Autoimmune Diseases in Epidermolysis Bullosa

Gene therapy serves as a promising therapy in the pipeline for treatment of epidermolysis bullosa (EB). However, with great promise, the risk of autoimmunity must be considered. While EB is a group of inherited blistering disorders caused by mutations in various skin proteins, autoimmune blistering diseases (AIBD) have a similar clinical phenotype and are caused by autoantibodies targeting skin antigens. Often, AIBD and EB have the same protein targeted through antibody or mutation, respectively. Moreover, EB patients are also reported to carry anti-skin antibodies of questionable pathogenicity. It has been speculated that activation of autoimmunity is both a consequence and cause of further skin deterioration in EB due to a state of chronic inflammation.

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1. Introduction

Autoimmune blistering diseases (AIBD) are rare diseases with significant morbidity and mortality [1][2]. AIBD are caused by autoantibodies targeting various skin antigens. In contrast, epidermolysis bullosa (EB) is a group of inherited blistering disorders caused by mutations in various skin proteins [3]. AIBD and EB often have the same protein targeted through antibody or mutation, respectively (Table 1).

Table 1. Select autoantigens shared between pemphigoid diseases and epidermolysis bullosa.

Antigen	EB Subtype	AIBD Subtype
BP230 (dystonin)	EBS	Bullous pemphigoid
Collagen XVII (BP180)	JEB	Bullous pemphigoid, Pemphigoid gestationis
Laminin 332	JEB	Mucous membrane pemphigoid
α6β4 integrin	JEB	Mucous membrane pemphigoid
Collagen VII	DEB	Epidermolysis bullosa acquisita

AIBD = autoimmune blistering disease; EBS = epidermolysis bullosa simplex; JEB = junctional epidermolysis bullosa; and DEB = dystrophic epidermolysis bullosa.

EB encompasses a group of inherited skin fragility diseases marked by blisters which may erode and lead to ulcers in the skin and mucous membranes [4]. Recently, EB has been grouped into four expansive categories based on the location of tissue separation within the basement membrane zone (BMZ) [4]. These categories include the simplex forms (EBS), the junctional forms (JEB), the dystrophic forms (DEB), and the newest subtype, known as the Kindler syndrome [4]. In this review, we will focus on the JEB and DEB forms which involve tissue separation, namely within the lamina lucida, within and below the lamina densa confined to the upper papillary dermis, respectively [4].

While EB and AIBD are distinct entities, there are reports of spontaneous development of AIBD in patients with pre-existing EB. Likewise, several groups have noted the prevalence of anti-skin antibodies in EB patients. Although the pathogenicity of these antibodies remains elusive, some speculate that chronic inflammation in EB combined with antigen unmasking can lead to a break in immune tolerance, resulting in development of AIBD.

Gene therapy serves as a promising therapy in the pipeline for treatment of EB. Through various vectors, different technologies lead to correction or expression of functional portions of collagen [3][5]. However, with great promise, the risk of autoimmunity must be considered. For example, corrections of highly immunogenic portions of protein may confer a greater risk towards developing AIBD. For illustration, Figure 1 details the location of mutations in collagen VII and

