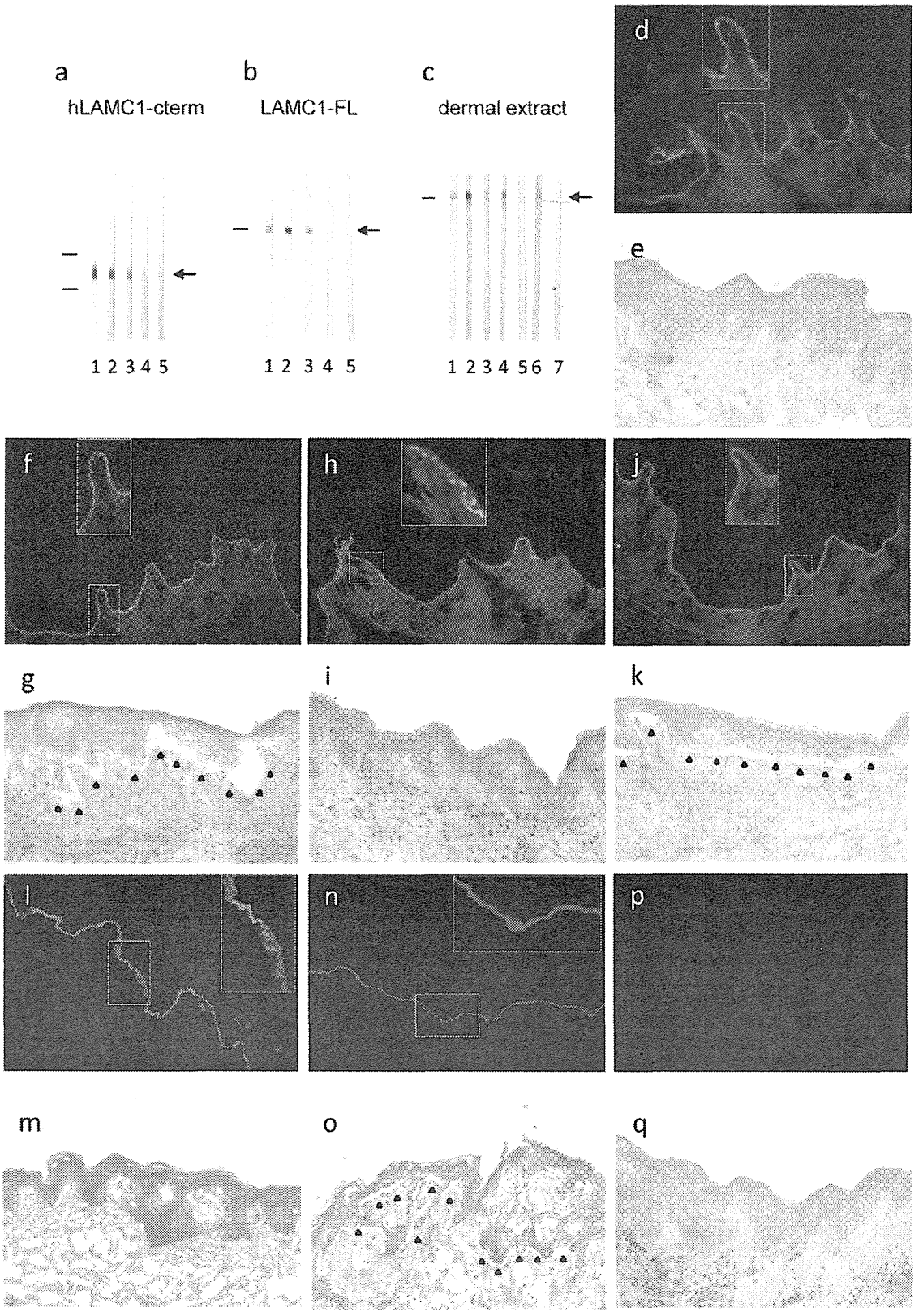
a
hLAMC1-cterm
1 2 3 4 5
b
LAMC1-FL
1 2 3 4 5
c
dermal extract
1 2 3 4 5 6 7
d
e
f
h
j
g
i
k
l
n
p
m
o
q

Figure 3. Human IgG specific to laminin γ1 is not pathogenic ex vivo. Using recombinant forms of the C-terminus of laminin γ1 (hLAMC1-cterm) and full length laminin γ1 (LAMC1-FL) IgG specific for hLAMC1-cterm (a, c; lane 3) and LAMC1-FL (b, lane 3; c lane 5) was generated from anti-p200 pemphigoid serum (a, b, c; lane 2), as well as serum depleted from anti-hLAMC1-cterm (a, c, lane 4) and LAMC1-FL reactivity (b, lane 4; c lane 6) reactivity, respectively, as shown by immunoblotting with recombinant hLAMC1-cterm (a), LAMC1-FL (b), and extract of human dermis (c). Interestingly, serum depleted from anti-hLAMC1-cterm and LAMC1-FL reactivity, respectively, (a, b; lane 4) still labeled the p200 protein in dermal extract (c, lane 4, lane 6). Monoclonal antibody against LAMC1 (a, b, c; lane 1) and serum from a healthy volunteer (a, b; lane 5; c lane 7) were used as controls. Arrows indicate the positions of the proteins and bars the molecular weight markers (a, 34 kD and 26 kD; b, 200 kD; c, 200 kD). hLAMC1-cterm-specific (h, i) and LAMC1-FL-specific patients IgG (l, m) and the monoclonal anti-LAMC1 antibody (d, e) labeled the dermal-epidermal junction (DEJ) by indirect immunofluorescence (IF) microscopy but did not induce dermal-epidermal separation (DES). In contrast, serum depleted from reactivity against hLAMC1-cterm (j, k), and LAMC1-FL (n, o), respectively, as well as patient serum (f, g) resulted in DES (black triangles mark base of the split). While untouched patient serum (f) as well as serum depleted from anti-hLAMC1-cterm (j) and LAMC1-FL reactivity (n), respectively, stained the DEJ of human skin in a linear pattern, the monoclonal anti-hLAMC1-antibody (d), hLAMC1-cterm-specific patient IgG (h), and hLAMC1-FL-specific patient IgG showed an additional staining of basal keratinocytes. Serum from a healthy volunteer was used as control (p, q). Magnification: x200.
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pemphigoid sera, including 21 sera that recognized hLAMC1-cterm, were probed for reactivity with the 5 laminin γ1 fragments by immunoblotting. Only weak reactivity outside hLAMC1-cterm was detected in 32% of patients' sera. Highest reactivity was found with fragment 5 in 4 (16%) of 25 sera (Table 2).

