https://doi.org/10.31925/farmacia.2021.4.4 **REVIEW**

CHALLENGES AND LIMITATIONS IN DEVELOPING AN ANIMAL MODEL OF EPIDERMOLYSIS BULLOSA ACQUISITA: A MINIREVIEW

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Abstract

Epidermolysis bullosa acquisita (EBA) is a rare autoimmune pathology that affects the skin and the mucous membranes, presenting as clinical signs skin fragility, cutaneous vesicle and bullae, blisters, erosions with residual scarring and milia formation. Despite the advances recorded in the field of EBA pathogenesis molecular mechanism, diagnostic and therapeutically approaches, this topic is far from being elucidated. The present study aimed to offer a comprehensive overview of the advances recorded in the field of EBA in terms of animal murine models, key players in understanding EBA, highlighting the challenges and limitations encountered during the development of this type of models.

Rezumat

Epidermoliza buloasă dobândită (EBD) este o patologie autoimună rară care afectează pielea și membranele mucoaselor, prezentând ca semne clinice fragilitate cutanată, vezicule și bule, pustule, eroziuni cu cicatrici reziduale și formarea unor protuberanțe de culoare albă. Deși s-au înregistrat progrese semnificative în ceea ce privește EBD prin prisma mecanismelor moleculare implicate în patogeneză, a diagnosticului și a abordării terapeutice, acest subiect este departe de a fi elucidat. Studiul de față a avut drept scop sumarizarea în mod comprehensiv a datelor existente privind modelele animale murine pentru EBD, elemente cu rol major în înțelegerea acestei patologii, evidențiind provocările și limitările întâlnite în timpul dezvoltării unui astfel de model animal.

Keywords: epidermolysis bullosa acquisita, immunization, animal models, collagen type VII

Introduction

Epidermolysis bullosa acquisita (EBA) is a severe chronic skin and mucous membranes-specific auto-immune pathology, member of the rare diseases' family, that presents as characteristic features skin fragility, cutaneous vesicle and bullae, blisters, erosions with residual scarring and milia formation [19, 24, 36, 44]. The mechanism involved in EBA development is considered to be a loss of tolerance followed by a particular immune response consisting of the production of autoantibodies and T cells that target collagen type VII [34]. Collagen type VII (COL7) is a 290 kDa adhesion protein located in the sub-lamina densa of the skin and mucous membranes, synthetized and secreted by epidermal keratinocytes and dermal fibroblasts, that represents the primary constituent of anchoring fibrils responsible for the insurance of a resilient attachment of epidermis to the dermis [19, 23, 28]. Disturbances within the anchoring fibrils' function are associated with skin fragility, blisters and wound healing with scarring, the specific features of EBA [28].

On the basis of the clinical diagnostic, EBA presents two major subtypes: i) noninflammatory EBA, also known as classical or mechanobullous (specific signs: skin fragility, tense blisters, scaring and milia formation at the trauma prone sites, nail dystrophy and scarring alopecia) and ii) inflammatory EBA (specific signs: extensive vesiculobullous eruptions located on the trunk, extremities, skin folds, and central body, and pruritus) [19, 23]. As rare disease, EBA has an incidence lower than 0.5 cases *per* million/year, but still represents an attractive subject for the researchers due to the substantial impact on the quality of life of the patients diagnosed with this disease. Moreover, several systemic illnesses were associated with EBA, including rheumatoid arthritis, multiple endocrinopathy syndrome, thyroiditis, etc., data that are debatable at present, but a direct link was established between EBA and chronic inflammatory bowel diseases, as Crohn's disease [16, 41]. The scantiness of EBA patients hinders and limits the

knowledge regarding the pathogenesis, diagnostic and treatment options, data that are crucial for advances in this direction [19, 23]. A useful method to remedy these drawbacks is represented by the development of experimental animal models. Animal models are considered reliable alternatives to get insights into the human rare diseases' pathogenesis molecular mechanisms, diagnostic, and novel treatment options [3, 14, 31]. Two main methods were established to obtain experimental mouse models of EBA, as follows: (i) by passive transfer of rabbit or human antibodies against collagen type VII into mice and (ii) by active immunization using autologous collagen type VII [19, 34-37]. In the last years, EBA mouse models provided relevant knowledge about the molecular basis of EBA pathogenesis [11, 18, 24, 25, 27, 36, 38, 49], therapeutically targets and potential novel therapies [13, 15, 42], but this subject is far from being exhausted.