collagen XVII with respect to the immunogenic epitopes in AIBD. As such, we reviewed the literature concerning the development of AIBD and anti-skin in EB patients.
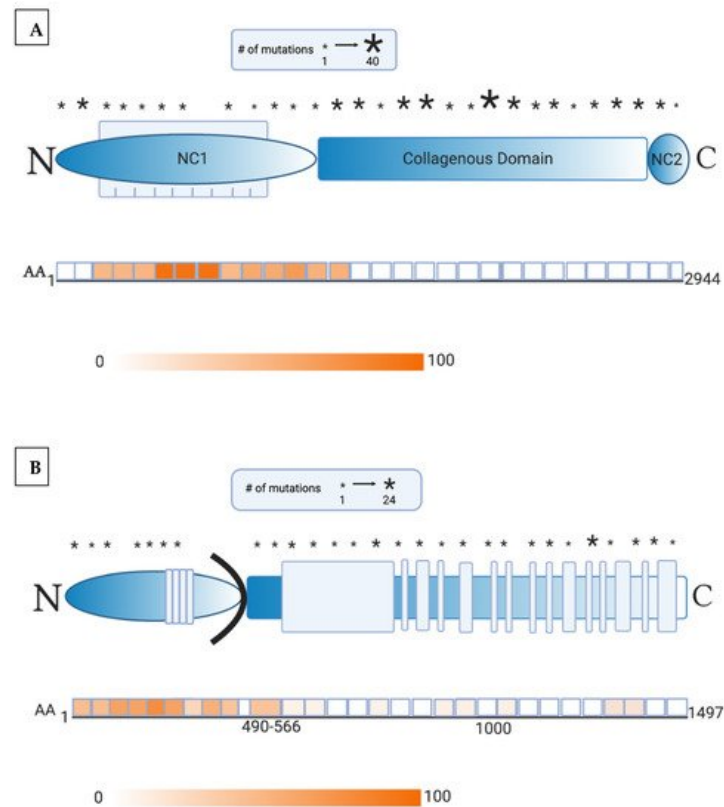

Figure 1. (**A**) Collagen mutation profile and immunogenic domains of epitope reactivity. This schematic representation of collagen VII consists of noncollagenous-1 (NC1, shown as a blue elliptical bar), triple-helix collagenous (shown as a blue rectangular bar), and noncollagenous-2 domains (NC2, shown as a blue oval bar). A crucial region within NC1 is the fibronectin-III-like domains 1–9 (shown as blue vertical bars). The asterisks indicate the approximate location of the mutations alongside intervals of 100 amino acids within the collagen VII polypeptide chain. The size of the asterisk corresponds to the number of mutations detected within the specified interval. The second half of this image depicts the combined results of studies measuring the reactivity of sera from patients with epidermolysis bullosa acquisita to epitopes alongside collagen VII. The intensity of the color relates to the percent reactivity identified within the individual domains. The areas of highest immunogenicity in a majority of patients include fibronectin-III-like domains 4–6 of collagen VII (approximately AA 500–800), and various regions within the NC1 and collagenous domains. As such, gene therapy must target the greatest number of mutations, while avoiding highly immunogenic areas of epitope binding [6][7][8]. (**B**) In this schematic representation of collagen XVII, the extracellular domain consists of stretches of noncollagenous domains and a series of 15 collagenous domains (shown as blue vertical bars). Also represented are the intracellular (shown as a blue elliptical bar) and transmembrane domains (shown as a black curved bar). The asterisks indicate the approximate location of the mutations alongside intervals of 50 amino acids within the collagen XVII polypeptide chain. The size of the asterisk corresponds to the number of mutations detected within the specified interval. The second half of this image depicts the combined results of studies measuring the reactivity of sera from patients with bullous pemphigoid to epitopes alongside collagen XVII. The intensity of the color relates to the percent reactivity identified within the individual domains. The NC16a region of collagen XVII (AA 490–566) was identified as having the highest immunogenicity in the majority of patients but reactivity to various subdomains within the intracellular region must be considered. As such, gene therapy must target the greatest number of mutations, while avoiding highly immunogenic areas of epitope binding [9][10][11][12][13].

2. Presence of Anti-Skin Antibodies in EB

Although EB is an inherited disorder caused by genetic defects, autoantibodies to skin antigens have been demonstrated in the sera of patients with EB [14][15][16][17]. Overall, patients with EB have significantly higher antibody titers against components of molecules responsible for cell adhesion, such as desmoglein 1, desmoglein 3, collagen XVII, BP230, and collagen VII compared to controls (Table 2) [7]. Similarly, 40.6–90.6% of patients with EB have significant antibody titers against collagen type III, IV, and V, as well as laminin [14]. Different subtypes of EB have varying prevalence of these circulating autoantibodies [14][15][16]. For instance, patients with the generalized forms of EB had significantly higher antibody titers (2–5-fold) against desmoglein 1, desmoglein 3, collagen XVII, BP230, and collagen VII compared to patients with other EB forms [15][16]. This difference in antibodies concentrations was accentuated when the sera of

patients with recessive DEB (the most severe EB subtype clinically) was compared to that of patients with EBS, as recessive DEB patients had 7–11-fold higher antibody titers against collagen XVII, BP230, and collagen VII [15]. Furthermore, a direct correlation was found between disease severity (Birmingham EB Severity scores) and the concentrations of the antibody titers against collagen XVII, BP230, and collagen VII [15][16].

