Epidermolysis Bullosa Acquisita: From Pathophysiology to Novel Therapeutic Options

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Epidermolysis bullosa acquisita (EBA) is a prototypic organ-specific autoimmune disease induced by autoantibodies to type VII collagen causing mucocutaneous blisters. In the inflammatory (bullous pemphigoid-like) EBA variant, autoantibody binding is followed by a lesional inflammatory cell infiltration, and the overall clinical picture may be indistinguishable from that of bullous pemphigoid, the latter being the most common autoimmune bullous disease. The last decade witnessed the development of several mouse models of inflammatory EBA that facilitated the elucidation of the pathogenesis of autoantibody-induced, cell-mediated subepidermal blistering diseases and identified new therapeutic targets for these and possibly other autoantibody-driven disorders.

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INTRODUCTION

Epidermolysis bullosa acquisita (EBA) is a prototypic autoimmune disease in which recalcitrant blisters on the skin and mucous membranes develop through binding of autoantibodies to type VII collagen (COL7), a constituent of anchoring fibrils of the dermal-epidermal junction (Schmidt and Zillikens, 2013; Woodley et al., 1984, 1988). COL7 is a homotrimer with three α-chains, and each consists of one central collagenous domain flanked by two noncollagenous (NC1 and NC2) domains. The N-terminal 145-kDa NC1 domain, which harbors subdomains comprising a cartilage matrix protein domain, nine fibronectin III-like domains, a collagen binding von Willebrand factor A-like domain, and a cysteine- and proline-rich domain, is the immunodominant region recognized by autoantibodies in almost all EBA patients (Chen et al., 1997, 2007; Gammon et al., 1993; Lapiere et al., 1993; Wegener et al., 2014). The recombinant NC1 domain was subsequently used in highly sensitive and specific assays for serologic diagnosis of the disease (Komorowski et al., 2013; Saleh et al., 2011).

EBA has two major clinical subtypes, the mechanobullous and inflammatory variants. Whereas the first presents with skin fragility, blisters, scarring, and dystrophic changes on trauma-prone areas with minimal clinical or histologic inflammation, the latter resembles other autoimmune bullous diseases such as bullous pemphigoid (most commonly), mucous membrane pemphigoid, Brunsting-Perry pemphigoid, and linear IgA dermatosis (Schmidt and Zillikens, 2013).

Multiple lines of evidence show that anti-COL7-NC1 autoantibodies are pathogenetically relevant in EBA: (i) circulating autoantibodies parallel disease activity in patients (Kim et al., 2013; Saleh et al., 2011), (ii) transplacental autoantibody transfer causes transient skin blistering in the newborn (Abrams et al., 2011), (iii) autoantibodies recruit and activate leukocytes ex vivo, resulting in dermal-epidermal separation in human skin cryosections (Recke et al., 2010, 2014; Sitaru et al., 2002), and (iv) injection of antibodies against COL7 or (v) immunization with autoantigen leading to autoantibody production in mice results in skin inflammation that duplicates important aspects of the human disease, especially the inflammatory (bullous pemphigoid-like) EBA variant (antibody transfer- and immunization-induced EBA, respectively; Table 1). Pathogenicity may not be limited to the skin, as mucosal morbidity in EBA patients as well as autoantibody-induced intestinal inflammation and weight loss in mice with experimental EBA associated with alterations in metabolic pathways similar to those found in inflammatory bowel diseases has been described (Ishii et al., 2011; Luke et al., 1999; Schönig et al., 2013).

Different animal models of blistering disorders other than EBA leading to subepidermal (bullous pemphigoid, mucous membrane pemphigoid, linear IgA dermatosis, and dermatitis herpetiformis) or intraepidermal (pemphigus vulgaris, pemphigus foliaceus, and paraneoplastic pemphigus) blistering have been reported. In some of these models, blisters were induced using additional methods, such as transfer of antigen-specific lymphocytes and antigen-based genetic modifications (e.g., COL17 and desmoglein 3 in bullous pemphigoid and pemphigus vulgaris, respectively)

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Abbreviations: Breg, immunoregulatory B cell; EBA, epidermolysis bullosa acquisita; Flii, Flightless I; Hsp90, heat shock protein 90; IVIG, intravenous immunoglobulins; MHC, major histocompatibility complex; MMP, matrix metalloproteinase; NC, noncollagenous; Th1, T helper type 1; Th2, T helper type 2; Treg, immunoregulatory T cell

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Table 1. Experimental models of epidermolysis bullosa acquisita