Pathogenicity of Rabbit IgG Against Recombinant Murine LAMC1-cterm (mLAMC1-cterm)

Rabbit anti-mLAMC1-cterm IgG reacted with a 200 kDa protein in the extract of murine dermis by immunoblotting (Fig. 4a lane 2). The same band was labelled by the commercial polyclonal anti-murine LAMC1 antibody H190 (Fig. 4a lane 1) that also recognized the recombinant mLAMC1-cterm used for immunization of rabbits (data not shown). Furthermore, rabbit anti-mLAMC1-cterm IgG stained the DEJ of mouse skin at a titre of 1:100 by indirect IF microscopy. The staining of the basal keratinocytes (Fig. 4c) was similar to the pattern seen with patient hLAMC1-cterm specific IgG on human skin (Fig. 3e, insert). Faint binding was also observed with the polyclonal anti-LAMC1 antibody H190 by indirect IF microscopy on murine skin (Fig. 4b). When used in the cryosection model, rabbit anti-mLAMC1-cterm IgG did not recruit neutrophils at the DEJ and did not induce DES even when applied at very high titres of $>1:10^5$ by immunoblotting with dermal extract. (Fig. 4g). Same findings were obtained using the monoclonal LAMC1 antibody H190 (Fig. 4f) and preimmune rabbit IgG (Fig. 4h). In contrast, as shown previously [10], rabbit anti-mBP180 NC15A IgG (reactive against the immunodominant region of BP antigen) resulted in DES (Fig. 4e).

Passive transfer of IgG to neonatal mice. To explore the pathogenic effect of IgG from rabbits immunized against mLAMC1-cterm *in vivo*, neonatal C57BL/6 mice (n = 8) were injected with rabbit anti-mLAMC1-cterm IgG and preimmune rabbit IgG, respectively, at a concentration of 10 mg/g body weight. In none of the mice, clinical disease was observed and histopathological examinations of back skin did not reveal dermal inflammation or subepidermal blisters (Fig. 5a). Direct IF microscopy of back skin showed linear deposits of rabbit IgG in 2 out of 8 mice, while in none of the mice, deposits of murine complement C3 were detected at the DEJ (Fig. 5b, c). All sera taken on day 12 showed IgG staining of basal keratinocytes along the DEJ by indirect IF microscopy of normal mouse skin at titres between 1:30 and 1:40 (Fig. 5d). In all mice, strong anti-mLAMC1-cterm reactivity was also detected by ELISA (Fig. 5e) and immunblotting with recombinant mLAMC1-cterm and the 200 kDa protein in extract of murine dermis (Fig. 5f, g). Also anti-mLAMC1-cterm specific IgG affinity-purified with recombinant mLAMC1-cterm from rabbits immunized with mLAMC1-cterm were injected into neonatal mice (n = 3) at a concentration of 1 mg/g of body weight with similar outcome (data not shown).

Passive transfer of IgG into adult mice. C57BL/6 (n = 5) and BALB/c (n = 5) adult mice were injected, every second day, for 12 days, with 15 mg of rabbit anti-mLAMC1-cterm IgG. None of the mice treated showed blisters clinically or histopathologically (Fig. 6a, e). IF microscopy of perilesional mouse skin revealed weak, linear, deposits of rabbit IgG in 2 C57BL/6 and one BALB/c mice (Fig. 6b, f), while murine complement C3 at the DEJ was negative in all cases (Fig. 6c, g). All mice showed serum autoantibodies against the DEJ with titers ranging from 1:30 to 1.40 by indirect IF microscopy (Fig. 6d, h), to p200 by immunoblotting (titre $1:10^5$) (Fig. 6k), and to mLAMC1-cterm (titre $1:10^5$) by ELISA and immunoblotting (Fig. 6 i, j).

Table 2. Serum autoantibody reactivity in anti-p200 pemphigoid patients with overlapping fragments of laminin γ1 covering the whole molecule.

	fragments outside hLAMC1-cterm					hLAMC1-cterm
	1	2	3	4	5	
positive sera	1 of 25 8 of 25	2 of 25	3 of 25	1 of 25	4 of 25	21 of 25

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Immunization Against mLAMC1-cterm Induces Autoantibody Production in mice of Different Strains but no Blisters

Following previous protocols [17,18,22], C57BL/6 (n = 5), BALB/c (n = 5), and SJL (n = 5) mice were immunized 4 times with mLAMC1-cterm. Control C57BL/6 (n = 1), BALB/c (n = 1), and SJL (n = 1) were immunized with an emulsion containing PBS and TiterMax®. After 16 weeks, no clinical or histopathological changes were detected in mLAMC1-cterm-immunized mice of the 3 strains (Fig. 7 a, e, i). By direct IF microscopy, 2 C57BL/6 showed faint deposits at the DEJ (Fig. 7 b). Murine C3 deposition at the DEJ was not observed (Fig. 7 c, g and k). In contrast, all mouse sera contained antibodies against recombinant mLAMC1-cterm (titre $1:10^8$), the p200 protein in extract of murine dermis (titre 1:5.000), and the DEJ (titer 1:30) by ELISA, immunoblotting, and indirect IF microscopy, respectively (Fig. 7d, h, l-o).

Discussion

In the present study, we showed that serum autoantibodies in anti-p200 pemphigoid are pathogenic while antibodies against laminin γ1 did not mediate this pathogenic effect in different experimental models.

