]. Antibodies specific to type VII collagen fail to induce the disease when injected into C5-deficient mice [19]. A more detailed study analyzed the specific role of each pathway using C1q-deficient mice, MBL expression-lacking mice and factor-B-deficient mice for analysis of the classical, the lectin and the alternative pathway, respectively. As a result, mice that did not express MBL showed a similar EBA phenotype as wild-type controls. However, C1q-deficient mice showed weak and partial protection, and factor-B-deficient mice showed strong protection from EBA induction [78], indicating a significant contribution of the complement system in the pathogenesis of autoantibody-induced tissue injury in EBA [80].

Various studies have shown that after extravasation into the skin, **(iii) myeloid effector cells bind to the skin-bound immune complexes** in a restricted Fcg receptor fashion. *In vitro*, only IgG but not F(ab)2 fragments, directed to COL7 caused dermal-epidermal separation when incubated on cryosections of human skin, followed by the addition of neutrophils from healthy donors [81]. On the other hand, F(ab′)2 fragments of rabbit IgG specific to COL7 were not pathogenic when injected into mice [19]. The importance of Fc-Fc receptor interactions for blister formation in experimental EBA is further supported by the absence of skin lesions in mice injected with chicken anti-mouse COL7 IgY, which does not bind murine complement and Fc receptors [82], and the therapeutic effects observed when blocking these interactions, i.e., using soluble CD32 [83]. In addition to these findings, IgG glycosylation has been shown to have preventive and therapeutic effects in inflammation models including EBA

[84]. Further analysis has described the roles of different Fc receptors in mice: three different activating Fc gamma receptors (FcgRs) and one inhibitory FcgR. FcgRI, FcgRIII, and FcgRIV activate FcgR but are different in their Fc-binding avidity. Currently, FcgRIIB is the only inhibitory FcgR described in mice [85]. Expression profiling of lesional EBA skin of mice showed an increased expression of FcgRIV [71]. Subsequent studies identified FcgRIV as the key mediator of tissue injury in EBA; however, a blockade of FcgRI, FcgRIII, or both receptors in combination had no effect on the EBA manifestation. Blockade of the FcgRIIB showed that this FcgR protects mice from EBA development; i.e., its absence leads to enhanced blistering in antibody transfer models of pemphigoid disease [71, 86].

This pro-inflammatory milieu promotes **(iv)** CD18- and ICAM-1-mediated **recruitment of myeloid effector cells** (predominantly neutrophils) into the skin [83, 87]. This process is tightly regulated and primarily mediated by adhesion molecules and cytokines, such as IL-1, CXCR1/2, GM-CSF and IL-6 [69, 88-90]. In general, leukocyte extravasation is initiated by the interactions of endothelial adhesion molecules of the selectin family with their leukocyte counterpart. [91]. Regarding the contribution of adhesion molecules to the pathogenesis of blistering in EBA, currently, only CD18 and ICAM-1 have been shown to contribute to (or be associated with) blistering [92, 93]. Furthermore, in AIBD, very little is known about how leukocyte migration is directed to the immune-complexes located at the DEJ after extravasation.

Once the neutrophils are activated by binding to the immune complexes (see above), a **multifaceted signaling cascade** occurs within the myeloid effector cells. This complex pathway involves the activation of PI3K beta [94], p38, AKT, ERK1/2 [95] and Lyn kinases [96]. The exact temporal and spatial order of these signaling events is completely unknown. Ultimately, the signaling cascade leads to the **activation of the myeloid effector cells**, exemplified by release of reactive oxygen species (ROS) and proteases. ROS production has

been demonstrated to be crucial for blister formation [93]. In addition to ROS, MMPs such as elastase and gelatinase B have been identified as crucial mediators of dermal-epidermal separation in EBA [97]. Dermal-epidermal separation, induced by incubating cryosections of normal human skin with IgG from EBA patient serum and neutrophils from healthy blood donors, could be completely abolished by broad-range protease inhibitors, as well as inhibitors of serine and matrix metalloproteases. This finding demonstrates that subepidermal blistering in EBA is a protease-mediated process. Interestingly, protease inhibition abolished dermal–epidermal separation induced by sera from patients with both the classic and inflammatory phenotype of EBA. When characterizing the proteases involved more specifically, selective inhibition of human leucocyte elastase or gelatinase B/MMP-9 suppressed blistering. While inhibition of either serine or matrix metalloproteases completely blocked dermal–epidermal separation induced by EBA or BP autoantibodies, split induction was only slightly diminished by a cysteine protease inhibitor. Interestingly, inhibition of aspartic proteases with pepstatin A enhanced blister formation. This unexpected finding may be related to the granulocyte-activating and chemotactic properties of pepstatin A. As a conclusion, these findings strongly suggest that elastase and gelatinase B are essential for granulocyte-mediated proteolysis resulting in dermal–epidermal separation in EBA patients' skin and might serve as a high-ranking treatment modality [97].