Model	Reproduction	Method	Reference
In vitro human EBA model	Neutrophil activation-associated ROS production	Incubation of isolated human neutrophils with immune complexes of human COL7 and recombinant monoclonal anti-COL7 IgG or IgA	Recke et al., 2010, 2014
Ex vivo human EBA model	Molecular phenotypes of the effector phase of disease	Incubation of cryosections of human skin with anti-COL7 antibodies (patient serum, total patient IgG, affinity-purified patient IgG, or monoclonal anti-COL7 IgG or IgA) and isolated human polymorphonuclear leukocytes or neutrophils	Recke et al., 2010, 2014; Sitaru et al., 2002
In vivo antibody transfer-induced EBA mouse model	Clinical and molecular phenotypes of the effector phase of disease	(i) Repeated transfers of rabbit anti-mouse COL7 IgG into C57Bl/6, BALB/c, or BALB/c^{nude} mice (ii) Repeated transfers of rabbit anti-human COL7 IgG into SKH1 mice (iii) Repeated transfers of human anti-human COL7 IgG into SKH1 mice (iv) Repeated transfers of human affinity-purified (CMP subdomain) anti-human COL7 IgG into SKH1 mice (v) Repeated transfers of human affinity-purified (Fn3-like subdomain) anti-human COL7 IgG into SKH1 mice (vi) Repeated transfers of rabbit anti-mouse COL7 IgG (vWFA2-like subdomain) into several in- or outbred mice (vii) Repeated transfers of rabbit affinity-purified (multiple NC1 domain fragments) anti-mouse COL7 IgG into BALB/ mice (viii) Repeated transfers of rabbit anti-human COL7 IgG into mice carrying null mutations of COL7 and the human COL7 transgene	Chen et al., 2007; Csorba et al., 2014; Iwata et al., 2013; Sitaru et al., 2005; Vorobyev et al., 2015; Wang et al., 2011; Woodley et al., 2005, 2006
In vivo immunization-induced EBA mouse model	Clinical and molecular phenotypes of both the initiation and effector phase of disease	(i) Repeated immunizations of SJL/J, BALB/c, and FcγRIIB-deficient mice with a portion of the Fn3-like subdomain (ii) One-time immunization of SJL/J, B6.SJL-H2s, C57Bl/10.s, and MRL/MpJ mice with a portion of the Fn3-like subdomain (iii) One-time immunization of SJL/J and B6.SJL-H2s mice with vWFA2-like subdomain (iv) Repeated immunizations of SJL/J mice with multiple NC1 domain fragments	Csorba et al., 2014; Iwata et al., 2013; Kasperkiewicz et al., 2010; Ludwig et al., 2011; Sitaru et al., 2006

Abbreviations: CMP, cartilage matrix protein; COL7, type VII collagen; EBA, epidermolysis bullosa acquisita; Fn3, fibronectin III; NC1, noncollagenous 1; ROS, reactive oxygen species; vWFA2, von Willebrand factor A2.

(Iwata et al., 2015a). Especially with the available experimental models of inflammatory EBA, much progress has been achieved in elucidating (i) initiation of autoimmunity to the target antigen, (ii) maintained autoantibody production, and (iii) autoantibody-induced tissue damage, although the precise order of single events within this pathophysiologic cascade is currently rather hypothetical. Here, we summarize the most current information on EBA pathophysiology and discuss recent insights into emerging therapeutic strategies targeting the different pathogenic events (Figure 1, Table 2).

PATHOPHYSIOLOGIC EVENTS AND EMERGING TREATMENTS

Induction (afferent) phase: loss of tolerance to COL7

Genetic factors. In patients, EBA is associated with the major histocompatibility complex (MHC) class II haplotype, in particular HLA-DR2 (Gammon et al., 1988). In addition, people of African descent carrying the risk allele HLA-DRB1*15:03 seem to be at increased risk to develop EBA, because black-skinned patients were significantly overrepresented (as compared with other autoimmune bullous diseases) in a large EBA patient cohort (Zumelzu et al., 2011). Whether this finding translates into a higher risk of black-skinned patients to develop EBA is uncertain, because respective prospective studies have not been carried out. Yet, this observation strongly points toward genes outside the MHC locus contributing to EBA susceptibility. In immunization-induced EBA, 75% of mice carrying the MHC haplotype H2s (SJL/J, C57Bl/10.s) developed clinical lesions, whereas only 5% of inbred non-H2s mouse strains were prone to develop both autoantibodies and disease. This underscores the essential role of the MHC locus in disease pathogenesis.

On the other hand, EBA incidence and clinical disease severity in immunization-induced EBA are divergent among strains sharing the H2s locus but differing genetically regarding genes outside this locus (Ludwig et al., 2011). Accordingly, several non-MHC quantitative trait loci linked to specific chromosomes that control susceptibility to EBA could be identified (Ludwig et al., 2012). Although these genetic data were derived from immunization-induced EBA, variations in skin blistering between mouse strains were also