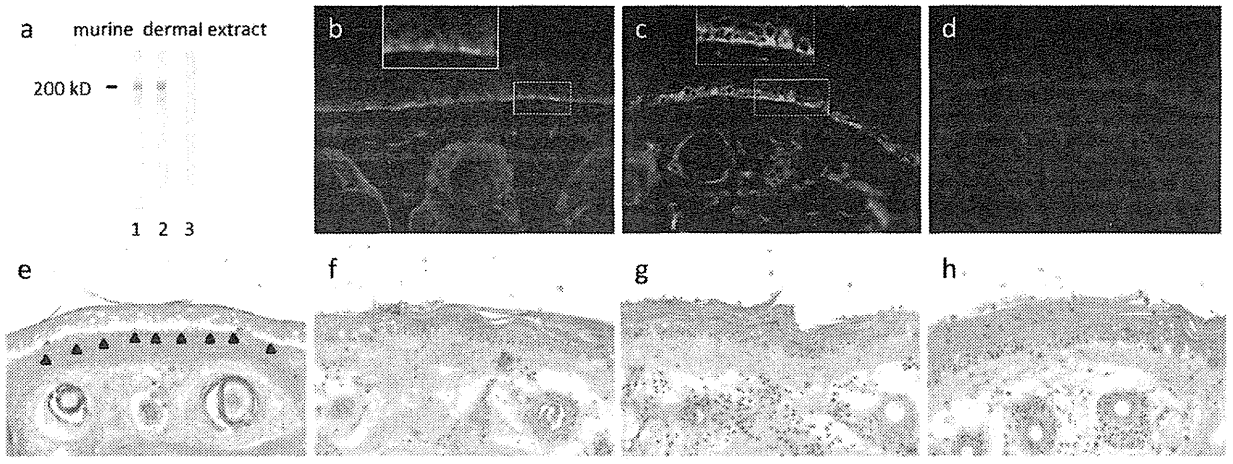

Figure 4. A high concentration of rabbit anti-mLAMC1-cterm IgG is not pathogenic ex vivo. Rabbit IgG generated against the murine laminin γ1 C-terminus (mLAMC1-cterm) did not induce DES in cryosections of mouse skin. Rabbit anti-mLAMC1-cterm (a, lane 2; c, g; dilution 1:1000) and commercial rabbit antibody H-190 against mLAMC1 (a, lane 1; b, f, dilution 1:200) recognized the p200 protein by immunoblotting with extract of murine dermis (a) and labeled the DEJ of murine skin (b, c) but did not induce DES in cryosections of mouse skin (f, g). Of note, anti-mLAMC1-cterm rabbit IgG stained the basal layer of keratinocytes at the murine DEJ (b, c, inserts) in a similar pattern as seen with monoclonal anti-hLAMC1-antibody and hLAMC1-cterm-specific patient IgG (Fig. 3, c-f). Rabbit IgG against murine BP180 NC15A (e) and preimmune rabbit IgG (d, h) were used as positive and negative controls, respectively. Magnification: x400.
doi:10.1371/journal.pone.0041769.g004

In the first set of experiments, an ex vivo model of autoantibody-mediated leukocyte-dependent neutrophil activation and DES was applied [21]. As previously shown for IgG from patients with BP and EBA [10,16,23], all 7 anti-p200 pemphigoid patients sera induced DES. Recently, the C-terminus of laminin γ1 has been described as the immunodominant region in anti-p200 pemphigoid [6]. To dissect the impact of autoantibody reactivity against this autoantigenic site in our ex vivo model, IgG affinity-purified with the recombinant human C-terminus of laminin γ1 (hLAMC1-cterm) and serum depleted from

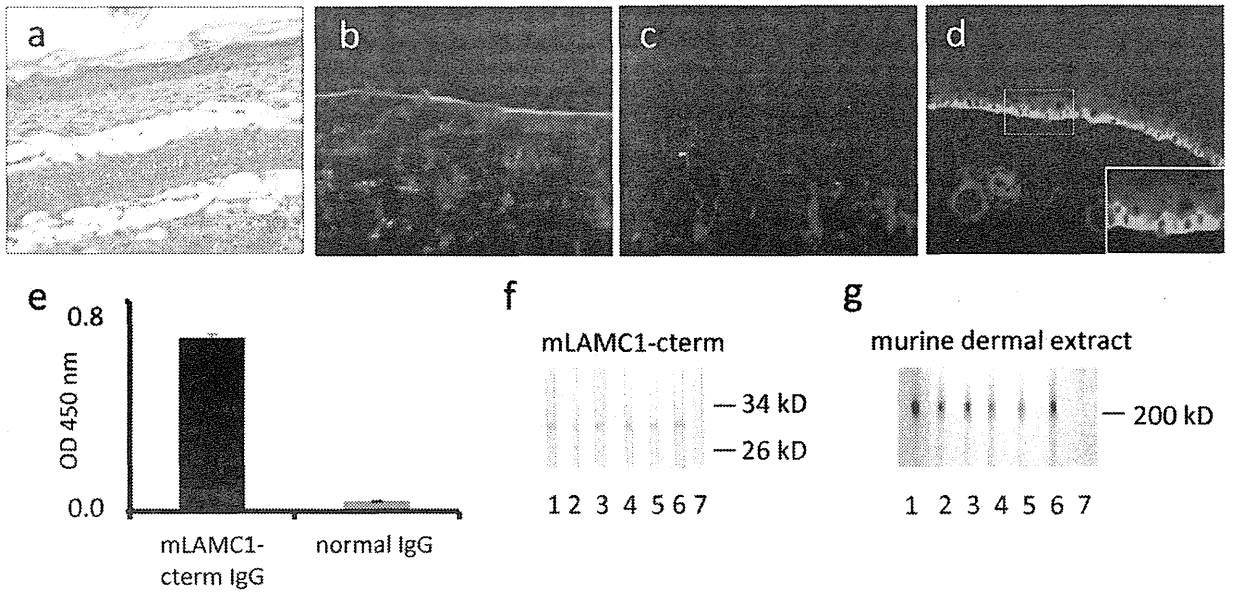

Figure 5. Passive transfer of rabbit anti-mLAMC1-cterm IgG into neonatal mice does not reproduce the human disease. Rabbit IgG against the murine laminin γ1 C-terminus (mLAMC1-cterm) was not pathogenic when passively transferred into neonatal C57BL/6 mice. Injection of rabbit anti-mLAMC1-cterm IgG at a concentration of 10 mg/g body weight every second day for 10 days did not induce histopathological lesions on day 12 (a). Linear deposition of rabbit IgG at the DEJ was only observed in 2 of 8 mice (b), while staining of murine C3 was always negative (c). At day 12, in sera of all mice, rabbit IgG stained the basal keratinocytes at the DEJ of normal mouse skin (d), reacted with recombinant mLAMC1-cterm by ELISA (e) and immunoblotting (f, lanes 2–6) and with the 200 kDa p200 protein in extract of murine dermis (g, lanes 2–6). Polyclonal rabbit antibody H-190 against mLAMC1 (f, g, lane 1) and normal mouse serum (f, g, lane 7) was used as controls.
doi:10.1371/journal.pone.0041769.g005