While the previous mechanisms focus on the initiation and perpetuation of blistering, recent evidence also indicated that **(v) resolving tissue damage and/or inflammation** are also crucial in determining the extent of blistering. Flightless I (Flii), an actin remodeling protein, has recently been shown to contribute to the resolution of skin blistering in experimental models of EBA. *In vivo*, the induction of EBA leads to increased cutaneous Flii expression, resulting in impaired Claudin-1 and Claudin-4 tight junction protein expression, as well as a delay in the recovery from blistering [98, 99]. Reduced Flii expression in Flii+/− mice or

caused by the topical application of Flii neutralizing antibodies significantly impaired blister formation in experimental EBA and enhanced the healing of blistered skin in this model [100]. These observations point towards a significant contribution of pathways that are involved in the resolution of cutaneous involvement. Therefore, EBA may manifest not only when many proinflammatory stimuli are present but also when the balance of proinflammatory, anti-inflammatory, and resolving pathways are in unbalanced, driving the cells toward proinflammatory mechanisms.

2.5. The role of cytokines in EBA

Recent evidence implied that cytokines govern blistering in EBA. Because biologics targeting these pathways are already in clinical use - albeit for other indications [101] - we emphasize their role in EBA in this review.

Several cytokines have been shown to contribute to blister formation in experimental EBA (Table 2). IL-6 acts as a pro-inflammatory cytokine in various autoimmune diseases; therefore, it is a relevant target for treatment of autoimmune diseases. In EBA patients and in experimental EBA models, serum IL-6 levels are significantly elevated. Unexpectedly, mice lacking IL-6 showed a significantly increased clinical phenotype in experimental EBA compared with the controls. Furthermore, the administration of recombinant IL-6 led to a dose-dependent reduction of experimental EBA. In conclusion, IL-6 acts as an anti-inflammatory cytokine in experimental EBA in mice [88]. On the molecular level, the protective effect of IL-6 was mediated by the IL-6 dependent release of Il-1ra and prophylactic IL-1ra administration prevented blistering. An elevated serum concentration of IL-1b was further demonstrated in both experimental EBA-mice and EBA patients [89].

In contrast, IL-1R–deficient or wild-type mice treated with the IL-1R antagonist Anakinra or anti–IL-1b were significantly less affected by experimental EBA compared with wild-type mice. These findings suggest that IL-1 contributes to the recruitment of inflammatory cells into the skin. Accordingly, IL-1R–deficient mice and Anakinra-treated mice injected with anti-COL7 antibodies showed a decreased expression of ICAM-1. This effect appeared to be specifically attributable to IL-1: Anakinra blocked the upregulation of different endothelial adhesion molecules on IL-1–stimulated, but not on TNF-a–stimulated, cultured endothelial cells. Interestingly, the injection of caspase-1/11–deficient mice with anti-COL7 IgG led to skin lesions to the same extent as in wild-type mice. Collectively, IL-1, independent of caspase-1, contributes to the pathogenesis of EBA [89].

Furthermore, the evaluation of cytokines affecting neutrophil functions such as IL-8 (CXCL1 and CXCL2 in the mouse) and GM-CSF in experimental EBA showed increased expression. Moreover, oral administration of allosteric CXCR1 and 2 inhibitors (DF2156A) impaired the induction of skin blistering induced by the transfer of anti-COL7 in mice.

In a therapeutic setting, administration of DF2156A improved clinical EBA manifestation after the disease onset in immunization-induced EBA [90]. As for the evaluation of GM-CSF, anti-COL7 IgG was injected into GM-CSF-deficient mice or wild type mice treated with a function-blocking GM-CSF antibody. Induction of experimental EBA was impaired if GM-CSF function was blocked compared to appropriate controls. *In vitro* studies demonstrated the requirement of GM-CSF for neutrophil recruitment from bone marrow into the blood and from the blood into the skin. Furthermore, GM-CSF preactivated the neutrophils, leading to an enhancement of immune complex-induced neutrophil activation. In a therapeutic setting, the blockade of GM-CSF in mice with already established immunization-induced EBA showed beneficial therapeutic effects [69, 89].