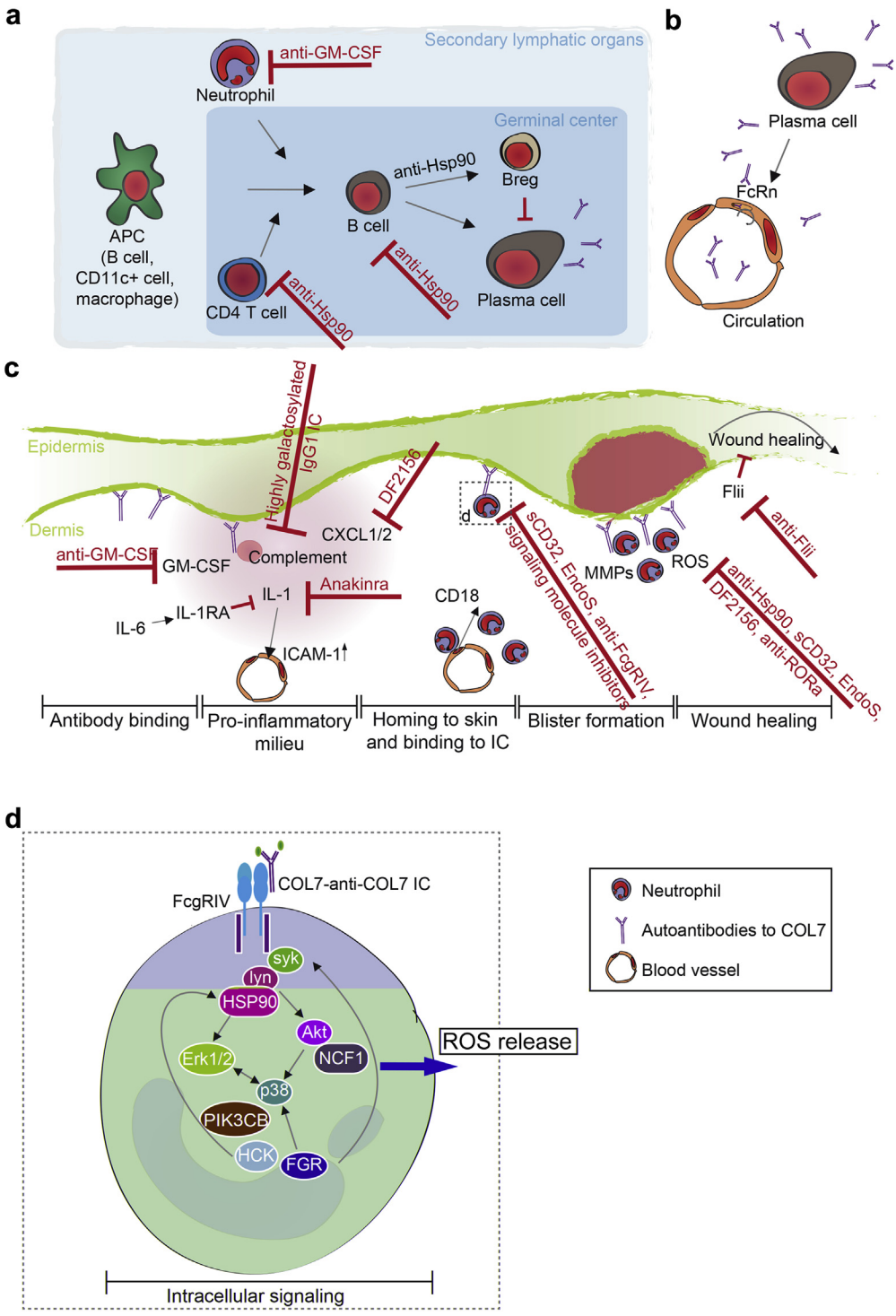

Figure 1. Pathophysiology and treatment of experimental epidermolysis bullosa acquisita. Proposed pathophysiologic events of the (**a**) induction (afferent), (**b**) autoantibody maintenance, and (**c**) effector (efferent) phases and their targeting by novel treatments based on experimental evidence. (**d**) Inset shows immune complex-initiated signal transduction pathways in neutrophils using information from the STRING database (https://string-db.org/). Signaling involving retinoid-related orphan receptor α (RORα) may cooperatively play a role in experimental epidermolysis bullosa acquisita, as it relates to this pathway shown here, through linkage between RORα and Akt1-interacting LXR nuclear receptor (Sadeghi et al., 2015b). APC, antigen-presenting cells; Breg, regulatory B cells; COL7, type VII collagen; DF2156, CXCL1/2 inhibitor; EBA, epidermolysis bullosa acquisita; FcRn, neonatal Fc receptor; Hsp90, heat shock protein 90; IC, immune complexes; IL-1RA, IL-1 receptor antagonist; MMPs, matrix metalloproteinases; ROS, reactive oxygen species; sCD32, soluble CD32.

observed in antibody transfer-induced EBA that reflects the effector phase of the disease (Iwata et al., 2013; Sitaru et al., 2005), suggesting that also later disease stages are genetically controlled. Besides genetic factors, gene-microbiota interactions have been described to influence disease development in immunization-induced EBA (Srinivas et al., 2013).