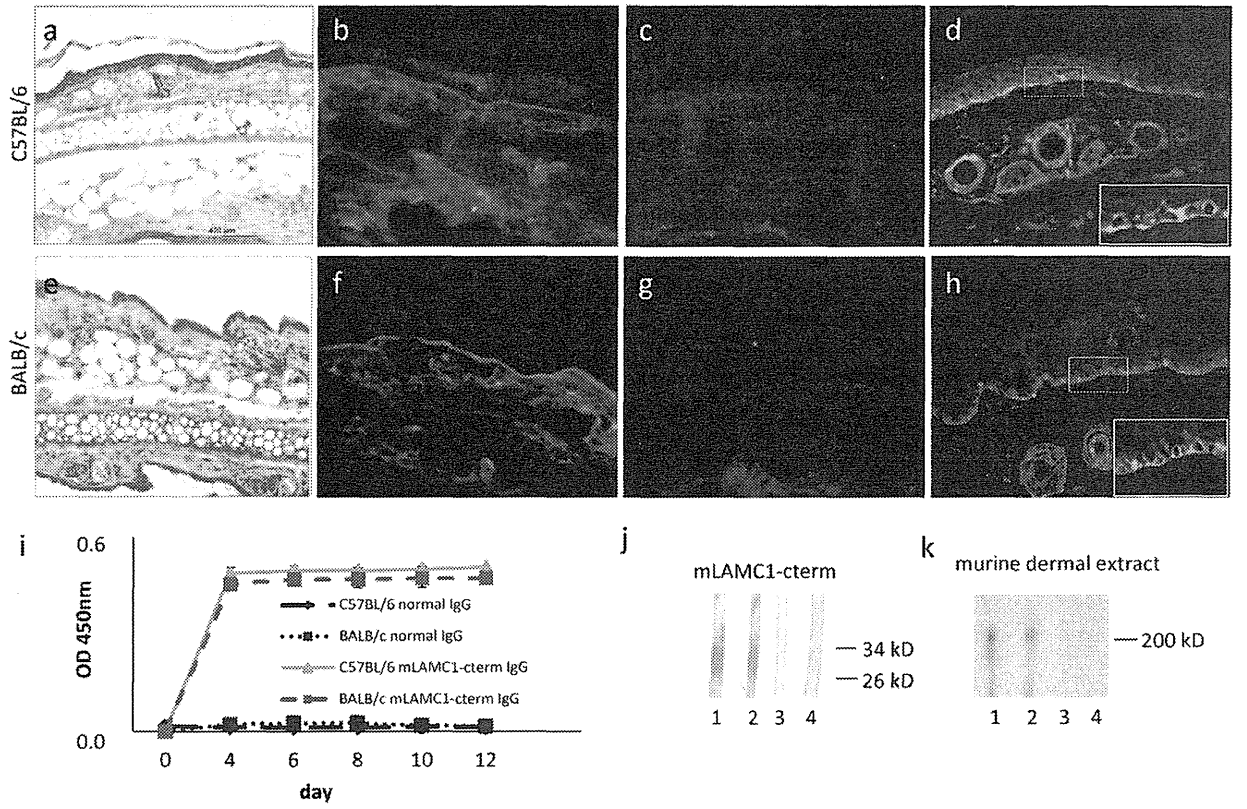

Figure 6. Passive transfer of rabbit anti-mLAMC1-cterm IgG into adult mice is not pathogenic. Rabbit IgG against the murine laminin γ1 C-terminus (mLAMC1-cterm) was not pathogenic when passively transferred into adult C57BL/6 and BALB/c mice. Injection of 15 mg rabbit anti-mLAMC1-cterm IgG every second day for 10 days did not result in clinical or histopathological (a, e) lesions on day 12. Linear deposition of rabbit IgG at the dermal-epidermal junction (DEJ) was only observed in 2 of 5 C57BL/6 (b) and one of 5 BALB/c mice (f), while staining of murine C3 was negative in all mice (c, g). At day 12, in sera of all 10 mice, rabbit IgG labeled the basal keratinocytes at the DEJ of normal mouse skin (d, h) and reacted with recombinant mLAMC1-cterm by ELISA (i) and immunoblotting (j, C57BL/6, lanes 1; BALB/c, lane 2) and the 200 kDa p200 protein in extract of murine dermis (k, C57BL/6 lane 1; BALB/c, lane 2). Normal mouse sera (j and k, C57BL/6, lane 3; BALB/c, lane 4) were used as controls.
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hLAMC1-cterm-reactivity were prepared from anti-p200 pemphigoid sera. While latter serum fractions induced DES, hLAMC1-cterm-specific IgG was not pathogenic. The same results were obtained using IgG specific to the recombinant eukaryotic E8 fragment, a C-terminal portion of laminin 111 and specific to LAMC1-FL. When sera were completely depleted of IgG no DES occurred. This result clearly points to antibody-mediated tissue damage in anti-p200 pemphigoid.

The following hypotheses to explain inefficacy in our ex vivo model were compiled: i) the concentration of anti-LAMC1-cterm-IgG was too low, ii) glycosilation-dependent epitopes mediate pathogenicity, iii) the elution process of hLAMC1-cterm-specific IgG during the affinity purification procedure impaired pathogenicity, iv) antibodies against a heterotrimeric form of laminin γ1 are pathogenic, v) epitopes on laminin γ1 outside LAMC1-cterm are pathogenetically relevant, vi) the cryosection model is not suitable to demonstrate the pathogenic effect of anti-laminin γ1 antibodies, and vii) laminin γ1 is recognized by autoantibodies but not the autoantigen in anti-p200 pemphigoid.

To address the first hypothesis, a 5-fold higher IgG concentration compared to the corresponding patient sera as well as high concentrations of both rabbit IgG generated against murine LAMC1-cterm and commercially available anti-laminin γ1 IgG were used in the cryosection model, but no DES was observed. Since both the p200 protein and laminin γ1 were shown to be N-glycosylated [5,6], we then investigated whether glycosilation-dependent epitopes mediate DES in our ex vivo model. When patient IgG, affinity purified against eukaryotic expressed hLAMC1-cterm was applied also no effect was observed. The third hypothesis claiming a potential damage of IgG following the elution process was excluded since hLAMC1-cterm-specific IgG showed strong reactivity with recombinant hLAMC1-cterm, the p200 protein in human dermis, and the human DEJ by immunoblotting and indirect IF microscopy, respectively. In addition, both rabbit serum and concentrated rabbit IgG generated against mLAMC1-cterm as well as a monoclonal anti-laminin γ1 antibody (all not subjected to affinity purification) were ineffective in the ex vivo model.

To test the fourth hypothesis we used the E8 fragment of laminin 111. This E8 fragment was used by Dainichi *et al.* for the detection of anti-laminin γ1 reactivity in anti-p200 pemphigoid sera [6]. Similar to experiments with hLAMC1-cterm E8 fragment-specific IgG was ineffective in the cryosection assay and serum depleted from anti-E8 antibodies was still reactive with the p200 protein in dermal extract and induced DES in our ex vivo model. To address the fifth hypothesis, epitope mapping experiments were performed that revealed the binding of only a minority of anti-p200 pemphigoid sera outside LAMC1-cterm.