The aim of the present study consisted in portraying a comprehensive overview of the EBA murine models highlighting the challenges and limitations encountered in the process of developing this specific animal models. In addition, it was presented the molecular mechanisms involved in EBA pathogenesis and the main murine strains used to obtain reproducible EBA animal models.

Molecular mechanisms involved in EBA pathogenesis

The concept and the name of "epidermolysis bullosa acquisita" dates for more than a century being established by Kablitz in 1904 [22, 32], but only later, in 1984, Woodley and his colleagues identified the COL7, a structural protein found within the dermal-epidermal junction (DEJ) of human skin, as target for the specific autoantibodies of EBA [47, 48].

The role of anti-collagen type VII (anti-COL7) antibodies in the development of EBA is well-defined, but the pathogenic mechanism of this rare disease is complex and involves a cascade of events, events that despite the considerable progress recorded in this direction, still need further elucidation.

The results of previous epidemiological, but also of *in vitro* and *in vivo* studies, enabled the understanding of the molecular mechanisms involved in EBA pathogenesis, process that comprises several phases, as: (i) the afferent/the induction phase - correspondent to the loss of tolerance to COL7 or the validation of COL7 as autoantigen; (ii) the production and the maintenance of an increased half-life of the autoantibodies, and (iii) the efferent phase - correspondent to an immune response and to the blisters' formation (the appearance of the clinical specific signs) [12, 16, 22, 23].

According to recent knowledge, the loss of tolerance to COL7 (the first potential event of EBA pathogenic process) is considered to be genetically controlled since it was noticed that most patients diagnosed with EBA present the MHC (major histocompatibility complex) class II haplotype with HLA (human leukocyte antigen) - DR2 and HLA-DRB1 ∗15:03 particularities [12, 16, 22, 23, 50]. These data are endorsed by animal studies that indicate a clear link between the susceptibility to EBA development and the presence of H2s haplotype in different inbreed mouse strains (SJL/L, C57Bl/10.s) [16, 21, 22]. Besides the MHC genes, that proved to be key players in EBA development, another gene was identified to interfere with this process, the inhibitory FcγR (Fc gamma receptor) IIB receptor gene that must display a scarce expression [16, 22, 37]. In addition to the genetic risk factors, Srinivas *et al*. [40] highlighted the role of the interactions between the host genes and skin resident microbial communities as risk factors for EBA susceptibility.

Based on previous experimental data, it could be stated that the afferent phase of EBA is characterized by the following events: recognition of the COL7 as antigen followed by the activation of antigen presenting cells featured by autoreactive B cells, dendritic cells, and macrophages, leading to the synthesis of CD4+ T cells that proved to be crucial for the production of autoantibodies against COL7. A major role in the regulation of T cells proliferation was played by the heat-shock protein 90 (Hsp 90) [16, 23].

The production of autoantibodies against COL7 and their presence in the bloodstream is considered the second phase of the pathogenic mechanism involved in EBA development. The vast majority of the anti-COL7 antibodies identified in EBA patients belong to immunoglobulin G (IgG) class (all four subclasses were detected in the following order: IgG4 > IgG1 > IgG2 > IgG3) [16, 22], but other classes of immunoglobulins as IgA, IgE and IgM were also mentioned [16]. In *in vivo* experimental models it was shown that the anti-COL7 autoantibodies are exclusively produced in the peripheral lymph nodes by autoreactive B cells that differentiate in plasma cells and are located at this level [12, 16, 23]. A parameter of great interest that was studied is represented by the half-life of the anti-COL7 autoantibodies. After the release of the autoantibodies into the bloodstream, the neonatal Fc receptor (FcRn), a heterodimeric protein of the MHC class I, controls the autoantibodies' half-life by suppressing IgG catabolism, converting this

receptor into a target for novel anti-EBA potential drugs [4, 12, 16, 23].