Autoreactive T cells. Autoreactive T cells recognizing immunodominant regions of COL7 have been identified in EBA patients (Müller et al., 2010). Cell-depleting studies in immunization-induced EBA indicated that various antigen-presenting cells could support a COL7-specific CD4 T cell response, but that B cells are required for this process. This autoantigen presentation was found to be an ongoing event, as indicated by its presence in several antigen-presenting cells in draining lymph nodes for up to 4 weeks after immunization (Iwata et al., 2013). The direct requirement of T cells for autoantibody production was demonstrated by protection from disease induction in T cell-deficient mice and restoration of disease susceptibility through lymphocyte

Table 2. Successfully in vivo-applied novel pharmacologic approaches in mouse models of epidermolysis bullosa acquisita

Drugs	Targets	Main directly or indirectly affected factors underlying clinical efficacy	Drug side-effect profiles
17-DMAG, TCBL-145	Hsp90	T cells, B cells, neutrophils, and autoantibody production (Tukaj et al., 2015b)	17-DMAG: Fatigue, nausea, diarrhea, as well as liver, lung, cardiac, renal, and ocular toxicities (Garcia-Carbonero et al., 2013)[1,2] TCBL-145: Not available (preclinical status)[2]
Anti-GM-CSF antibody	GM-CSF	Neutrophils and (in GM-CSF–deficient mice) autoantibody production (Samavedam et al., 2014)	Nasopharyngitis, upper respiratory infections, and pulmonary function abnormalities (Di Franco et al., 2014)[1,2]
Anakinra	IL-1	Neutrophils (Sadeghi et al., 2015a; Samavedam et al., 2013)	Headache, nausea, diarrhea, injection site reactions, infusion reactions, and infections (Rubbert-Roth, 2012)[1,2]
DF2156	CXCR1/2	Neutrophils (Hirose et al., 2013)	No side effects reported so far (Citro et al., 2012; Opfermann et al., 2015)[1,2]
U0126	Erk1/2	Neutrophils (Hellberg et al., 2013)	Rash, fatigue, diarrhea, and ocular toxicity (Zhao and Adjei, 2014)[1,2]
SB203580	p38	Neutrophils (Hellberg et al., 2013)	Rash, dizziness, diarrhea, and transaminasemia (Salgado et al., 2014)[1,2]
sCD32	IgG	Neutrophils and autoantibody production (Iwata et al., 2015b)	Not available (currently in phase II clinical trials for idiopathic thrombocytopenia and lupus erythematosus; http://www.controlled-trials.com/isrctn/search.html?srch=SM101)[2]
Highly galactosylated IgG1 immune complexes	FcγRIIB/dectin-1	Complement and neutrophils (Karsten et al., 2012)	Not available (preclinical status)[2]
EndoS	N-linked glycans of native IgG	Neutrophils (Hirose et al., 2012)	Not available (preclinical status)[2]
Anti-FcγRIV antibody	FcγRIV (human orthologue: FcγRIIIA)	Neutrophils (Kasperkiewicz et al., 2012)	Not available (preclinical status)[2]
SR3335	Retinoid-related orphan receptor α	Neutrophils (Sadeghi et al., 2015b)	Not available (preclinical status)[2]
Anti-Flii antibody	Flii	Wound healing (Kopecki et al., 2013)	Not available (preclinical status)[2]

Abbreviations: 17-DMAG, 17-dimethylaminoethylamino-17-demethoxygeldanamycin; Flii, Flightless I; Hsp90, heat shock protein 90; sCD32, soluble CD32.
[1]Commonly reported adverse drug events (compound- or drug family-related) derived from clinical studies in patients with diseases other than epidermolysis bullosa acquisita.
[2]No overt toxicity noted in epidermolysis bullosa acquisita animal studies.

reconstitution in immunization-induced EBA (Sitaru et al., 2010). Depletion of CD4, but not of CD8, T cells around the immunization period delayed anti-COL7 autoantibody and blister formation in mice (Iwata et al., 2013). Additionally, a T helper type 1 (Th1)-like cytokine profile with an increased IFN-γ/IL-4 ratio in draining lymph nodes has been linked to skin blistering in experimental EBA, whereas a Th2-like cytokine gene expression determined resistance to disease induction in mice (Hammers et al., 2011).

Regulatory T and B cells. Only two studies investigated the role of immunoregulatory T and B cells (Tregs, Bregs) in experimental EBA. Chen et al. (2006) found that Treg depletion by anti-CD25 antibody did not affect autoantibody production in SKH1 mice, suggesting that development of autoimmunity to COL7 is independent of Treg function. We recently demonstrated that splenic Bregs from COL7-immunized mice were able to blunt autoantibody production in autoantigen-restimulated isolated draining lymph node cells from immunized mice. Interestingly, splenic Breg frequencies were higher in immunized mice compared with nonimmunized controls (Tukaj et al., 2014a). Similarly, blood Bregs from patients with autoimmune diseases, including autoimmune bullous disorders, have been reported to be expanded in comparison with healthy controls for an unclear reason (Iwata et al., 2011). Hence, the role of Tregs and Bregs in the induction and progression of EBA remains in need of further elucidation.