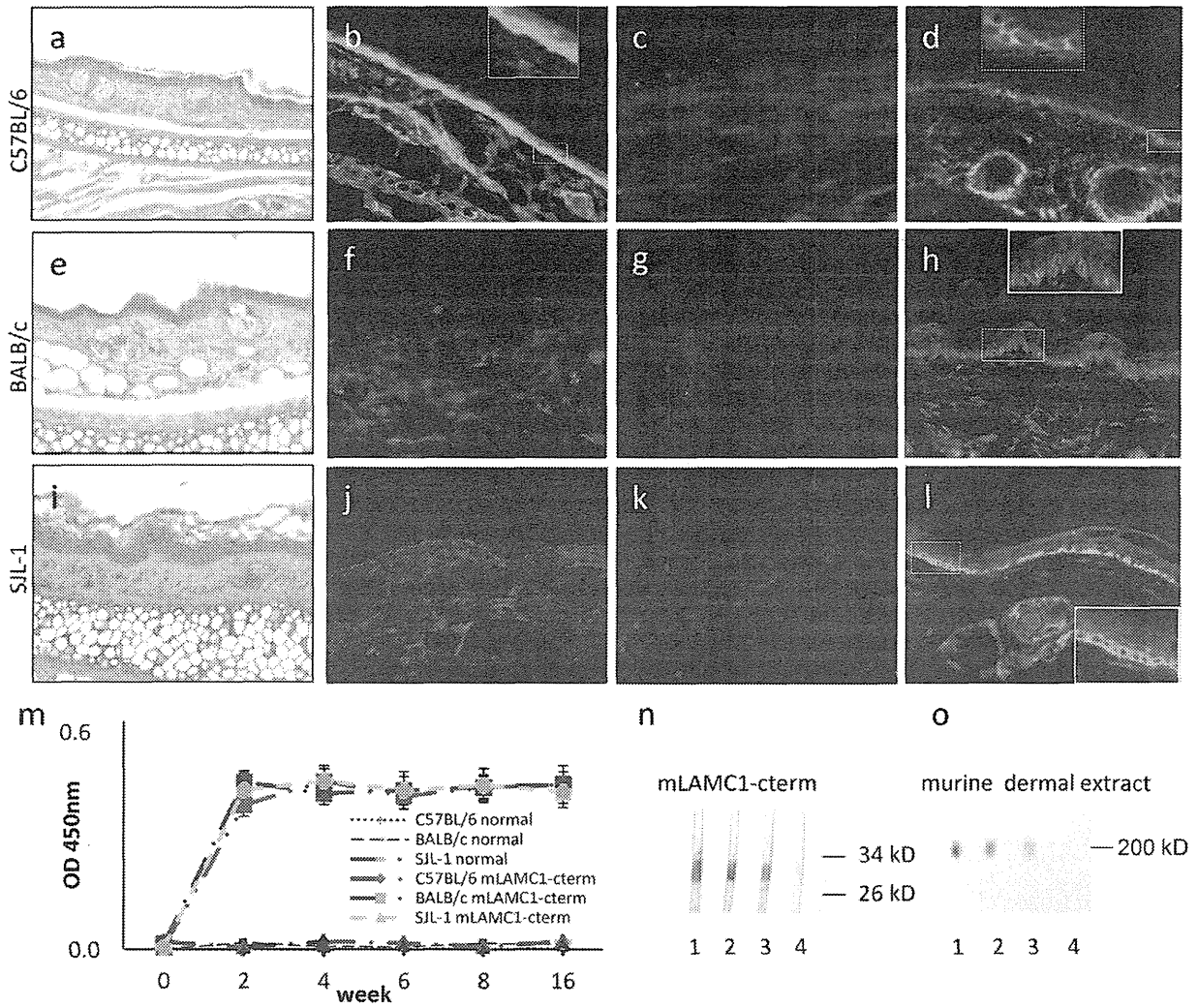

Figure 7. Immunization of mice with mLAMC1-cterm induces high serum levels of anti-mLAMC1-cterm antibodies but no skin lesions. Immunization of different mouse strains with the recombinant murine laminin γ1 C-terminus (mLAMC1-cterm) did induce high levels of anti-mLAMC1-cterm antibodies but no macro- or microscopic disease. Three different mouse strains (B57BL/6, BALB/c, and SJL; n = 5/strain) were immunized 4 times with 60 μg of recombinant mLAMC1-cterm in conjunction with TiterMax®. After 16 weeks, no clinical or histopathological changes (a, e, i) were seen. Deposition of mouse IgG at the dermal-epidermal junction (DEJ) was found in only 2 C56BL/6 mice (b). In all other mice, no IgG or C3 deposition was detected at the DEJ (f, j, c, g, k). In all mice, serum autoantibodies labeled the basal layer of keratinocytes at the murine DEJ (d, h, l) and reacted with recombinant mLAMC1-cterm by ELISA (m) and immunoblotting (n; C57BL/6, lane 1; BALB/c, lane 2; SJL, lane 3) and the 200 kDa p200 protein in extract of murine dermis (o; C57BL/6, lane 1; BALB/c, lane 2; SJL, lane 3). Serum of a mouse immunized with TiterMax® alone served as control (n and o; lane 4).
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Thus, major pathogenic sites on laminin γ1 outside the LAMC1-cterm domain were excluded. Since the recombinant fragments used in the epitope mapping studies were overlapping by only 0 to 5 amino acids and some B-cell epitopes could thus have been missed patients' IgG affinity-purified with the entire laminin γ1 chain was employed in the cryosection model. Again, the anti-full length laminin γ1 IgG did not induce DES while IgG depleted from anti-full length laminin γ1 retained its DES-inducing capacity.