3. Emerging treatments for EBA

The current (unsatisfactory) treatment options for EBA have been outlined above. An increasing understanding of EBA pathogenesis indicates new potential therapeutic targets that interfere in different phases of disease progression, including (i) the generation of autoantibodies, (ii) maintaining autoantibodies in the circulation and (iii) autoantibody-induced tissue injury.

3.1. Emerging treatments predominantly targeting generation of autoantibodies

(i) T cells

T cells are essential for the generation of autoantibodies in experimental EBA [65, 67, 102]. Furthermore, COL7-specific T cells can be detected in EBA patients [102]. Therefore, T cell activation- and interaction-targeting strategies are a promising option for severe and treatment-refractory cases. These treatments include monoclonal antibodies against CD3, CD4, CD40L and IL-2R, some of which have already been successfully used in mouse models and in single patients with autoimmune bullous diseases [103-105].

(ii) B cells

Whereas antigen-specific B cells can only be detected in peripheral lymph nodes in mice, the location of autoreactive B cells human EBA patients remains unknown. Treatment with rituximab, a monoclonal antibody targeting CD20 depletes mature, autoreactive B cells. Reported rituximab treatment in cases of EBA patients either led to complete remission or very good partial remissions [106, 107]. Rituximab can safely be combined with high-dose

IVIG, which may exert a synergistic effect and simultaneously protect against serious infection-related adverse events [108, 109]. Rituximab and Ofatumumab, another antibody targeting CD20, are currently being studied for the treatment of other AIBD in multi-centered, randomized controlled clinical trials and might be an important therapeutic option for EBA once their safety profiles and treatment regimens are more definitively understood.

(iii) Neutrophils and GM-CSF

As mentioned above, there is evidence that GM-CSF and neutrophils contribute to adaptive immune functions in experimental EBA: both GM-CSF-deficient mice and neutrophil-depleted mice had lower autoantibody titers compared to wild-type mice after immunization with COL7. Thus, both GM-CSF and neutrophils play a role in the generation of autoantibodies in experimental EBA, presumably by mediating the immigration of antigen-presenting cells into the peripheral lymph nodes [69]. Although the precise mechanisms have not been elucidated, GM-CSF and neutrophil pathways represent further potential targets for the modulation of autoantibody production in EBA patients.

(iv) HSP90

Because a blockade of HSP90 prevented the onset of immunization-induced EBA and induced clinical recovery in established disease [66], the blockade of HSP90 is an attractive therapeutic approach. Due to its inhibitory effects on malignant cells [110], anti-HSP90 treatment is currently being tested in clinical trials for cancer therapy. It also acts as a potent immunomodulatory agent, which is why it is also increasingly becoming a focus of research in autoimmune diseases including blistering disorders [66, 111, 112]. A study on the mechanisms that lead to immunomodulation revealed that HSP90 inhibition leads to a reduction in autoreactive T cell proliferation [66]. This might be due to an inhibition of pro-inflammatory NF-kB, the up-regulation of anti-inflammatory HSP70, or the modulation of

Lck kinase-mediated T cell receptor signaling [113]. As for regulatory T cells, HSP90 inhibitors have also been shown to enhance Treg function in other *in vivo* models of inflammation and autoimmunity [111, 112]. As shown in an immunization-induced EBA model, anti-HSP90 treatment further modulates humoral B cell responses by inhibiting effector B cell subsets and promoting regulatory B cell subsets [114].

3.2. Emerging treatments predominantly targeting the maintaining autoantibody concentration

As previously mentioned, the neonatal Fc receptor (FcRn) strongly contributes to the regulation of circulating IgG levels including autoreactive IgGs by preventing their degradation [115]. As a heterodimer, it consists of an alpha-chain (FCGRT) and a beta-2-microglobulin (B2M) light chain. FCGRT must complex with the B2M light chain to exit the endoplasmic reticulum and for the efficient pH-dependent binding of IgG [73]. Therefore, both chains are necessary for a fully functional FcRn [116]. Similar to animal models of bullous pemphigoid and pemphigus [75, 76], reduced autoantibody levels were found in mice lacking FcRn compared to wild-type controls in the antibody-induced transfer model and the immunization-induced model of EBA. This resulted in protection from tissue injury that could be overridden by the transfer of high amounts of anti-COL7 antibodies [75, 76]. Currently, fully human monoclonal antibodies are tested in pre-clinical trials. Recently, Nixon et al. developed an anti-FcRn antibody that caused a significant reduction in circulating IgG with no changes in albumin, IgM, or IgA when administered to rodents and non-human primates [117]. Preliminary studies in rodents and non-human primates found the antibody to be well-tolerated. These results suggest anti-FcRn antibodies may represent a novel therapeutic approach to the treatment of IgG antibody-mediated autoimmune diseases [117].