GM-CSF and neutrophils. There is evidence that the cytokine GM-CSF and neutrophils contribute to adaptive immune functions in the induction phase of experimental EBA. GM-CSF–deficient mice had lower autoantibodies compared with wild-type mice in immunization-induced EBA, which was associated with reduced neutrophils in draining lymph nodes. A similar inhibitory effect on autoantibody production was observed in neutrophil-depleted mice, and combining GM-CSF inhibition and neutrophil depletion showed additive effects. Moreover, neutrophils co-localized with B cell activating factor in draining lymph nodes after immunization. Thus, both GM-CSF and neutrophils play a role in autoantibody formation in experimental EBA, presumably by mediating the influx of antigen-presenting cells into peripheral lymph nodes (Samavedam et al., 2014). This indicates that so-called B cell-helper neutrophils not only contribute to T cell-independent antibody production, but also to T cell-dependent antibody responses (Puga et al., 2011; Vinuesa and Chang, 2013).

Novel therapeutic implications to modulate the induction phase of the autoimmune response. Because of the documented central role of T cells in the generation of autoantibodies in human and experimental EBA (Iwata et al., 2013; Müller et al., 2010; Sitaru et al., 2010), T cell activation- and interaction-targeting strategies represent a promising therapeutic option for EBA patients. These include

monoclonal antibodies against CD3, CD4, IL-2R, and CD40L, some of which have already been successfully used in mouse models of and single patients with autoimmune bullous diseases (Ujiie and Shimizu, 2012).

A novel strategy to inhibit autoreactive T cell responses is derived from experiments using inhibitors of the cell stress-inducible chaperone heat shock protein 90 (Hsp90) in experimental EBA (Kasperkiewicz et al., 2011). Currently tested in clinical trials for therapy of cancer patients due to its inhibitory effects on malignant cells (Solárová et al., 2015), anti-Hsp90 treatment is also increasingly becoming a research focus in autoimmune diseases, including blistering disorders, as it exerts potent immunomodulatory actions (Collins et al., 2013; de Zoeten et al., 2011; Kasperkiewicz et al., 2011; Tukaj et al., 2014a, 2014b, 2014c, 2015a, 2015b). Application of Hsp90 inhibitors before and after disease onset blocked EBA development and induced clinical recovery associated with suppressed autoantibody production compared with vehicle-treated animals in immunization-induced EBA, respectively. Autoreactive T cell proliferation was inhibited, as shown by a compromised response of isolated draining lymph node cells from immunized mice to restimulation with T cell-activating anti-CD3/CD28 antibody or COL7 in presence of Hsp90 antagonists (Kasperkiewicz et al., 2011). Inhibition of proinflammatory NF-kB, up-regulation of anti-inflammatory Hsp70, and/or blunting of Lck kinase-mediated T cell receptor signaling could likely account for this effect, as demonstrated by Hsp90 inhibition experiments with human T cells (Tukaj et al., 2014b). In addition, anti-Hsp90 treatment potently modulated humoral immune responses at the B cell level by inhibiting and promoting effector and regulatory B cell subsets in immunization-induced EBA, respectively (Tukaj et al., 2014a). Moreover, Hsp90 inhibitors have been also shown to enhance Treg function in other in vivo models of inflammation and autoimmunity (Collins et al., 2013; de Zoeten et al., 2011).

Because an increased IFN-γ/IL-4 ratio was linked to production of pathogenic autoantibodies in experimental EBA (Hammers et al., 2011), shifting the immune response toward a Th2 phenotype by a monoclonal anti−IFN-γ antibody and/or recombinant IL-4 could be considered as a novel therapeutic strategy. In this context, both reduced serum IFN-γ levels of COL7-immunized mice and a lowered IFN-γ expression on human Th1 cells has been found after anti-Hsp90 treatment (Tukaj et al., 2014a, 2014b). In addition, treatment of COL7-immunized mice with intravenous immunoglobulins (IVIG) resulted in ameliorated clinical disease severity, reduced levels of autoantibodies, and a shift toward Th2-mediated nonpathogenic autoantibodies (Hirose et al., 2015), which supports their previously described effective and safe use in patients with autoimmune bullous diseases, including EBA (Ahmed and Gürcan, 2012; Ishii et al., 2010). Although the precise mechanisms are largely unclear, targeting GM-CSF and neutrophil functions could represent further potential treatment options to modulate autoantibody production in EBA patients.

Maintained autoantibody production

Autoreactive plasma cells. Autoantibody-producing plasma cells have been found in higher numbers in EBA-susceptible than in disease-resistant mouse strains (Hammers et al., 2011). COL7-specific plasma cells were restricted to draining lymph nodes of immunized mice, which may at least partly be explained by their lack of homing-associated CXCR3 and CXCR4 chemokine receptor expression (Tiburzy et al., 2013).

