Natural Occurrence of Autoantibodies against Basement Membrane Proteins in Epidermolysis Bullosa

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TO THE EDITOR

Epidermolysis bullosa (EB) is a group of genetic blistering diseases characterized by lifelong trauma-induced blistering of the skin and mucosa and extracutaneous manifestations. Autoantibodies to a structural protein of the epidermal basement membrane zone (BMZ) such as dystonin (BP230), plectin, type XVII collagen (COL17/BP180), laminin-332, or type VII collagen (COL7) result in the same level of blister formation as in EB subtypes caused by mutations in their coding gene, such as in EB simplex (*DST* and *PLEC*), junctional EB (JEB) (*COL17A1, LAMA3, LAMB3,* or *LAMC2*), and dystrophic EB (DEB) (*COL7A1)* (Goletz et al., 2017; Has et al., 2020). The innate and adaptive immune systems are designed not to recognize the host's own cells and proteins owing to natural immunological tolerance and negative selection of host-specific T lymphocytes in the central lymphatic organs. However, the lack of one of the proteins due to inherited mutations can interfere with this process. When the missing protein is introduced later in life, it can be recognized as dangerous, and an immune response can occur (Alberts, 2002; Siprashvili et al., 2016).

Four previous publications presented results of serological tests, ELISA, and indirect immunofluorescence (IIF) on monkey esophagus in patients with EB (DEB and EB simplex) (Annicchiarico et al., 2015; Esposito et al., 2016; Tampoia et al., 2013; Woodley et al., 2014). Circulating antibodies against BMZ proteins were present in the serum, and the authors suggested the

need for further ex vivo experiments to assess their pathogenicity. Although three publications lacked direct immunofluorescence (DIF) on a skin biopsy specimen and IIF on salt split skin (SSS) for detection of tissue-bound autoantibodies, Woodley et al. (2014) additionally performed DIF and IIF on SSS in patients with DEB with a positive ELISA.

Treatment approaches for EB are being investigated, and progress has been recently made; however, they can be threatened by pre-existent circulating antibodies (Eichstadt et al., 2019; Gaucher et al., 2020; Hirsch et al., 2017). In these studies, their presence was assessed before transplantation only by ELISA, IIF, and western blot. In the study of Eichstadt et al. (2019), DIF was performed but only on the transplanted sites after the transplantation and not before. IgG deposition was found in one of the transplanted sites in one of the treated subjects at 3 months and 2 years after transplantation; however, circulating antibodies were only detectable at months 1 and 3 and until month 6 after transplantation. Therefore, they suggested that the humoral immune response was provoked by the transplantation site rather than that the circulating antibodies were pre-existing. For the diagnosis of pemphigoid diseases, Meijer et al. (2019) recently proposed that DIF and IIF on SSS and not ELISA or blot are essential, and therefore, these techniques should be used to illustrate whether pre-existing antibodies can bind to the skin (Schmidt and Zillikens, 2009). Because these data are missing in the

literature, we have investigated skin biopsies and serum of 37 patients with EB with a wide variety of techniques, including DIF and IIF on SSS to assess the presence of circulating antibodies.

Of the 37 patients, 12 were affected with JEB due to mutations in *LAMB3* and *COL17A1*, and 25 were affected with DEB due to mutations in *COL7A1* (Table 1). A total of 10 of the 37 included patients had revertant mosaicism (6 with JEB and 4 with recessive DEB [RDEB]) (Supplementary Table S1), that is, healthy, natural skin patches due to correcting somatic mutations that occurred during embryo development or later in life (Pasmooij et al., 2012). We analyzed the already stored punch biopsies from 35 of the 37 patients. Serum samples from all the patients were obtained with permission from medical ethical committees in the Netherlands (University Medical Center Groningen 2013/317) and Spain (Code Hospital Universitario La Paz: PI1359 and PI1595). All patients or their parents provided written informed consent. Furthermore, 14 sera from 13 patients with severe burn wounds were used as the control for ELISA, blotting, and IIF. For a detailed methods description, see previous publications (Groth et al., 2011; Vodegel et al., 2004). The age of the patients at the time of biopsy and serum sampling varied from 0 to 61 years (Supplementary Table S1) for the patients with EB and from 6 to 86 years for the burn wound patients (Supplementary Table S2). DIF was performed on all available skin specimens (1−3 biopsies per patient) to detect human IgG and IgA. Furthermore, we performed IIF on two substrates, monkey esophagus, and SSS; keratinocyte footprint assay for laminin-332 (Giurdanella et al., 2020); and ELISA for COL17 (NC16A), BP230, and COL7. In addition, immunoblot was performed on keratinocyte cell extract