3.3. Emerging treatments predominantly targeting autoantibody-induced tissue injury

(i) Binding to the immunocomplexes: SM101

As previously mentioned, Fc receptor–dependent binding of neutrophils to immune complexes in the skin is a key feature for autoantibody-mediated blister formation in EBA [19]. Therefore, the inhibition of the binding emerges as a potential therapeutic target. SM101, a soluble FcγRIIB, competes with FcgR expressed in immune cells for pathogenic immune complexes [118] and thereby inhibits their interaction. Treatment of lupus-prone mice with SM101 significantly delayed the onset of proteinuria, reduced histopathological damage and improved survival [119]. SM101 has completed Phase 2a studies in idiopathic thrombocytopenic purpura (ITP) and systemic lupus erythematosus (SLE). The Phase 2a data presented during the American College of Rheumatology's annual meeting in 2014 indicated a dose response for multiple endpoints in patients with SLE treated with two different doses of SM101 for six months [119]. Therefore, SM101 might be a potential therapeutic option for other autoimmune diseases such as EBA.

(ii) Therapeutics targeting the sugar moiety of the Fc portion of anti-COL7 IgG

An exciting new therapeutic avenue has recently been described by changing the sugar moiety of the Fc portion of anti-COL7 IgG. Multiple studies have demonstrated that the glycosylation status of IgG has a great impact on its function [120]. Alterations in the Fc glycosylation patterns appear in several chronic inflammatory diseases [121] and modification of IgG glycosylation has shown preventive and/or therapeutic effects in models of inflammation, including EBA [122-127]. Decreased levels of galactosylation and sialylation of IgG have been found to associate with disease onset and severity in inflammatory autoimmune

conditions [128, 129]. Consistently, highly galactosylated IgG1 has been shown to promote cooperative signaling of Fcγ receptor (FcγR)IIb with dectin-1, resulting in anti-inflammatory effects [80]. The glycosylation status of the Fc portion of IgG is important for interaction with FcγRs. In fact, hydrolysis of IgG glycan reduces the binding ability to FcγRs in both mice and humans [130, 131]. EndoS is an endoglycosidase from the human pathogen Streptococcus pyogenes that hydrolyzes the conserved N-linked glycan on IgG heavy chains [132]. To date, EndoS is the only endoglycosidase that is exclusively known to hydrolyze the glycan of native IgG [132]. EndoS has been successfully applied in experimental autoimmune diseases, including immune-mediated thrombocytopenia [132] and rheumatoid arthritis [123]. Regarding EBA, EndoS significantly reduced the pathogenic effect of anti-COL7 IgG in 3 different experimental models. In the immunization-induced mouse model of EBA, EndoS suppressed disease activity even when applied after anti-COL7 antibodies had bound to the skin and clinical disease had developed. Furthermore, EndoS hydrolyzed already bound pathogenic autoantibodies in vivo and differentially modulated the expression of activating and inhibiting FcγRs [127].

(iii) Targeting cytokines: Anakinra, anti-GM-CSF, CXCL1/2

As previously mentioned, certain cytokines and chemokines such as CXCL1 and CXCL2 are novel therapeutic targets in EBA. Reparixin is an example of a compound that blocks CXCL1 and/or CXCL2 and enhanced pancreatic islet survival after transplantation in a phase II randomized, open-label pilot study [133]. The oral administration of similar allosteric CXCR1 and 2 inhibitors (DF2156A) impaired the induction of skin blistering in mice induced by transfer of anti-COL7. In a therapeutic setting, the administration of DF2156A improved clinical EBA manifestation after disease onset in immunization-induced EBA [90]. Inhibition of CXCL1/2 by receptor-blockade therefore seems to be an emerging therapeutic approach for patients with treatment-refractory EBA.