It was further demonstrated that murine COL7-specific plasma cells resemble intermediates between short-lived (few days) and long-lived (many months or years) plasma cells with half-lives of several weeks (Tiburzy et al., 2013), fitting to the calculated 4- to 8-week turnover time of circulating and skin-bound anti-COL7 autoantibodies in mice (Kasperkiewicz et al., 2010). This knowledge could provide an explanation for the frequently observed relatively slow decline (8−12 weeks) of autoantibody titers in patients with autoimmune bullous diseases successfully treated with B cell-targeting immunosuppressants (Schmidt et al., 2008).

Neonatal Fc receptor. The neonatal Fc receptor plays a major role in the regulation of circulating IgG levels, including autoreactive IgGs, by preventing their degradation (Challa et al., 2014). Similar to what was demonstrated in animal models of bullous pemphigoid and pemphigus (Li et al., 2005), reduced autoantibody levels were found in animals lacking neonatal Fc receptor compared with wild-type controls in both antibody transfer- and immunization-induced EBA. This resulted in protection from tissue injury, but could be overridden by transfer of excessive amounts of anti-COL7 antibodies (Sesarman et al., 2008a).

Novel therapeutic implications to modulate autoantibody maintenance. Although proteasome inhibitors such as bortezomib have the potential to directly target autoantibody-producing long-lived plasma cells (Neubert et al., 2008), their application is limited due to toxicity (Broyl et al., 2012), and they have not been used in autoimmune bullous disorders so far. Nevertheless, depletion of both plasma cell precursors by B cell-targeting monoclonal anti-CD20 antibodies and high levels of circulating autoantibodies by immunoadsorption or plasmapheresis has been successfully used for treatment of these diseases, including individual patients with EBA (Meyersburg et al., 2012; Niedermeier et al., 2007; Schmidt et al., 2006, 2008). Improvement of such methods using recombinant forms of the autoantigen would offer essential advantages over the currently performed unspecific global elimination of all B cells and antibodies. Additionally, autoantibody levels can be lowered by saturation of neonatal Fc receptor binding sites, which represents one of the proposed underlying mechanisms of the immunomodulatory action of IVIG, as it has been shown in mouse models of bullous pemphigoid and pemphigus (Li et al., 2005).

Effector (efferent) phase: autoantibody-induced tissue damage

Complement. After autoantibody deposition in the skin, occurring within 24 hours after transfer of anti-COL7 IgG into mice (Ishii et al., 2011), generation of a proinflammatory cutaneous environment including complement activation presumably represents one of the first steps of the effector phase of experimental EBA. Autoantibody-induced tissue

injury in experimental models of inflammatory EBA strictly depends on the Fc fragment of IgG. F(ab)2 fragments or chicken anti-COL7 IgY, both of which are unable to active murine complement and bind to murine FcRs, neither induced blistering ex vivo in presence of neutrophils nor induced skin lesions in mice (Sesarman et al., 2008b; Sitaru et al., 2002, 2005). It has been shown that human IgG1 and IgG3 anti-COL7 subclass antibodies activate complement and induce dermal-epidermal separation ex vivo (Recke et al., 2010). Moreover, deposits of complement-fixing IgG2a and IgG2b autoantibodies and C3 are commonly observed at the dermal-epidermal junction in immunization-induced EBA (Sitaru et al., 2006). In antibody transfer-induced EBA, C5-deficient mice were completely resistant to blister formation (Sitaru et al., 2005). Mice lacking the alternative (factor B-deficient mice) and, to a lesser extent, the classical complement system exhibited an ameliorated disease activity, whereas those with defective lectin pathway of complement showed a similar phenotype compared with controls (Mihai et al., 2007). The frequently observed C3 deposition at the skin basement membrane zone of patients with mechano-bullous EBA points toward a yet to be defined involvement of complement also in this noninflammatory disease variant (Buijsrogge et al., 2011; Delgado et al., 2011).

Neutrophil recruitment. Cleavage products of complement activation, such as C5a, may then mediate the recruitment of neutrophils into the skin. In fact, CD18-deficient mice lacking expression of all neutrophil adhesion-related β2 integrins and mice treated with neutrophil-depleting anti-Gr-1 antibody were completely protected from skin blistering in antibody transfer-induced EBA (Chiriac et al., 2007). In addition, expression of GM-CSF, CXCL1/2, and IL-1α/β is up-regulated in the skin and linked to neutrophil-dependent blister formation in experimental EBA (Hirose et al., 2013; Sadeghi et al., 2015a; Samavedam et al., 2013, 2014). Conceivably, both a partly autocrine secretion of these chemotactic and proinflammatory cytokines from neutrophils and their release from yet-to-be identified cells lead to perpetuation of neutrophil recruitment. Although contribution of additional resident immune cells to this proinflammatory skin environment of the effector phase can be assumed, these, however, do not involve mast cells, because conditional depletion of this cell type had no impact on the blistering phenotype in antibody transfer-induced EBA (Kasprick et al., 2015).