Table 1. Detailed Data Per Patient of all Tests Conducted for Autoantibodies

NR	EB Type	Database NR	Mutation (DNA)	Mutation (Protein)	Protein Expression in Nonblistered Skin	Biopsy Direct IF	IIF MO	IIF SSS	KFA Lam332	ELISA MBP Groningen BP180	ELISA MBP Groningen BP230	ELISA MBP Groningen COL7	ELISA Euroimmune Lübeck BP180	ELISA Euroimmune Lübeck BP230	ELISA Euroimmune Lübeck COL7	Immunoblot Groningen BP180	Immunoblot Groningen BP230	Immunoblot Groningen LAD	Immunoblot Lübeck COL7	Immunoblot Lübeck DermExtr	Immunoblot Lübeck Lam-γ1
COL17A1 Patients																					
1	JEB-intermed	EB02501	COL17A1: c.2237delG / c.2237delG	p.Gly746Alafs*53 / p.Gly746Alafs*53	Negative (COL17)	−	−	−	−	18	17	9	−	−	−	−	−	−	−	−	−
2	JEB-intermed	EB02601	COL17A1: c.3676C>T / c.1601delA	p.Arg1226* / p.Asp534Alafs*19	Negative (COL17)	−	BMZ	−	−	−	−	−	−	−	−	−	−	−	−	−	−
3	JEB-intermed	EB03502	COL17A1: c.2237delG / c.3676C>T	p.Gly746Alafs*53 / p.Arg1226*	Negative (COL17)	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
4	JEB-intermed	EB13401	COL17A1: c.1365delC / c.3600_3601delCT COL17A1: c.1260delC / c.3495_c.3496delCT	p.Thr421Leufs*72 / p.Ser1166Leufs*6	Negative (COL17)	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
5	JEB-intermed	EB20801	COL17A1: c.2237delG / c.2237delG	p.Gly746Alafs*53 / p.Gly746Alafs*53	Negative (COL17)	−	BMZ	−	−	−	−	−	−	−	−	−	−	−	−	−	−
6	JEB-intermed	EB08601	COL17A1: c.1772-2 A>C / c.3327delT	in-frame exon skipping / p.Pro1110Argfs*21	Negative (COL17)	−	−	IgA roof	−	−	−	−	−	−	−	−	Weakly positive	−	−	−	−
7	JEB-loc	EB16801	COL17A1: c.4320delT / c.4320delT	p.Gln1442Lysfs*70 / p.Gln1442Lysfs*70	Strongly reduced (COL17)	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
8	JEB-loc	EB09801	COL17A1: c.2251C>T / c.3327delT	p.Gln751* / p.Pro1110Argfs*21	Strongly reduced (COL17)	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
LAMB3 Patients																					
9	JEB-intermed	EB02901	LAMB3: c.628G>A / c.628G>A	p.Glu210Lys / p.Glu210Lys	Strongly reduced (lam332)	−	BMZ	−	−	−	−	−	−	−	−	−	−	−	−	−	−
10	JEB-intermed	EB29901	LAMB3: c.1106dup / c.1106dup	p.Ala370Serfs*13 / p.Ala370Serfs*13	Normal (lam332)	−	−	−	Positive	−	−	−	−	−	−	−	−	−	−	−	−
11	JEB-intermed	EB13201	LAMB3: c.1063T>C / c.1903C>T	p.Cys355Arg / p.Arg635*	Strongly reduced (lam332)	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
12	JEB-loc	EB25401	LAMB3: c.628G>A / c.1903C>T	p.Glu210Lys / p.Arg635*	Strongly reduced (lam332)	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
COL7A1 patients																					
13	RDEB-sev	EB26501 (P116)	COL7A1: c.887delG / c.6527dup	p.Gly296Valfs*5 / p.Gly2177Trpfs*113	Strongly reduced (type XII collagen)	−	ECS	−	−	−	−	−	−	24	42	−	−	−	−	−	−
14	RDEB-sev	EB29701 (P108)	COL7A1: c.2142A>G / c.6527dup	Altered splicing resulting in out-of-frame transcript p.Gly2177Trpfs*113	Negative (COL7)	−	−	−	−	−	−	−	−	−	−	−	−	−	Positive	EBA/p200	Positive
15	RDEB-sev	EB02201	COL7A1: c.3G>T / c.4997dup	Start-lost / p.Pro1668fAlafs*4	Negative (COL7)	−	−	−	−	29	19	6	−	−	−	−	−	−	−	−	−
16	RDEB-sev	EB07701	COL7A1: c.925_944dup / c.1264dup	p.Ile315Metfs*12 / p.Arg422Profs*19	Negative (COL7)	−	−	−	−	60	37	13	23	40	−	−	−	−	−	−	−
17	RDEB-sev	EB09001	COL7A1: c.4767delA / c.4767delA	p.Asp1590Thrfs*120 / p.Asp1590Thrfs*120	Negative (COL7)	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
18	RDEB-sev	P10	COL7A1: c.6527dup / c.6527dup	p.Gly2177Trpfs*113 / p.Gly2177Trpfs*113	Strongly reduced (COL7)	Not tested	−	−	−	10	12	−	164	30	30	−	Dubious	−	−	−	−