The final step of EBA pathogenic mechanism is the efferent phase that concurs with the immune inflammatory reaction, the separation of epidermis from dermis and the appearance of subepidermal tissue injury (blisters, skin fragility, erosions, etc). This process comprises a cascade of events as follows: (i) binding of the IgG anti-COL7 autoantibodies to the epitopes found on the non-collagenous 1 (NC1) domain of COL7 at DEJ level; (ii) induction of a pro-inflammatory environment and complement activation; (iii) recruitments of various leukocytes cells, mainly neutrophils; (iv) the recruited neutrophils bind to the Fc domain of the IgG anti-COL7 autoantibodies via the receptors present on cells' surface - FcgRs leading to neutrophils activation; (v) neutrophils activation determines the synthesis of elastase and gelatinase (activators of metalloproteinases - molecules responsible for the injury of extracellular matrices located at basement membrane zone, loss of dermal-epidermal adhesion and subepidermal formation) and the production of reactive oxygen species (ROS) that trigger an impairment of basement membrane zone and enhance blister formation; (vi) production of various pro-inflammatory cytokines by keratinocytes and mast cells, and (vii) the overexpression of actin remodelling cytoskeletal protein Flightless I (Flii) that is correlated with an impaired wound healing process. All the events mentioned above were clearly and comprehensibly explained in previous articles [10, 12, 16, 17, 22, 23].

Experimental design of EBA's murine animal models

In the light of EBA's status as rare autoimmune disease and of the scarce number of cases, animal models proved to be very useful in understanding the pathogenesis of this disorder and, also, to discover novel molecular targets for an efficient treatment. In the following paragraphs it will be discussed the murine animal models of EBA.

After the identification of COL7 as autoantigen in EBA, two approaches were used to develop animal models: (i) the passive transfer of anti-COL7 antibodies (of human or rabbit origin) into mice and (ii) the active immunization using COL7 as antigen [34, 35, 46].

The passive transfer method was applied to obtain insights into the EBA's efferent phase specific mechanism. This method of developing EBA animal models consists of the passive transfer of anti-COL7 IgG isolated from rabbits and purified that were repeatedly immunized against human or murine COL7 using sera from EBA patients and the appearance of blisters in inoculated mice [2, 35]. This procedure proved to be successful in adult mice, whereas neonatal mice were resistant to develop EBA by passive transfer. A limitation of this model is represented by the increased amounts of high-titred anti-COL7 Ig samples required for injection [39, 45, 46]. Moreover, the differences between human and murine collagen could determine binding at different epitopes of COL7 leading to an inactivation of complement and neutrophil cascade and to a lack of subepidermal tissue injury. To overcome these drawbacks are used for the passive transfer the anti-murine COL7 antibodies [1, 19].

The development of EBA mouse models by active immunization requires the administration of COL7 as antigen to trigger an autoimmune reaction in mice that has as result the appearance of EBA pathology (blisters, erosions, skin fragility). The immunization can be done subcutaneously in the footpad of the mouse or at the tail base, and the emulsion injected must contain recombinant autologous murine COL7 mixed with a specific non-ionic copolymer TiterMax used as adjuvant. The immunization with COL7 autoantigen must be repeated two to three times at an interval of 3 weeks [34, 35]. This type of animal model offers insights regarding the loss of tolerance to COL7, the autoantibody production, and the immune response and tissue injury, all phases of EBA development, in terms of cellular and molecular targets and could be useful for the discovery of targeted immunotherapies for EBA's treatment [31, 35].

In Table I are presented several examples of EBA murine models obtained both by passive transfer as well as by active immunization.