Table 2. Presence of autoantibodies in patients with EB.

| | EB Subtype | n | Autoantigen | | | | | | | FN | LAM | Dsg1 | Dsg3 | Collagen XVII/ BP180 | BP230 |
| | | | Collagen | | | | | | | | | | | | |
			I	II	III	IV	V	VI	VII						
[14]	EBA	2	0.0%	0.0%	100.0%	0.0%	100.0%	0.0%		0.0%	50.0%				
	EBS	20	0.0%	0.0%	85.0%	60.0%	85.0%	0.0%		0.0%	40.0%				
	JEB	4	0.0%	0.0%	100.0%	50.0%	100.0%	25.0%		0.0%	0.0%				
	DEB	6	0.0%	16.7%	83.3%	33.3%	100.0%	16.7%		16.7%	66.7%				
	Total	32	0.0%	3.1%	87.5%	50.0%	90.6%	6.3%		3.1%	40.6%				
[15]	RDEB	19							4.96 U/mL			5.62 U/mL	6.14 U/mL	14.2 U/mL	12.7 U/mL
	Other EB	23							1.08 U/mL			2.67 U/mL	2.8 U/mL	5.7 U/mL	3.7 U/mL
	Healthy Controls	38							0.26 U/mL			2.12 U/mL	1.58 U/mL	1.82 U/mL	1.68 U/mL
[16]	RDEB	17							88%					combined percentage of 88%	
	EBS	10							10%					combined percentage of 50%	

Summary of studies assessing the seropositive (%) or quantity (U/mL) of autoantibodies against skin antigens. Abbreviations: Dsg 1 = desmoglein 1; Dsg 3 = desmoglein 3; EBA = epidermolysis bullosa acquisita; EBS = epidermolysis bullosa simplex; FN = fibronectin; JEB = junctional epidermolysis bullosa; and LAM = laminin; RDEB = recessive dystrophic epidermolysis bullosa.

The implications of elevation in serum concentrations of autoantibodies in EB are not yet known, but autoantibodies against collagen VII have been shown to induce blistering in humans and experimental models [18]. IgG4 dominated the autoimmune response in patients with collagen-VII-specific antibodies [19]. High levels of IgG4 autoantibodies and other circulating anti-collagen VII autoantibodies have been detected in a majority of recessive DEB patients, independent of the *COL7A1* mutation type or quantitative collagen VII levels [16][20][21]. In epidermolysis bullosa acquisita (EBA), autoantibody-induced tissue damage against collagen VII contributes to blistering [22]. Complement activation through both the classical and alternative pathways have been implicated in disease pathogenesis but the alternative pathway appears to be predominant [23][24]. Autoreactive IgG and immune complex-FcγR binding initiate an inflammatory complement cascade resulting in extravasation of neutrophils, release of proteolytic enzymes, and reactive oxygen species [25]. Moreover, T cells may perpetuate tissue damage in EBA through association with immune complexes and neutrophils [26].

Anti-collagen XVII antibodies trigger subepidermal blistering in this bullous pemphigoid (BP) model via complement activation and non-complement patterns [27][28]. The IgG4 subtype was found to induce inflammation by activating leukocytes in a non-complement fixing pattern or by binding collagen XVII in a Fc-independent manner causing dermo–epidermal junction (DEJ) separation in BP [29]. Elevated anti-collagen XVII autoantibodies are even linked to more active and severe disease, as well as poorer prognosis [30][31][32]. However, negative direct and indirect immunofluorescence test results in most cases suggest that circulating autoantibodies are not pathogenic [33].