In a final set of experiments to address the sixth hypothesis, the effect of anti-LAMC1-cterm antibodies was explored in various *in vivo* models. Since patient antibodies do not cross-react with murine skin [19], antibodies against murine LAMC1-cterm were raised by immunization of rabbits and different mouse strains. High doses of total IgG from these rabbits and IgG affinity-purified using recombinant mLAMC1-cterm were then injected into adult and neonatal mice, respectively. While high levels of circulating anti-mLAMC1-cterm antibodies were present in all animals, binding of IgG at the DEJ was weak, did not attract C3 at the DEJ, and did not result in clinical or histopathological lesions. Similar findings were obtained when mice from different strains were immunized with mLAMC1-cterm. In contrast, previously, injection of rabbit IgG raised against the immunodominant regions of type XVII collagen (BP180) into neonatal mice reproduced the human disease (BP) within 48 hours [9]. Also, injection of rabbit or human IgG against the immunodominant

regions of type XVII collagen or type VII collagen into adult mice recapitulated the human diseases BP and EBA, respectively [11,13,24]. Recently, we successfully induced clinical disease mimicking the human disorders BP and EBA by immunizing susceptible mice strains with the immunodominant regions of type XVII collagen and type VII collagen, respectively, following a similar protocol as applied in the present study [11,18]. Autoimmunity against laminin 332, the target antigen in a subgroup of patients with mucous membrane pemphigoid was induced in mice by the passive transfer of human and rabbit anti-laminin 332 IgG [14,25]. These data show that autoimmunity against target antigens including collagens and laminin could be mounted by both passive transfer of autoantibodies and immunization-induced autoantibodies mimicking the clinical disease of the corresponding subepidermal blistering diseases. We speculate that the failure to induce clinical disease in our mouse models, although high levels of serum anti-mLAMC1-cterm IgG were present, may be attributed to the weak binding of autoantibodies at the DEJ. This notion is fuelled by the peculiar binding pattern of anti-LAMC1-cterm IgG at the basal keratinocytes of the DEJ that differs from the exclusively linear binding observed in patients' skin and of patients' serum autoantibodies labeling human skin. Furthermore, the unique reactivity of patient autoantibodies with the DEJ, but not with any other tissues which express the laminin γ1 chain, remains enigmatic.

Based on these data, we conclude that while the C-terminus of laminin γ1 is a major target of autoantibodies in anti-p200 pemphigoid, we failed to demonstrate the pathogenicity of anti-laminin γ1 antibodies using different approaches. A somehow similar autoantibody constellation is known in BP. While BP230 had been described as the major target antigen recognized by the majority of BP patients [26,27], its pathogenic relevance could not be unequivocally demonstrated. Subsequently, antibodies against type XVII collagen were described [28,29] and numerous evidence for their pathogenic relevance has been gathered [9,10,20,24,30,31].

In summary, in this study, the pathogenic potential of autoantibodies in anti-p200 pemphigoid was shown for the first time. The specificity of the pathogenically relevant autoantibody awaits further elucidation.

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Author Contributions

Conceived and designed the experiments: KV SG ES DZ AR RJL. Performed the experiments: KV SG TB JD MH. Analyzed the data: KV SG ES DZ. Contributed reagents/materials/analysis tools: TH. Wrote the paper: KV SG ES DZ TH.

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Viewpoint

How do keratinizing disorders and blistering disorders overlap?

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Abstract: Inherited keratinizing disorders are caused by mutations in the genes encoding cornified cell envelope proteins, enzymes and their inhibitors, adhesion molecules, cytoskeletal proteins and others in the epidermis. These molecules are known to regulate differentiation, proliferation and cell adhesions. Intriguingly, some keratinizing disorders show blistering skin lesions, while some inherited blistering disorders show abnormal keratinization. Therefore, hereditary keratinizing and blistering diseases are closely related and show overlapping genetic backgrounds. In this review, we overviewed keratinizing and blistering disorders in terms of overlapping of the two disease groups. Gene mutations in desmosomal components cause striate keratoderma, Naxos disease, epidermolytic palmoplantar keratoderma and plakophilin deficiency, which first show skin fragility and blisters and later hyperkeratosis. Gene mutations in hemidesmosomal components cause various forms of epidermolysis bullosa, some of which show hyperkeratosis on the nails, palms and soles, in addition to blister formation. Diseases with gene mutations in calcium pump proteins are Darier disease and Hailey–Hailey disease, which show clinicopathological overlaps and develop both keratinizing and blistering skin lesions. Finally, gene mutations in epidermal keratins cause epidermolysis bullosa simplex, epidermolytic ichthyosis, superficial epidermolytic ichthyosis, epidermolytic palmoplantar keratoderma and pachyonychia congenita/focal palmoplantar keratoderma, which show thickening of the palms and soles with underlying blister formation. In general, responsible proteins for diseases developing both keratinizing and blistering conditions are adhesion molecules, calcium pump proteins and keratins, but not connexins, cornified cell envelop proteins, enzymes or inhibitors. It is still unknown how particular keratinizing diseases develop blisters and vice versa.

Key words: epidermal differentiation – genodermatosis – inherited skin disorder

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Overview of keratinization

Keratinocytes undergo keratinization, which is a continuous process of proliferation and terminal epidermal differentiation (1). Differentiation of keratinocytes starts from basal cells located at the lowermost basal layer of the epidermis. Basal cells attach basement membrane through relatively stable anchoring device, hemidesmosomes. Keratinocytes attach to each other mainly by desmosomes. Basal cells, then, push divided daughter cells up into the epidermis, which become spinous cells in the second layer, spinous layer. Spinous layer is composed of approximately 10 layers. In spinous layer, keratinocytes keep going up and become flatter and finally becomes granular cells in granular layer. Granular cells show different features from those of spinous cells. Granular cells are flat and have cornified cell envelope in the cytoplasm, which provides a vital physical barrier (2–4). Cornified cell envelope is a 10-nm-thick layer composed of highly cross-linked insoluble proteins, to which ceramide lipids covalently bound (2–4). At the molecular level, keratin, loricrin, filaggrin, involucrin and transglutaminase 1 are the major components of cornified cell envelope (2,3). Finally, granular cells become corneocytes in cornified layer, which are dead cells and contain abundant lipids. Keratinized skin surfaces are protected from external stimuli by combination of extracellular lipids (lipid envelopes), corneodesmosomes and cornified cell envelop. Among them, corneodesmosomes attract more attention as a major player in the skin surface protection (5). In addition, keratinocytes exchange ions through gap junctions. Keratinocytes in granular layer also attach to each other firmly by tight junctions.

Keratinization is observed in the skin, tongue and external half of the lips. The final step of keratinization is called cornification. As mentioned previously, cornification is a process to form epidermal barrier in stratified squamous epithelial tissues, in which terminal differentiation and programmed cell death of keratinocytes occur (6).

Mutations in genes encoding these proteins cause abnormal keratinization, which leads to keratinizing disorders and occasionally blistering disorders. Furthermore, blistering diseases caused by mutations in adhesion molecules may show abnormal keratinization, in addition to blister formation.

In the following sections, we summarize inherited skin disorders caused by gene mutations of keratinization-related proteins, as well as adhesion molecules (Table 1, Fig. 1). We do not catalogue all keratinizing disorders and blistering disorders. Instead, we focus on the interface disorders between the two groups and discuss about the mechanisms how abnormal keratinization and blisters simultaneously occur from the same gene mutations in these diseases.