Strains of mice used to develop EBA murine models

EBA pathogenesis process involves a complex mechanism that makes it difficult to reproduce. A crucial step in the development of EBA model is represented by the induction of autoantibodies against collagen type VII, which, according to recent knowledge is a genetically controlled step, the selection of the adequate strain of mice playing a critical role [23]. Sitaru *et al*. showed that the susceptibility of mice to develop EBA depends on the strain, the highest response being observed in SJL-1 mice (82%), followed by Balb/c (56%), whereas other strains as SKH-1 and C57BL/6 mice showed no incidence after immunization against collagen type VII [37]. Further studies proved that T cells are needed for the induction of collagen VII autoantibodies in EBA mouse models, SJL nude mice lacking T cells were resistant to clinical disease development following active immunization [36]. It was also noticed that most of the inbred strains of mice used for EBA animal model developed anti-collagen VII antibodies after a single immunization, but only several strains presented clinical signs of the

disease, as: SJL/J and B6.SJLH2s - presented a severe form of EBA, C57Bl/10.s and MRL/MpJ - mild form of EBA), and NOD/Shilt/J and C57Bl10.q mice - resistant to antibodies induction and to EBA development [21, 23].

Table I

Examples of EBA murine models using passive transfer and active immunization methods

Mouse strain	*Passive transfer/ active immunization*	*Outcome*	*Reference*
SKH1	Passive transfer - patients' sera	Skin fragility, blisters, erosions, nail loss; human EBA autoantibodies are pathogenic	[46]
C57Bl/6, BALB/c, BALB/c nude	Passive transfer - rabbit anti-mouse COL7	subepidermal skin blisters similar to human disease	[39]
SKH1	Passive transfer - human affinity-purified (CMP) anti-human COL7 IgG	Skin lesions present on the body surface	[5]
SJL/J, BALB/c, and Fc gamma receptor IIB-deficient mice	Active immunization - repetitive administration	Autoantibody production, skin lesions	[37]
SJL/J, B6.SJL-H2s, C57Bl/10.s, and MRK/MpJ mice	Active immunization - single administration	Autoantibody production, skin lesions	[9, 21]
B6.SJL-H2^s C3^c/1CyJ	Active immunization - recombinant murine COL7^{vWFA2}	Active disease development; FcRn – target for EBA treatment	[13]
B6.SJL-H2s C3c/1CyJ (B6.s), C57Bl/6J (B6.j) and Fcgr2b−/− on the B6.SJL (B6.s-Fcgr2b−/−) or C57Bl/6J (B6.j-Fcgr2b−/−) genetic background	Active immunization - recombinant murine COL7 vWFA2	Severe clinical phenotype in B6.s-Fcgr2b−/−	[18]
BALB/cJ, C57BL/6J, DBA1/J, and MRL/ MpJ,); B6.129P2-Fcer1gtm1Rav/J (g-chain deficient), B10.D2-Hc0 H2d H2-T18c/oSnJ (C5-deficient), and B10.D2-Hc1 H2d H2-T18c/nSnJ (C5- sufficient); and MHC-congenic B6.C-H2d/bByJ (B6.d), B6.SJL-H2s C3c/ 1CyJ (B6.s), and B6.AK-H2k/FlaEgJ (B6.k)	Active immunization - repetitive injection of anti-COL7^{vWFA2} IgG,	Skin blisters; insights into the efferent phase of EBA pathogenesis	[11]

SJL/J inbred mice strain presents several particularities in terms of immunological status, like: (i) an inverted B to T lymphocytes ratio (17% versus 79%) as compared to other strains, as C57BL/6 mice (67% versus 21%); (ii) a reduced innate natural killer function; (iii) a low Ig E responsiveness; (iv) a high IL-17 production capacity, and (v) a hypertrophied thymus [8, 33]. In addition, it was noticed that SJL/J mice are resistant to the infection induced by rabies virus, measles virus, mouse hepatitis virus strains A59, JHM, and MHV-S, mouse-adapted influenza A virus strains, and pneumonia virus of mice and exhibit a high susceptibility to murine cytomegalovirus, mouse adenovirus type 1, Theiler's murine encephalomyelitis virus and Friend's viral leukemogenesis [8].