(continued)

Table 1. Continued

NR	EB Type	Database NR	Mutation (DNA)	Mutation (Protein)	Protein Expression in Nonblistered Skin	Biopsy Direct IF	IIF MO	IIF SSS	KFA Lam332	ELISA MBP Groningen BP180	BP230	COL7	ELISA Euroimmune Lübeck BP180	BP230	COL7	Immunoblot Groningen BP180	BP230	LAD	Immunoblot Lübeck COL7	DermExtr	Lam-γ1
19	RDEB-sev	P31	COL7A1: c.6527dup / c.6527dup	p.Gly2177Trpfs*113 / p.Gly2177Trpfs*113	Strongly reduced (COL7)	–	–	–	–	16	–	–	–	–	–	–	Dubious	–	–	–	–
20	RDEB-sev	P46	COL7A1: c.325_326insCG / c.3277-1G>C	p.Glu109Alafs*39 / Altered splicing resulting in out-of-frame transcripts	Strongly reduced (COL7)	–	BMZ	–	–	48	35	14	–	–	–	–	Positive	–	–	–	–
21	RDEB-sev	P52	COL7A1: c.7756dup / c.7930-1G>C	p.Gln2586Profs*12 / In-frame exon skipping	Negative (COL7)	–	–	–	–	–	–	–	–	–	–	–	–	–	–	EBA/p200	–
22	RDEB-sev	P104	COL7A1: c.6618+1G>A / c.6618+1G>A	p.K2206_G2207insMSL_E220Gfs*86/ p.K2206_G2207insMSL_E220Gfs*86 Altered splicing resulting in out-of-frame transcripts / Altered splicing resulting in out-of-frame transcripts	Strongly reduced (COL7)	–	–	–	–	39	–	–	30	–	–	–	–	–	–	EBA/p200	–
23	RDEB-sev	P14	COL7A1: c.6527dup / c.336C>G	p.Gly2177Trpfs*113 / p.Tyr112*	Negative (COL7)	IgG	–	–	–	–	–	–	–	–	25	–	–	–	–	–	–
24	RDEB-sev	P18	COL7A1: c.6527dup / c.2984dup	p.Gly2177Trpfs*113 / p.Gly996Trpfs*44	Negative (COL7)	Not tested	–	–	–	–	–	–	–	25	73	–	–	–	–	–	–
25	RDEB-sev	P61	COL7A1: c.6527dup / c.6527dup	p.Gly2177Trpfs*113 / p.Gly2177Trpfs*113	Strongly reduced (COL7)	–	–	–	–	96	40	–	60	83	64	–	–	–	–	–	–
26	RDEB-sev	P104.I	COL7A1: c.6618+1G>A / c.6618+1G>A	p.K2206_G2207insMSL_E220Gfs*86/ p.K2206_G2207insMSL_E220Gfs*86 Altered splicing resulting in out-of-frame transcripts / Altered splicing resulting in out-of-frame transcripts?	Strongly reduced (COL7)	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
27	RDEB-sev	P78	COL7A1: c.6527dup / c.5130_5131insCTCAC	p.Gly2177Trpfs*113 / p.Thr1711Leufs*132	Negative (COL7)	–	–	IgA Floor	–	24	20	8	–	–	–	–	–	–	–	–	–