Because the blockade of GM-CSF confers beneficial effects both in antibody transfer- and immunization-induced EBA, it is tempting to speculate that it might be an important future therapeutic target. Currently, GM-CSF has emerged as a target for rheumatoid arthritis, and three different anti-GM-CSF antibodies are currently being evaluated in phase II clinical trials for this indication.

Because the serum levels of IL-6 are found to be significantly higher in EBA patients, IL-6 is a potential target in EBA: Tocilizumab is a humanized monoclonal antibody against the interleukin-6 receptor (IL-6R) that is commonly used as a treatment for rheumatoid arthritis (RA) and systemic juvenile idiopathic arthritis [134]. Because the effects of IL-6 are at least partially mediated by the regulation of IL-1ra, this cytokine is a further potential target. The interleukin-1 (IL-1) receptor antagonist Anakinra is commonly applied in severe cases of rheumatoid arthritis [135]. Furthermore, Anakinra is very effective in patients with Schnitzler's syndrome [136]. Currently, Anakinra has not been used to treat AIBD or EBA patients. However, based on the efficacy of the compound for other inflammatory conditions and the preclinical data in experimental EBA, compounds targeting IL-1 are expected to have beneficial effects on EBA patients.

(iv) Extravasation: CD18, α4 integrin, CD11a and heparins

Because a lack of CD18 expression leads to the reduction of neutrophil extravasation into the skin and subsequent clinical disease manifestation in experimental EBA [93], leukocyte extravasation is another potential target in EBA patients. Currently, the humanized anti-α4 integrin antibody Natalizumab is an effective treatment for relapsing-remitting multiple sclerosis [137]. The side effects of Natalizumab treatment, including leukoencephalopathy [138] make it necessary to monitor the patients regularly and the individual risk and benefit of the treatment must thoroughly be weighed. Other treatments targeting leukocyte extravasation, for example, anti-CD11a (Efalizumab), have either been withdrawn, have not

been successful in sufficiently controlling skin inflammation [139, 140], or have not been tested in inflammatory skin conditions [141]. Modified heparins could be an alternative to the targeted disruption of leukocyte extravasation by use of recombinant antibodies. In addition to its potent anti-coagulant activity, heparin has potent anti-inflammatory activity [142]. Structural modifications of structurally defined glycan sulfates obtained by chemical modifications of neutral homoglycans produced by algae, bacteria, or fungi allowed the production of compounds with reduced anticoagulant activity but stronger anti-inflammatory and/or antimetastatic activity compared to heparin [143]. One of these modified homoglycans, termed PS3, inhibits cutaneous inflammation [143].

(v) Inhibitors of cell signaling

As previously mentioned, targeting signaling (Src, PI3Kβ, Akt, Erk1/2, p38) and effector (ROS, MMPs) molecules related to neutrophil activation and the blister wound-associated protein Flii proved to be effective in experimental EBA and could therefore also be a future therapeutic approach in EBA patients [83, 90, 93-95, 97, 144-146].

(vi) IVIG

The treatment of COL7-immunized mice with intravenous immunoglobulins (IVIG) resulted in ameliorated clinical disease severity, reduced levels of autoantibodies, and a shift towards Th2-mediated non-pathogenic autoantibodies [68], which supports their previously described effective and safe use in patients with autoimmune bullous diseases including EBA [54, 147].

3.4. Limitations

Without doubt, the major limitations of these findings is the fact that most of these have been obtained in mouse models of EBA, which are unfortunately only established in few laboratories [47, 148-150]. Yet, due to the rarity of the disease identification and validation of

novel therapeutic targets is in our opinion the only possible way to do so. Therefore, wide use of EBA animal models in many different laboratories would certainly significantly contribute to our understanding of the disease, as well as identify numerous validated therapeutic targets. To achieve a wider use of EBA animal models we are very open to share our reagents and expertise. At least as important is the establishment of an international patient registry for EBA patients. Based on the epidemiological data obtained herein, which must include treatment outcomes, valuable data can be gathered to plan international controlled clinical trials.

Another limitation of the above mentioned emerging treatments for EBA are their potential adverse events. Most of the presented therapeutics have immunosuppressive effects, which they share with the current treatment modalities used for EBA. The efficacy as well as the safety of these treatments needs to be evaluated in clinical trials. Potentially, adverse events of (at least some) could be less compared to systemic corticosteroid treatment for several reasons: First, they target pathways that are upregulated in EBA. Hence, the majority of inhibition would predominantly modulate the EBA-related increased expression of certain cytokines, while affecting less the concentrations required for immune homeostasis. Second, some of the compounds, i.e. small molecules, could potentially be topically applied. Third, one could imagine a personalized combination treatment, which would be tailored to target pathways specifically altered in an individual patient. Yet, these considerations need to be put to the test in clinical trials.