The process of developing an EBA mouse model could be real challenging, since multiple factors (genetical and environmental) can interfere and change the outcome of the experiment. Our research group conducted an experiment using SJL female mice to develop an EBA model by active immunization. The mice were immunized in footpads and at tail base with 100 µg recombinant mouse type VII collagen (GST-mCVIICr) followed by a boost injection at day 21 post-immunization. The mice presented oedema and inflammation at inoculation site following immunization, mostly the ones inoculated in the footpads (Figure 1), but no specific signs for EBA development as blisters, erosions, alopecia, crusts, or scarring were noticed neither after the first immunization, nor after the boost injection (the mice were monitored for 10 weeks - Figure 1) (unpublished data).

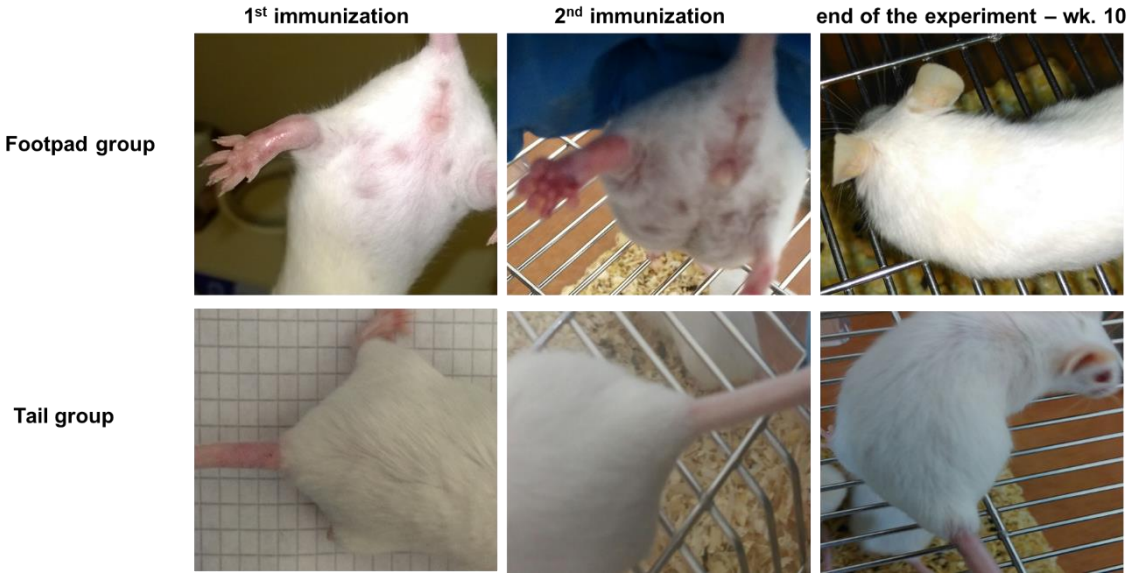

Figure 1.
Clinical evolution of SJL female mice immunized with recombinant mouse collagen type VII - GST-mCVIICr in TiterMax® after first (day 2) and second (day 22) immunization and at the end of the experiment (week 10). The mice presented oedema and inflammation at the site of injection, mainly the ones immunized in the footpads, but no signs of skin lesions were detected

A possible explanation for not developing EBA-specific lesions after immunization could be related to the genetic variations that occur within a mouse strain, mainly since the mice that we used were outbred, not inbred. Significant differences were described between the inbred and outbred mouse strains as regards the phenotypic variations and experimental behaviour [43].

Conclusions

The recent lines of evidence concerning the molecular and genetic pathways involved in EBA development and the existent animal models enhanced considerably our knowledge about this rare autoimmune disorder. Still, the complexity of the signalling pathways responsible for the immune response require further attention. The current data refer mostly to the inflammatory form of EBA, so as future perspectives, a greater interest should be attributed to the mechanobullous type of EBA in order to gather insights regarding its pathogenesis mechanism.

As regards the treatment options for EBA, the recent years brought a considerable number of novel promising agents both for curative and preventive properties [16]. A future perspective in this regard could be considered the evaluation of different compounds of natural origin that proved to be efficient in different pathological skin conditions (for e.g. pentacyclic triterpenes) [7, 26, 29, 30] or the use of nanoparticles in various formulations for topical use [6].

Conflict of interest

The authors declare no conflict of interest.

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