Moreover, immune-mediated complications and disease pathology have been described in EB patients, including celiac disease, amyloidosis, post-infectious glomerulonephritis, and IgA nephropathy [34][35][36][37]. Among cutaneous disease, a

few reported cases of autoantibodies causing concurrent AIBD in patients with inherited EB include: EBA in a patient with dominant DEB [20], EBA in a patient with recessive DEB [38], and BP in a patient with JEB [39] (Table 3). In each of these cases, the acquired AIBD was resistant to common therapy, patients had minimal clinical improvement, and one patient with dominant DEB even died from severe hypoalbuminemia and anemia [20]. The authors speculate that genetic modifiers or environmental factors may help explain why some patients with positive serology exhibit clinical disease while others do not. Although not fully understood, the authors propose that chronic blistering and inflammation due to altered protein synthesis and structure in EB [16][38] contribute to the immunologic recognition of "self." Alternatively, it is plausible that activation of autoimmunity is both a consequence and cause of further skin deterioration in EB due to a state of chronic inflammation. Herein, we review the factors that facilitate the initiation of autoimmune and inflammatory responses to help understand the pathogenesis and therapeutic implications of the overlap between EB and AIBD.

Table 3. Reported cases of confirmed cases of AIBD arising in patients with EB.

Year	Author	EB Type	AIBD Type	Workup
2016	Hayashi	DDEB	EBA	**DIF:** Linear deposits of IgG and C3 at the DEJ **IIF:** Linear deposition of IgG at the dermal side of the DEJ **Immunoblot analysis:** Reactive to collagen type VII and its NC1 domain. Non-reactive to laminin 322 **Mutations:** c.7868G > A in the COL7A1 gene
2018	Guerra	RDEB	EBA	**DIF:** Linear deposition of IgG with a u-serrated pattern along the cutaneous BMZ **IIF:** IgG binding to the dermal side of the salt-split skin **ELISA:** Positive for anti-collagen type VII, anti-BP180, and anti-BP230 **Immunoblot Analysis:** Reactive to laminin 332 **Mutations:** c.410G > A and c.3674C > T in the COL7A1 gene
2019	Fania	JEB	BP	**DIF:** Linear IgG and C3 deposits in an n-serrated pattern at the DEJ **IIF:** Epidermal staining of the salt-split skin **ELISA:** Positive for anti-BP180. Negative for anti-BP230 **Immunoblot analysis:** Reactive to BP180 and its LAD-1 domain. Not reactive to laminin 332 **Mutations:** c.1132 + 5G > A in the LAMB3 gene

AIBD = autoimmune blistering disease; DDEB = dominant dystrophic epidermolysis bullosa; RDEB = recessive dystrophic epidermolysis bullosa; JEB = junctional epidermolysis bullosa; EBA = epidermolysis bullosa acquisita; BP = bullous pemphigoid; DIF = direct immunofluorescence; IIF = indirect immunofluorescence; DEJ = dermal epidermal junction; BMZ = basement membrane zone; and EB = epidermolysis bullosa.

3. Dysregulated Inflammatory Response and Blister Formation

When subjected to trauma, keratinocytes display increased sensitivity to autoantibodies [40]. Collagen XVII is a known inhibitor of keratinocyte migration, while its shed ectodomain leads to stabilization and cell immobilization [41]. In nonlethal JEB, the absence of collagen XVII or dysfunctional interaction between laminin-332 and collagen XVII is speculated to promote keratinocyte migration [41][42][43]. Thus, the weakened attachment of keratinocytes to the basement membrane, increased keratinocyte sensitivity to circulating autoantibodies, and subsequent expression of eosinophil chemotactic factors enhance deposition of antibodies and facilitate blister formation [44].

However, blister formation has a much more complex etiology where the interplay between cytokines, chemokines, and MMP is important. In patients with JEB, anti-collagen XVII autoantibodies trigger the release of inflammatory cytokines that may exacerbate DEJ separation [45]. In vitro, when JEB-derived (collagen-XVII-deficient) epidermal keratinocytes are exposed to inflammatory stimuli (ultraviolet B radiation, lipopolysaccharide, phorbol 12-myristate 13-acetate, and tumor necrosis factor), an abnormally high IL-8 response is seen [45]. As a chemotactic agent, IL-8 contributes to neutrophil recruitment [46]. In turn, re-induction of collagen XVII expression normalizes this response (against lipopolysaccharide and ultraviolet B radiation), suggesting that it may serve as a pathway for inflammation and subsequent lesion formation in the

skin of collagen-XVII-deficient EB patients [45]. Although not fully elucidated, antibody-mediated disruption of interactions between collagen XVII and other components of the BM may result in a pro-inflammatory response.