Inherited skin disorders of desmosomes

Desmosomes are the major cell–cell adhesion devices in epithelial cells including epidermal keratinocytes and myocardial cells (7).

Table 1. Inherited skin diseases that share characteristics of both keratinizing and blistering disorders

Diseases	Mode of inheritance	Ichthyosis/keratoderma	Blister formation	Target genes/proteins	Specific features
Inherited skin disorders of desmosomes					
Plakophilin deficiency (ectodermal dysplasia/skin fragility syndrome)	AR	PPK	+	*PKP1*/plakophilin 1	Abnormal ectodermal development, hypotrichosis, nail dystrophy
Dilated cardiomyopathy with wooly hair and keratoderma	AR	Striate PPK	+	*DSP*/desmoplakin 1	Nail dystrophy, enamel dysplasia
Naxos disease	AR	Striate PPK	+ (skin fragility)	*JUP*/plakoglobin	Wooly hair
Inherited skin disorders of hemidesmosomes					
EBS (Dowling-Meara, other generalized, localized)	AD	Focal or diffuse PPK	+	*KRT5, KRT14*/keratin 5 or keratin 14	
EBS with muscular dystrophy	AR	Focal PPK (rare)	+	*PLEC1*/plectin	Nail dystrophy (late-onset case)
Non-Herlitz JEB, generalized	AR	Focal PPK	+	*COL17A1*/BP180, type XVII collagen, BPAG2	Nail, hair and tooth dystrophy, scalp abnormalities
Kindler syndrome	AR	PPK	+	*FERMT1*/Kindlin-1	Poikiloderma, photosensitivity
Inherited skin disorders of calcium pump					
Darier disease	AD	Keratotic papules/plaques in seborrhoeic areas	+	*ATP2A2*/SERCA2	Neuropsychiatric features
Hailey–Hailey disease	AD	–	+	*ATP2C1*/SPCA1	Lesions are restricted mainly to the neck, axillae, groin and perineum
Inherited skin disorders of epidermal keratins					
Epidermolytic ichthyosis (BCIE, epidermolytic hyperkeratosis)	AD	Ichthyosiform erythroderma, diffuse PPK	+	*KRT1, KRT10*/keratin1 or keratin 10	
Epidermolytic palmoplantar keratoderma	AD	Diffuse PPK	+	*KRT9*/keratin 9	
Pachyonychia congenita	AD	Focal PPK	+	*KRT6A-C, KRT16, KRT17*/keratin 6a-c, keratin 16 or keratin 17	
Superficial epidermolytic ichthyosis (ichthyosis bullosa of Siemens)	AD	Ichthyosiform erythroderma,	+	*KRT2*/keratin 2	

AD, autosomal dominant; AR, autosomal recessive; BCIE, bullous congenital ichthyosiform erythroderma; EBS, epidermolysis bullosa simplex; JEB, junctional EB; PPK, palmoplantar keratoderma.

Desmosomes are composed of members of several protein families, that is, desmosomal cadherin family transmembrane glycoproteins including desmogleins 1–4 (DSG1-4, genes; *DSG1-4*) and desmocollins 1–3 (DSC1-3, genes; *DSC1-3*), armadillo family proteins including plakoglobin (PG, gene; *JUP*) and plakophillins (PKP1-4, genes; *PKP1-4*), plakin family proteins including desmoplakin (DSP, gene; *DSP*), envoplakin and periplakin, and corneodesmosin (CDSN, gene; *CDSN*) (8).

Human gene mutations encompassing the nine components of desmosomes have already been reported. Among them, mutations in genes encoding DSG1, PG, PKP1, DSP and CDSN are reported to cause abnormal keratinization. Although mutations in these desmosomal protein genes are considered to show defect in cell adhesion, common clinical manifestation of these disease is palmoplantar keratosis. However, little is known about the mechanisms of how the gene mutations produce palmoplantar keratosis phenotype.

Prototypes of desmosome-related genetic disorders are striate palmoplantar keratoderma with mutations in the *DSG1* or *DSP* gene, Naxos disease with mutations in the *JUP* gene and plakophilin deficiency with mutation in the *PKP1* gene (9–11). Although mutations in desmosome-related genes cause mostly keratoderma, they sometimes induce blistering conditions; for example, Finnish female patient with compound heterozygous *DSP* mutations (c.6310delA/p.Ala2655Asp) showed epidermolytic palmoplantar keratoderma and extensive mucocutaneous blisters, as well as nail dystrophy, enamel dysplasia, sparse wooly hair and cardiomyopathy (12). In addition, plakophilin deficiency with *PKP1* mutations shows not only palmoplantar keratoderma, but also various abnormal conditions, including growth delay, hypotrichosis, hypohidrosis, nail dystrophy and erosive skin lesions, most of which are caused by ectodermal development abnormalities (13).

These diseases show overlapping symptoms of keratinizing and blistering disorders, suggesting that these two diseases are related to each other. In addition, mutations in these desmosomal genes are considered to demonstrate important roles in both keratinization and cell–cell adhesion in the epidermis, as well as development of hair, nail and heart.

Inherited disorders of hemidesmosomes

Hemidesmosomes are strong adhesion devices that connect epithelial cells to extracellular matrix proteins, including collagen IV, collagen VII and laminin-332. Intracellular hemidesmosomal component proteins include keratin 5/keratin 14 complex, plectin and BP230 (bullous pemphigoid antigen 1; BPAG1). BP180 (bullous pemphigoid antigen 2; BPAG2 or collagen XVII) and $\alpha 6\beta 4$ integrin are two transmembrane components of hemidesmosomes that tether cytoplasmic hemidesmosomal components to extracellular domain of hemidesmosomes. Binding of $\alpha 6\beta 4$ integrin to laminin-332 transduces signals, which are related to differentiation, proliferation, direction of cell movement and apoptosis in keratinocytes (14).