28	RDEB-sev	EB26001 (P17)	COL7A1: c.6527dup / c.6527dup	p.Gly2177Trpfs*113 / p.Gly2177Trpfs*113	Strongly reduced (COL7)	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
29	RDEB-sev	EB02401	COL7A1: c.6508C>T / c.6508C>T	p.Gln2170* / p.Gln2170*	Negative (COL7)	IgG/IgA/ C3c to revertant skin	BMZ	IgGfloor	–	9	–	10	–	–	27	–	–	–	–	–	–
30	RDEB-sev	EB06401	COL7A1: c.1573C>T / c.6508C>T	p.Arg525* / p.Gln2170*	Negative (COL7)	–	–	–	–	9	11	–	–	–	–	–	–	–	–	–	–
31	RDEB-intermed	EB00901	COL7A1: c.2699G>A / c.7237G>A	p.Trp900* / p.Gly2413Arg	Minimally reduced (COL7)	–	–	–	–	22	12	–	–	–	–	–	–	–	–	–	–
32	RDEB-intermed	EB34701	COL7A1: c.5272G>A / (unknown)	p.Gly1758Arg / unknown	Minimally reduced (COL7)	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
33	RDEB-intermed	P36	COL7A1: c.6527dup / c.7300G>A	p.Gly2177Trpfs*113 / p.Gly2434Arg	Reduced (COL7)	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
34	RDEB-intermed	P204	COL7A1: c.2722_2723delCA / c.5188 C>T	p.Gln908Valfs*45 / p.Arg1730*	Reduced (COL7)	–	–	–	–	10	–	–	–	–	43	–	–	–	–	–	–

(continued)

Table 1. Continued

Patient NR	EB Type	Database NR	Mutation (DNA)	Mutation (Protein)	Protein Expression in Nonblistered Skin	Biopsy Direct IF	IIF MO	IIF SSS	KFA Lam332	KFA BP180	KFA BP230	KFA COL7	ELISA MBP Groningen BP180	ELISA MBP Groningen BP230	ELISA MBP Groningen COL7	ELISA Euroimmune Lübeck BP180	ELISA Euroimmune Lübeck BP230	ELISA Euroimmune Lübeck COL7	Immunoblot Groningen BP180	Immunoblot Groningen BP230	Immunoblot Groningen LAD	Immunoblot Groningen COL7	Immunoblot Lübeck DermExtr	Immunoblot Lübeck Lam-γ1
35	RDEB-intermed	P120	COL7A1: c.5576_5577delAA / c.4012G>A	p.Lys1858Argfs*12 / p.Gly1338Arg	Strongly reduced (COL7)	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
36	RDEB-inversa	EB04701	COL7A1: c.8083G>A / c.8083G>A	p.Gly2695Ser / p.Gly2695Ser	Minimally reduced (COL7)	–	–	–	–	–	–	–	–	–	16	–	–	154	–	–	–	–	–	–
37	DDEB	P50	COL7A1: c.6182G>T / c.=	p.Gly2061Val / p.=	Reduced (COL7)	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	Weakly positive	–	–