Neutrophil activation. After migration to skin, neutrophils are activated through FcγR-mediated binding to immune complexes (Kasperkiewicz et al., 2012). Using different FcγR knockout mice and FcγRIV-blocking antibody in antibody transfer-induced EBA, we found that among activating FcγRs, FcγRIV is the only receptor required for tissue injury, whereas, in contrast, the inhibitory FcγRIIB counteracts skin inflammation (Kasperkiewicz et al., 2012).

Neutrophil cell surface receptors (e.g., integrin adhesion receptors and FcγRIV) trigger signal transduction pathways involving early upstream molecules such as the Src family kinases Hck, Fgr, and Lyn. Mice lacking these kinases are completely protected from blistering in antibody transfer-induced EBA, likely because of the contribution of these kinases in immune complex-induced activation of neutrophils (Kovács et al., 2014).

Immune complex-mediated neutrophil activation also involves other associated downstream signaling kinases, such as PI3Kβ, Erk1/2, p38, and Akt, the latter proposed to be linked to the retinoid-related orphan receptor α in experimental EBA. Targeting these molecules, including retinoid-related orphan receptor α, partially or fully protected from antibody transfer-induced EBA (Hellberg et al., 2013; Kulkarni et al., 2011; Sadeghi et al., 2015b).

Reactive oxygen species and proteases. One of the terminal pathophysiologic events in experimental EBA is believed to be the secretion of reactive oxygen species and matrix metalloproteinases (MMPs) from immune complex-activated neutrophils, which directly damage the dermal-epidermal junction (Chiriac et al., 2007; Shimanovich et al., 2004). Neutrophils from patients with chronic granulomatous disease and mice lacking cytosolic factor 1, both of which are unable to mount an oxidative burst, completely failed to induce dermal-epidermal separation induced by antibodies to type VII collagen ex vivo and in vivo, respectively (Chiriac et al., 2007). In vitro neutrophil reactive oxygen species production was suppressed by treatments targeting CXCR1/2, FcγRs, and Hsp90, all of which proved effective in EBA mouse models (Hirose et al., 2013; Iwata et al., 2015b; Sadeghi et al., 2015b; Tukaj et al., 2015a; Yu et al., 2014).

In addition, the use of broad-spectrum protease inhibitors or specific inhibition of MMP-9 (gelatinase B) and MMP-12 (elastase) completely abolished anti-COL7 autoantibody-induced dermal-epidermal separation ex vivo (Shimanovich et al., 2004). MMP-12 has been recently shown to be complexed by Hsp90 in EBA patients, implicating that it is a client protein of Hsp90 and that its activity is dependent on the function of this chaperone (Tukaj et al., 2015a).

Flightless I. The actin remodeling cytoskeletal protein Flightless I (Flii) has been implicated in the development and regulation of the epidermal barrier and associated with impaired wound healing (Kopecki and Cowin, 2008). In antibody transfer-induced EBA, its up-regulation correlated with the severity of blistering and resulted in impaired Claudin-1 and -4 tight junction protein expression as well as delayed recovery of functional barrier after blistering (Kopecki et al., 2011, 2014). Additionally, reduced Flii expression using Flii+/- mice or topical application of Flii neutralizing antibodies impaired blister formation and improved healing of blistered skin in this model (Kopecki et al., 2011, 2013). Because anti-Flii treatment did not affect neutrophils or macrophages in the skin (Kopecki et al., 2013), the contribution of Flii to tissue injury in experimental EBA is probably rather related to so far incompletely understood mechanisms associated with skin barrier function and wound healing than to immunologic processes per se.

Novel therapeutic implications to modulate the effector phase of the autoimmune response. Prevention of autoantibody binding to COL7 or activating FcγRs on effector cells could be one promising therapeutic option. This may likely be achieved by the already established treatment with IVIG,