4. Expert opinion

The identification of the autoantigens in AIBD triggered an in-depth understanding of the disease pathogenesis. This for example prompted the development of in vitro and in vivo experiments which have shaped our current understanding of processes that happen during EBA. However, AIBD and especially EBA are rare diseases. This complicates sampling and clinical research and the interest of drug-developing companies to work on this disease is rather scare. In contrast, research in the field of AIBD holds a great potential not only for drug-development of EBA itself, but also of other antibody-driven autoimmune diseases such as RA, EAE and multiple sclerosis. Since signaling pathways and inflammation in EBA analogously happen in those diseases, new-discovered therapeutic options might be transferred. The knowledge of COL7 as a specific and well-defined target for the autoantibodies in EBA provides a good basis for the identification and definition of specific signaling pathways. The ultimate goal of this research field is the development of specific, pathogenesis-based therapy. To achieve this goal, it is necessary to collect patient data in a controlled and standardized manner and to register treatment outcomes. Using the knowledge derived from drug testing in animal models, this would make it possible to apply therapy in a patient-orientated manner. Looking at the future, we expect therapy in EBA to become more and more precise and move towards a patient-based therapy, which is similar to what has been developed for certain types of cancer, e.g. BRAF in melanoma or Her2-expression in breast cancer. Through identification of specific mechanisms and identification of the leading cytokines it will be possible to specifically target the dominating pathway of a respective patient. In contrast to other diseases, biopsies are easy to access, which will facilitate patient-based therapy. An advantage of the variety of signaling pathways that ultimately lead to EBA is that each patient has some pathways which are up- and others which are downregulated. The result is a wide range of opportunities to interfere in the disease pathogenesis, resulting in a large number of targets. The idea will be to test each patient for dominating pathways,

create a network and then specifically target a respective pathway. A major advantage in the development of novel dermatological substances is the possibility of topical application through ointments. As a result, the ultimate goal of this research field is the identification of specific pathways involved in the pathogenesis of EBA to develop specific, pathogenesis-based therapeutic strategies. To reach this aim, it is necessary to refine existing animal models and advance precision medicine.

Since the first description of EBA more than 100 years ago, our understanding of the cellular and molecular mechanisms leading to the development of this severe disease has dramatically improved. This knowledge led to the development of new therapies, some of which have been successfully tested in preclinical models and now await confirmation in proof of concept trials. In the future new drug targets will emerge to improve the therapy and the living standard of people suffering from EBA.

Financial and competing interests disclosure

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

Article highlight box

- Epidermolysis bullosa acquisita is a difficult-to-treat autoimmune bullous disease with autoantibodies targeting COL7, the major component of anchoring fibrils

- Treatment of EBA is performed using standard immunosuppressive therapy that is accompanied by adverse effects
- Pathogenesis of EBA is divided into (i) the generation of autoantibodies, (ii) the maintenance of autoantibodies in the circulation and (iii) autoantibody-induced tissue injury
- Based on the latest knowledge of signaling pathways and mechanisms that lead to EBA, we here provide novel therapeutic targets that might enhance the development of more specific, personalized medicine.
- New-discovered therapeutic options of EBA can be transferred to in other antibody-driven autoimmune diseases such as RA, EAE and multiple sclerosis, since signaling pathways and inflammation in EBA analogously happen in those diseases

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Figure legends

Figure 1. Clinical presentation of epidermolysis bullosa acquisita (EBA). (A) Non-inflammatory type; tense blisters with scarring, milia and hyperpigmentation at the knee (B) tend to be localized to area of trauma. (B, C) Inflammatory-type of EBA; (B) Oral mucosal lesions; and (C) tense blisters with erythemas on the back in patients with inflammatory type EBA. (D)