Abbreviations: BMZ, basement membrane zone; COL, collagen; DDEB, dominant dystrophic epidermolysis bullosa; DermExtr, dermal extract; EB, epidermolysis bullosa; ECS, extracellular space; IF, immunofluorescence; IIF, indirect immunofluorescence; JEB-intermed, junctional epidermolysis bullosa intermediate; JEB-loc, junctional epidermolysis bullosa localized; KFA, keratinocyte footprint assay; Lam, laminin; MO, monkey esophagus; NR, number; RDEB-intermed, recessive dystrophic epidermolysis bullosa intermediate; RDEB-sev, recessive dystrophic epidermolysis bullosa severe; SSS, salt split skin. Only positive results above the cut-off value are given. Cut-off values for ELISA as used and evaluated by our diagnostic laboratories: For MBP, COL17 NC16A: >9 positive, = 9 doubtful, < 9 negative; BP230: > 20 positive, 9–20 doubtful, < 9 negative; COL7: > 6 positive, = 6 doubtful, < 6 negative; For Euroimmun, COL17 NC16A: > 20 positive, BP230: > 20 positive, COL7: > 20 positive. Positive results are indicated in bold, and doubtful results are indicated in italic.

to detect antibodies against BP230, COL17, and LAD-1 (Groningen, The Netherlands) (Pas, 2001), on dermal extract to detect antibodies against COL7, and on the recombinant C-terminus of laminin γ1 (Lübeck, Germany) to detect antibodies against the p200 protein. ELISA for the NC16A domain of COL17, BP230, and COL7 were performed in two different laboratories in Groningen (The Netherlands) and in Lübeck (Germany) using MBL and Euroimmun kits, respectively.

The most important finding of our study is that only two patients (2 of 35, 5.7%) showed linear binding of IgG along the BMZ in DIF. Both patients, #23 and #29, have severe RDEB and were negative for COL7 staining, although patient #29 also had a proven revertant patch. Patient #23 was negative for all serological tests except for one of the ELISA's for COL7 (Table 1). Because patient #23 was negative for the COL7 protein with LH7.2 in the skin, the IgG in the DIF was either not directed to COL7 or it is possible that the patient expresses small amounts of truncated COL7 protein to which the IgG is directed. Patient #29 DIF showed 3+ IgG staining to the BMZ in the revertant skin and negative in the mutant skin. Serology revealed dermal binding of IgG in SSS and positive anti-COL7 autoantibodies in both ELISAs, consistent with a diagnosis of EB acquisita (Figure 1 and Table 1). Both patients did not report any noticeable change of skin phenotype that would indicate the manifestation of EB acquisita, and in the case of patient #29, his revertant skin patch did not blister, even after inducing mechanical trauma (minimal skin rub test) (Figure 1b). This suggests that his general blistering was caused by RDEB and not by circulating autoantibodies as in a case published by Guerra et al. (2018), where EB acquisita occurred in a patient with a mild DEB phenotype.