because it has been proposed that the immunomodulatory effects of IVIG are mediated by both its Fab fragments (i.e., neutralization of pathogenically relevant epitopes) and Fc portion (i.e., inhibition of activating FcγRs) (Schwab and Nimmerjahn, 2013), although this mechanism has not been precisely elucidated in experimental EBA. Nevertheless, a decreased FcγRIV expression on Gr-1−positive cells was observed after IVIG treatment in immunization-induced EBA (Hirose et al., 2015). In a related context, IVIG has been described to inhibit effector cell activation by up-regulating FcγRIIB (Schwab and Nimmerjahn, 2013), and this inhibitory receptor was in turn required for the activity of IVIG in antibody transfer-induced EBA (Schwab et al., 2014). Alternatively, monoclonal antibodies against the human orthologue of mouse FcγRIV or recombinant nonpathogenic (fragments of) antibodies that competitively block the binding of pathogenic autoantibodies could be considered, as described in experimental bullous pemphigoid (Li et al., 2010; Schulze et al., 2014; Wang et al., 2010). It has been also shown that removal of terminal sugar residues on anti-COL7 IgG by the endoglycosidase EndoS, which disturbs their binding ability to activating FcγRs, affects the capacity of immune complexes to activate neutrophils in vitro and to cause EBA blistering ex vivo and in vivo (Hirose et al., 2012; Yu et al., 2014). EndoS treatment also decreased expression of activating FcγRs while up-regulating FcγRIIB and was capable of targeting in vivo skin-bound anti-COL7 IgG, which could prevent further disease progression when applied in mice that had already developed EBA lesions (Hirose et al., 2012). Similarly, IVIG activity in antibody transfer-induced EBA was lost if its terminal sialic acid residues were absent and, on the other hand, enhanced by tetra-Fc sialylation (Schwab et al., 2014; Washburn et al., 2015). Thus, targeting the glycosylation status of autoantibodies and enriching IVIG for terminal sialic acid residues are further promising novel therapeutic avenues for EBA patients. Moreover, recombinant soluble FcγRIIB is known to bind immune complexes and consecutively hinder their attachment to FcγRs on effector cells (Ierino et al., 1993). In experimental EBA, soluble FcγRIIB (CD32) successfully impaired blistering ex vivo and in vivo and was also associated with modulation of autoantibody production, suggesting that it interferes with different stages of the disease (Iwata et al., 2015b).

Complement inhibition by monoclonal anti-C5 antibodies or C5aR antagonists could represent another new treatment option for EBA patients (Mihai et al., 2007; Sitaru et al., 2005). In addition, highly galactosylated IgG1 immune complexes can block C5aR-mediated and other chemoattractant receptor-driven proinflammatory signaling events in neutrophils through binding to the inhibitory FcγRIIB and dectin-1. This effect is associated with suppression of skin blistering in antibody transfer-induced EBA (Karsten et al., 2012).

Because CD18-deficient mice were resistant to EBA induction (Chiriac et al., 2007), inhibition of leukocyte extravasation by antagonists of leukocyte adhesion molecules could be also considered. Further, suppressing the proinflammatory and chemotactic cytokine milieu to disrupt neutrophil accumulation in the skin might be achieved by targeting GM-CSF, CXCL1, CXCL2, or IL-1. Pharmacologic blockade of the functions of either GM-CSF or CXCL1/2 exhibited both preventive and therapeutic effects in experimental EBA in mice (Hirose et al., 2013; Samavedam et al., 2014). IL-6, however, had antiinflammatory effects in antibody transfer-induced EBA by inducing IL-1 receptor antagonist and thereby counteracting proinflammatory events triggered by IL-1 (Samavedam et al., 2013). Accordingly, application of the IL-1 receptor blocker anakinra prevented and ameliorated experimental EBA through down-regulation of ICAM-1 (Sadeghi et al., 2015a; Samavedam et al., 2013).

Finally, targeting receptor-regulated cellular signaling (retinoid-related orphan receptor α/Akt, Src, PI3Kβ, Erk1/2, p38) and effector molecules (reactive oxygen species, MMPs) related to neutrophil activation as well as the protein Flii associated with impaired healing of blisters, proved effective in experimental EBA and could also represent a future therapeutic approach for EBA patients (Chiriac et al., 2007; Hellberg et al., 2013; Hirose et al., 2013; Iwata et al., 2015b; Kopecki et al., 2011, 2013; Kulkarni et al., 2011; Sadeghi et al., 2015b; Shimanovich et al., 2004; Tukaj et al., 2015a; Yu et al., 2014).

CONCLUSIONS

The summarized studies have greatly advanced our understanding of the pathogenesis of the bullous pemphigoid-like EBA variant. With the identification of key factors of autoimmune-mediated blister formation, novel therapeutic strategies have emerged that can potentially help to overcome the frequently observed recalcitrance of the patients' disease to conventional immunosuppressive treatment in the future. All described novel in vivo-tested pharmacologic treatments, which mainly target efferent inflammatory events of EBA but partly also autoantibody production, are likely to be suitable for the inflammatory type of EBA in patients. It seems conceivable that especially the latter treatment approach (that is, anti-Hsp90, anti−GM-CSF, and sCD32) may be expected to be of value for the mechanobullous form of EBA, where blisters appear to form through a so far largely unknown direct autoantibody pathway probably not requiring downstream inflammation. Improving wound healing (that is, anti-Flii) could be advantageous for both EBA variants. The potent in vivo efficacy observed with many new pharmacologic compounds in experimental EBA generally supports their introduction into the clinical setting for treatment-refractory EBA and related autoantibody-mediated diseases, although safety profiles of some drugs first need to be established or further improved.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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