In the complement-independent pathway, the binding of autoantibody to collagen XVII and the subsequent internalization of these immune complexes causes a depletion of collagen XVII from cell surface [47]. This results in the formation of collagen XVII-deficient hemidesmosomes that weaken the adhesion in a patient's skin [47]. Thus, BP-IgG may induce or exacerbate skin fragility by itself [28].

4. Implications of Autoantibodies in Gene Therapy

Recent advancements in the understanding of EB pathogenesis have allowed researchers to identify novel treatment options, including gene therapy. Initial success in gene therapy was uncovered for JEB patients with a LAMB3 mutation, who received genetically engineered epidermal sheet grafts overexpressing an ex vivo, retroviral full-length LAMB3 transgenic product [48][49]. LAMB3 expression was maintained within the holoclonal epidermal stem cells and laminin 332 was found in the DEJ until 21 months [49]. However, gene therapy-mediated expression of a functional protein runs the risk of inducing autoimmunity. Fortunately, these study patients did not generate an immune reaction to the antigenic laminin β3 chain [50], likely because the selected patients had missense mutations involving a single amino acid or small deletions [48][49]. On the other hand, JEB patients with null mutations and fatal disease would be expected to develop immunoreactivity, and laminin-332 expression in skin via gene therapy would not correct severe upper respiratory, kidney, or internal disease [3]. Notably, autoantibodies against laminin β3 are also uncommon in patients with laminin-332 pemphigoid, occurring in less than 30% of patients [51]. However, autoantibodies against laminin α3 are present in close to 90%. This suggests that LAMB3 may be a less immunogenic target, thus contributing to the success of LAMB3 correction.

Gene therapy in recessive DEB patients is even more challenging due to the large size of *COL7A1* and the increased immunogenicity of NC1 [52]. Epidermal sheet grafting maintained collagen VII expression in the primary collagen-VII-deficient recessive DEB keratinocytes of immunodeficient mice [53]. However, in order to avoid the risk of autoimmunity, human trials excluded patients without positive expression to the NC1 domain of collagen VII, the most antigenic portion of this protein [6]. While NC1 is a highly targeted epitope in EBA, only approximately 30% of patient cell cultures fail to express NC1 [54]. Another important criterion was selection of patients with severe generalized recessive DEB showing the absence of expression of full-length collagen VII (near the NC2 domain) [55]. All patients tolerated grafting with collagen-VII-engineered autologous epidermal sheets without adverse events and skin biopsy demonstrated linear collagen VII expression [55][56]. Clinical improvement in wound healing was more profound in grafted sites and patient-reported pain, itch, and wound durability [55]. Only one patient in this study developed autoantibodies (specific to NC2 domain of collagen VII), but pre-therapy serum Western blot analysis showed low levels of transient collagen VII antibodies despite an initial negative screening with indirect immunofluorescence [55]. It is likely that immunoreactivity pre-existed in this patient, and gene therapy exacerbated the immune response. Screening data revealed that this patient expressed a collagen VII molecule containing NC1 domain but not NC2, suggesting that this patient's reaction was possibly an allo-reaction to the therapeutic gene product [55]. Thus, this patient did not experience increased blistering outside of the treated areas. Although low quantities of collagen VII antibodies in recessive DEB patients are considered nonpathogenic, as in this patient's case [56][57][58], caution is advised when these patients are treated with gene therapy.

Nonsense mutations, prevalent in 30% of recessive DEB patients, result in a premature termination codon that generates a truncated collagen VII product. In a recent trial of these recessive DEB patients treated with intravenous gentamicin, a premature termination codon readthrough was induced which created a new type VII collagen and anchoring fibrils that persisted for 3 months. Preliminary results demonstrate that none of the patients developed autoantibodies to collagen VII despite aminoglycoside-induced production of new collagen VII [59].

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