In 22 of the 37 patients (59.5%), we found at least one positive serological test (Table 1), and in three other patients, we found at least one serological test that was doubtful, meaning that 67.5% (25/37) of our cohort had circulating antibodies against BMZ proteins. Interestingly, the proportion of patients with at least one positive or doubtful serological test was highest in the severe RDEB subgroup (83%, 14/18
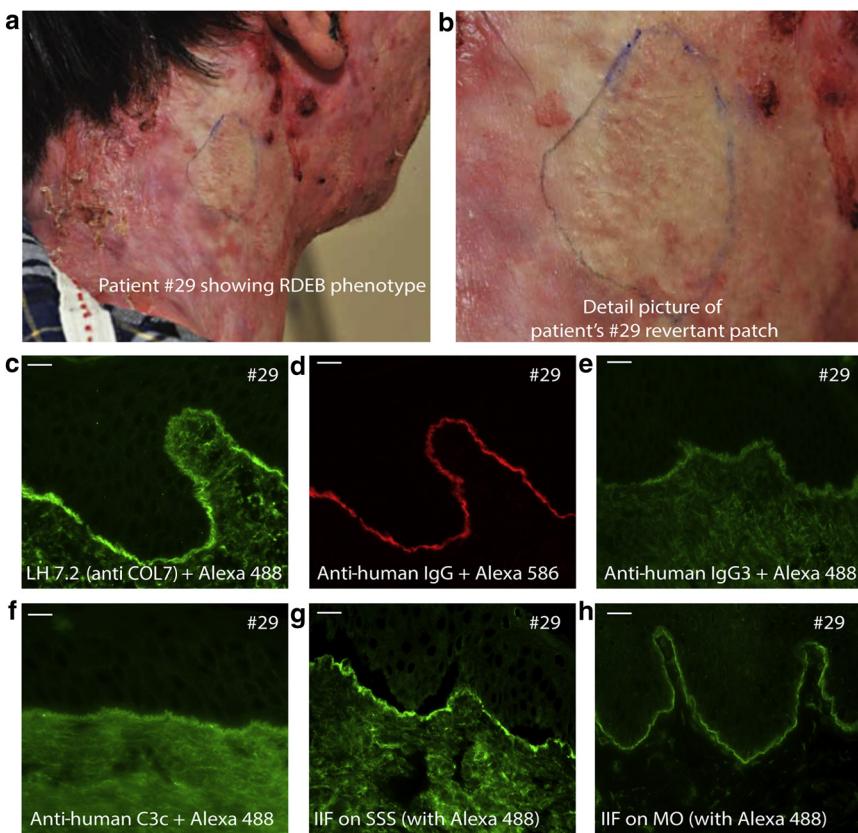
Figure 1. Clinical presentation of patient #29 and immunofluorescent linear staining of the patient's revertant patch. (**a**) Clinical presentation of patient #29 with RDEB due to homozygous *COL7A1*: c.6508C>T mutation. (**b**) Detail of revertant patch due to natural correction with a second-site mutation c.6510G>T, which removed the termination codon. (**c**−**e**) Immunofluorescent linear staining of revertant patch with LH7.2 for (**c**) COL7 (green; Alexa 488), (**d**) antihuman IgG (red, Alexa 586), (**e**) antihuman IgG3 (green, Alexa 488), and (**f**) antihuman C3c (green, Alexa 488) along the basement membrane zone in a u-serration pattern. (**g**) Indirect immunofluorescence on the salt-split skin from a healthy donor, with patient #29's serum showing dermal IgG (green) binding to the floor of the split. (**h**) Indirect immunofluorescence with patient #29's serum on MO showing positive IgG binding (green) to the basement membrane zone. Bar = 20 μm. The patient consented to the publication of the images. COL, collagen; IIF, indirect immunofluoroscence; MO, monkey esophagus; RDEB, recessive dystrophic epidermolysis bullosa; SSS, salt split skin.