Mutations in genes encoding hemidesmosome-related molecules result primarily in epidermolysis bullosa (EB) (15). EB disorders are classified into four major categories based on the ultrastructur-

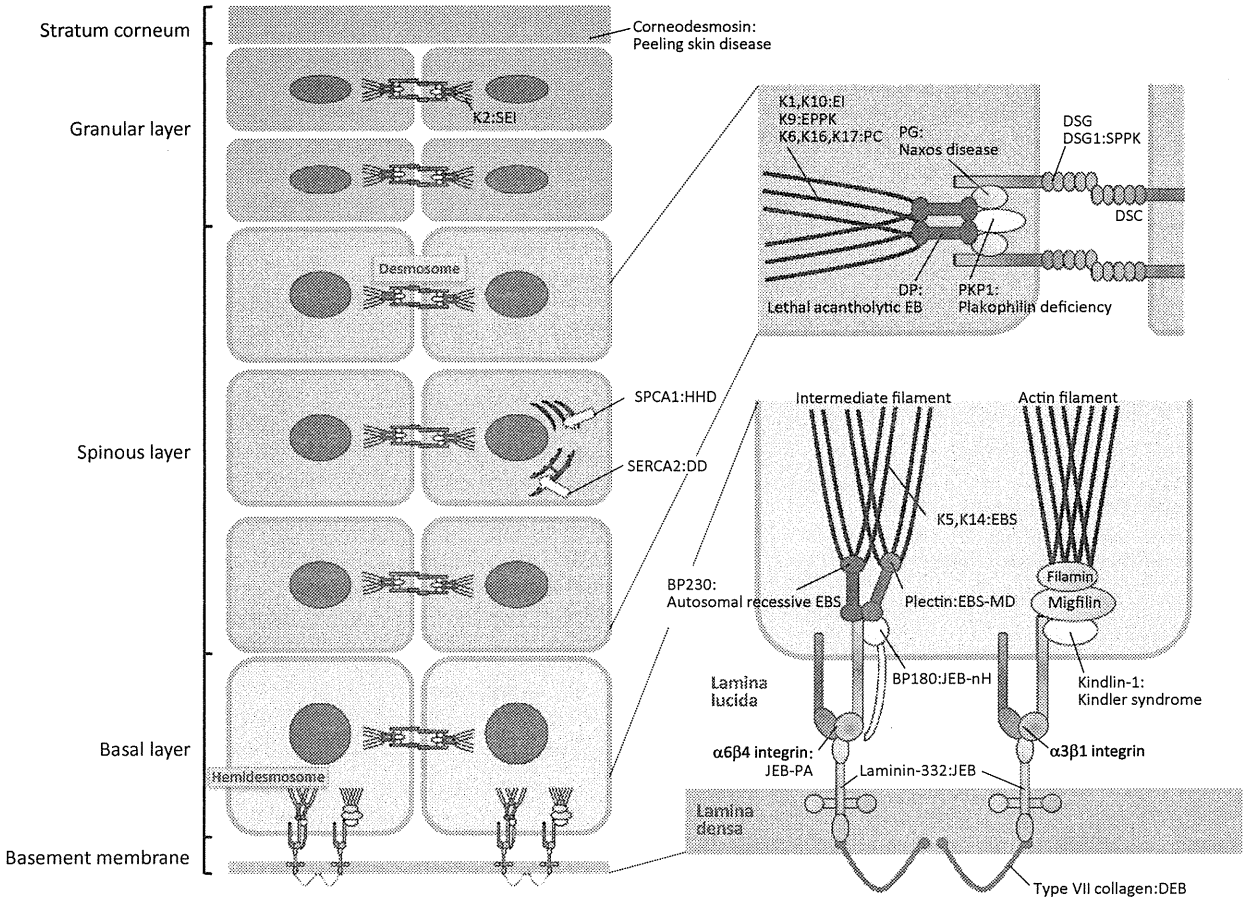

Figure 1. Structural organization of the epidermis and phenotypic consequences of molecular defects in inherited skin diseases that share characteristics of both keratinizing and blistering disorders. DEB, dystrophic epidermolysis bullosa; DD, Darier disease; DP, desmoplakin; DSC, desmocollin; DSG, desmoglein; EBS, EB simplex; EI, epidermolytic ichthyosis; EPPK, epidermolytic palmoplantar keratoderma; HHD, Hailey–Hailey disease; JEB, junctional EB; K, keratin; MD, muscular dystrophy; nH, non-Herlitz; PA, pyloric atresia; PC, pachyonychia congenita; PKP1, plakophilin 1; SEI, superficial epidermolytic ichthyosis; SERCA2, sacro/endoplasmic reticulum Ca^{2+}-ATPase isoform 2; SPCA1, secretory pathway Ca^{2+}/Mn^{2+}-ATPase protein 1; SPPK, striate palmoplantar keratoderma.

al blistering sites, that is, intraepidermal lysis (EB simplex), junctional type (junctional EB), dermolytic type (dystrophic EB) and mixed type (Kindler syndrome) (16).

Three major types of EB simplex are Dowling-Meara, Köbner and Weber–Cockayne types (17). Most severe Dowling-Meara type and moderate Köbner types manifest widespread blistering and erosions early in life. Intriguingly, patients with these two diseases form blister in the early childhood, but show decrease in blister formation and increase in palmoplantar keratoderma in adulthood.

Moreover, non-Herlitz junctional EB cases were reported to show palmoplantar keratoderma (18–20). One case revealed homozygous loss-of-function mutation in BP180 (20). Kindler syndrome is the first disease with mutations of focal contact-related gene. Focal contact is a minor attachment device at epidermal basement membrane zone and is composed of kindlin, migfilin, filamin and $\alpha 3\beta 1$ integrin (21). Kindler syndrome is characterized by acral blistering, poikiloderma, skin atrophy and photosensitivity (22). In addition, patients with Kindler syndrome show nail dystrophy and palmoplantar keratoderma (23). These findings strongly suggest a close relationship between hereditary blistering and keratinizing diseases, although abnormal keratinization is not associated with all patients with EB.

Inherited skin disorders of calcium pumps

There are two inherited skin disorders affecting calcium pumps, that is, Darier disease, which is a keratinizing disorder, and Hailey–Hailey disease, which is a blistering disorder. Darier disease is caused by mutations in the *ATP2A2* gene, which encodes a calcium pump protein, sacro/endoplasmic reticulum Ca^{2+}-ATPase isoform 2 (SERCA2) (24,25), whereas Hailey–Hailey disease is caused by mutations in the *ATP2C1* gene, which encodes a calcium pump protein, human secretory pathway Ca^{2+}/Mn^{2+}-ATPase protein 1 (SPCA1), located on the Golgi apparatus (26). Both types of Ca^{2+}-ATPase are key molecules in regulation of intracellular Ca^{2+} concentration in keratinocytes.

Darier disease is characterized by multiple keratotic papules and plaques on the seborrhoeic areas. Patients with Darier disease usually develop skin lesions in the second decade. Histopathology shows acantholysis and dyskeratotic cells, called grains and corps ronds, in the epidermis (7). In contrast, Hailey–Hailey disease is characterized by recurrent vesicular lesions with crusted erosions