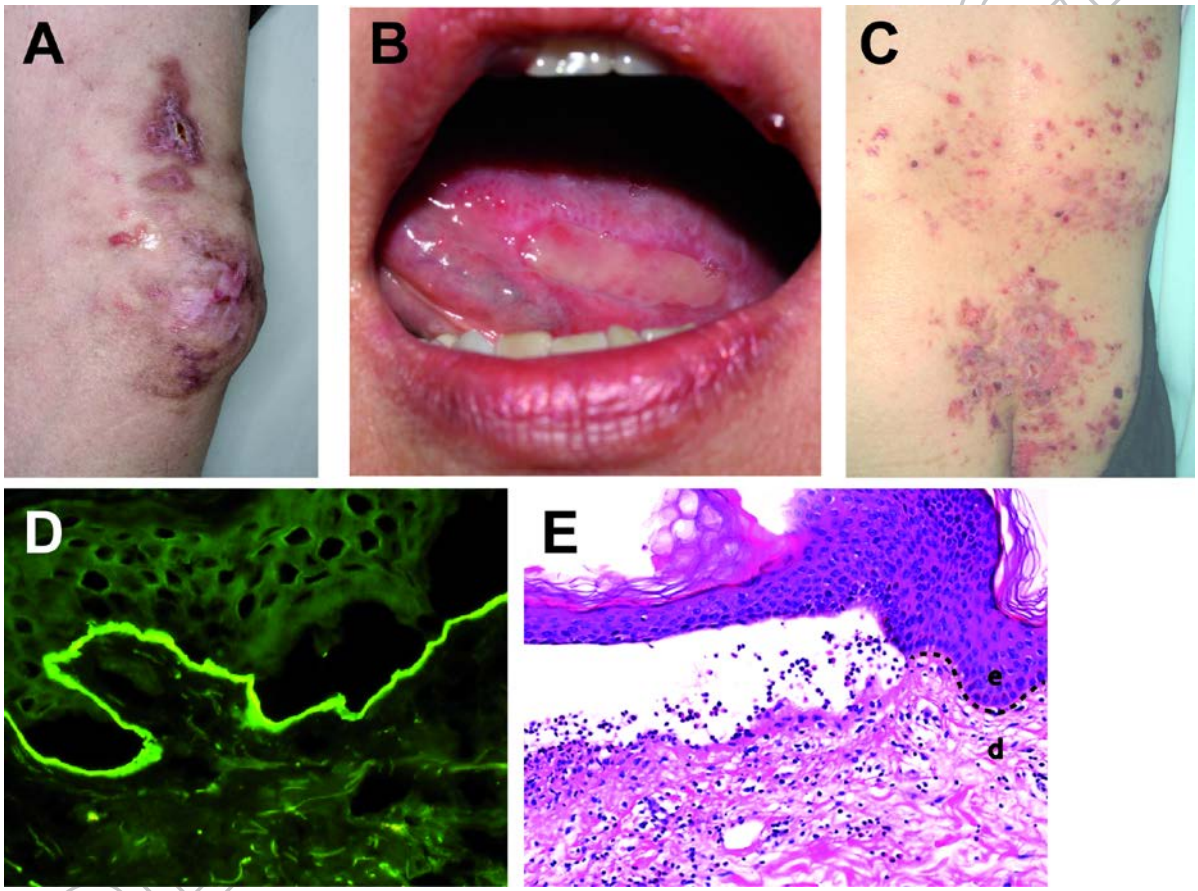

Figure 2. **Pathogenesis of EBA**. Loss of tolerance during EBA is mediated by the interaction of APCs with autoreactive B and T cells, leading to clonal expansion and differentiation into plasma cells. Autoantibodies against COL7 are released to the blood cycle and the effector organs. This leads to (i) binding of the autoantibodies to the DEJ at the skin, (ii) complement deposition, (iii) Fc-dependent effects on blister formation, (iv) neutrophil extravasation and activation, including the release of ROS and MMP, and (v) resolution of the autoantibody-induced tissue injury.

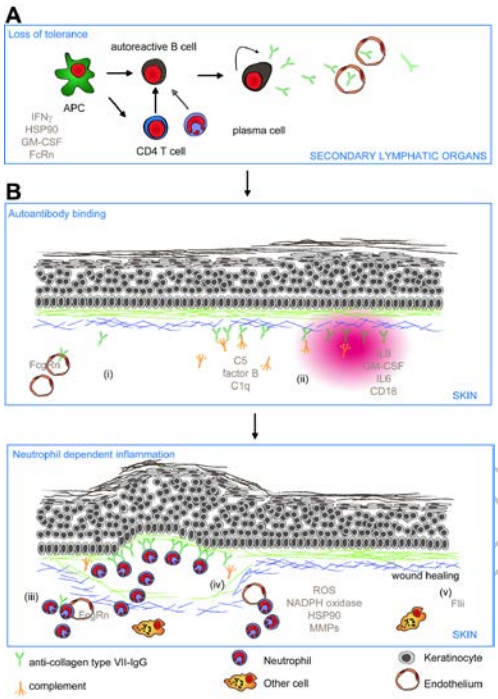

Table legends

Table 1. Functional relevance of cytokines during EBA.

Cytokine	Analytic method	Mouse	Human	Reference
IFNγ	lymph node mRNA expression	x	nd	[68, 88]
IL-4	lymph node mRNA expression, serum level detection	x	nd	[88]
IL-1ß	serum level detection, receptor blocking	x	x	[88]
IL-6	skin and serum level detection, IL-6 application and blockade	x	x	[88]
CXCL-1/2 (mouse) IL-8 (human)	skin and serum level detection, receptor blocking	x	x	[90]
GM-CSF	skin and serum level, knockout mice, pharmacological inhibition	x	x	[69]

Table 2. Emerging treatments for EBA.

	Target molecule	Functional relevance	Analytic method	Mouse EBA prophylactic	Mouse EBA therapeutic	Human autoimmune diseases	References
Generation and maintenance of autoantibodies	**CD3**	Pan T cell receptor	Blocking antibodies			x	[151, 152]
	CD4	T cell receptor	Blocking antibodies	x		x	[65, 104, 105, 153, 154]
	IL-2/IL-2R	Regulatory T cell activation- and interaction-targeting	IL-2-therapy, IL-2R targeting antibodies			x	[155-157]
	CD40/CD40 L	T cell activation- and interaction-targeting	Blocking antibodies			x	[158, 159]
	GM-CSF	Generation of autoantibodies	GM-CSF knockout mice, blocking antibodies	x	x	x	[69, 160]
	FcRn	Antibody maintenance	Blocking antibodies, FcRn deficient mice	x	x		[75, 117]
	HSP-90	Inhibition of T cell proliferation	HSP90 inhibitors	x	x		[146]

	CD20	B cell receptor	Rituximab treatment of EBA patients			x	[161]
Autoantibody-induced tissue injury	sFcγRIIb	Binding of immune complexes	Blocking with SM101 in SLE		x		[119]
	CXCL1	Leukocyte migration	Inhibition with DF2156A, treatment of BP patients	x	x	x	[90]
	CXCL2	Leukocyte migration	Inhibition with DF2156A, treatment of BP patients	x	x	x	[90]
	GM-CSF	Generation of autoantibodies	GM-CSF knockout mice, blocking antibodies	x	x	x	[69, 160]
	IL-1	Recruitment of inflammatory cells into the skin during EBA	Serum level quantification of IL-1β in EBA patients and mice, Anakinra administration in EBA-mice and RA-patients	x	x	x	[89]
	sCD32	Inhibition of the binding of immune complexes to cells expressing CD32	Blockage with sCD32		x	x	[83, 162]
	EndoS	Hydrolyzation of the N-linked glycan of native IgG	EndoS-treatment of EBA-mice and measurement of FcγR-expression	x	x		[127, 144]
	FcγRIV	Cell receptor	Knockout mice	x			[71]
	RORa	Cell receptor	Blocking antibodies and knock-out mice, quantification in EBA-mice	x			[92]
	HSP90	Inhibition of T cell proliferation	HSP90 inhibitors	x	x		[146]
	Complement	Initiation of the innate humoral immune response	Experimental EBA in C5-, C1q-, MBL- and factor B-deficient mice, Eculizumab therapy in hemolytic uremic syndrome	x		x	[19, 78, 80, 163]
	Flii	Actin remodeling protein	Neutralizing antibodies, knockout mice	x	x		[100]
	IL-6	Immunoregulatory cytokine receptor	Treatment of RA using tocilizumab			x	[164]
	CD18	Neutrophil extravasation into the skin	Natalizumab treatment in MS			(x)	[137, 138, 165]
	CD11a	Leukocyte extravasation	Efalizumab			(x)	[139, 140]
	Src	Cell signaling	Knockout mice	x			[96]
	PI3K	Cell signaling	Knockout mice and pharmacological inhibition	x			[94]
	Erk1/2	Cell signaling	Pharmacological inhibition	x			[95]
	p38	Cell signaling	Pharmacological inhibition	x			[95]
	IVIG	Intravenous immunoglobulins	Treatment of EBA-mice and patients with autoimmunity	x	x	x	[166]