positive and 1/18 doubtful) than in patients with JEB (50%, 4/12 positive and 2/12 doubtful) and in patients with other types of DEB (57%, 4/7 positive). However, the number of patients with JEB and other types of DEB was limited. Furthermore, besides patient #29, none of the other patients showed binding of IgG to the BMZ in SSS, whereas only two patients showed IgA binding (patient #6, positive and patient #27, doubtful), thereby resulting in 3 of 37 (8.1%) with positive or doubtful IIF on SSS. These positive findings on DIF and/or SSS, although only in three patients, are in contrast with the findings of Woodley et al. (2014). In their RDEB cohort, 11 of 22 patients had a positive ELISA. However, none of these 11 patients had a positive DIF or SSS. Finally, it is remarkable that several of the

patients have circulating autoantibodies against the protein that they are thought to be lacking owing to their mutations. This might indicate that patients are never truly null and that these patients still express a very small amount of the deficient protein, albeit in a truncated or altered form. An alternative explanation could be that these patients have revertant areas, which have not yet been identified.

In our cohort, we found positive Euroimmun ELISA for NC16A in 5 of 37 patients (13.5%) and positive MBP ELISA for NC16A in 12 of 37 patients (32.4%). van Beek et al. (2014) described that ELISA for NC16A in the elderly (aged >70 years) was positive in about 6.5% and 3.5% for Euroimmun and MBP ELISAs, respectively. This suggests more positive reactions in the

patients with EB than in the published elderly group.

An important question is why circulating autoantibodies are found so frequently in patients with EB and especially in patients with severe RDEB, which do not seem to be clinically relevant. Esposito et al. (2016) showed that patients with EB and especially those with RDEB have higher levels of proinflammatory cytokines than the levels in the control population. In addition, a recent review by Huitema et al. (2021) states that there is evidence that patients with RDEB have an underlying immunity defect. The high number of positive ELISAs in patients with EB may thus be caused by exposure to self-antigens due to repeated skin damage combined with a chronic immunological response or underlying immunity defect because all serological tests were negative in 13 patients with severe acute burn used as controls (data not shown). However, the exact reason is still unknown and warrants further investigation.

To summarize, the clinical relevance of autoantibodies in EB is disputable, especially those detected by ELISA or immunoblot because in the majority of patients, no in vivo binding of antibodies could be shown. Furthermore, because more than half of our cohort had a positive serological test without apparent clinical meaning, an exclusion for the therapy trials for EB based on ELISA causes a risk of omitting possible candidates. We suggest DIF combined with IIF on SSS because these methods have a better diagnostic and prognostic value. However, in EB, the clinical significance of reactivity even in DIF and/or IIF on SSS remains uncertain.

Data availability statement
No datasets were generated or analyzed during this study.

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CONFLICT OF INTEREST

The authors state no conflict of interest.

AUTHOR CONTRIBUTIONS

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SUPPLEMENTARY MATERIAL

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Supplementary Table S1. Included patients with age at the time of serum and biopsy sampling, and identification of revertant mosaicism

| Patient | | | | | | Age | | Revertant |
NR	EB type	Database NR	Mutation (DNA)	Mutation (Protein)	Protein Expression in Nonblistered Skin	Serum Sampling	Biopsy Sampling	Mosaicism Identified
COL17A1 Patients								
1	JEB-intermed	EB02501	*COL17A1*: c.2237delG / c.2237delG	p.Gly746Alafs*53/p.Gly746Alafs*53	Negative (type XVII collagen)	61	59	Yes
2	JEB-intermed	EB02601	*COL17A1*: c.3676C>T/c.1601delA	p.Arg1226*/p.Asp534Alafs*19	Negative (type XVII collagen)	46	44	Yes
3	JEB-intermed	EB03502	*COL17A1*: c.2237delG/c.3676C>T	p.Gly746Alafs*53/p.Arg1226*	Negative (type XVII collagen)	55	48	Yes
4	JEB-intermed	EB13401	*COL17A1*: c.1365delC/ c.3600_3601delCT *COL17A1*: c.1260delC/ c.3495_c.3496delCT	p.Thr421Leufs*72/p.Ser1166Leufs*6	Negative (type XVII collagen)	11	8	Yes
5	JEB-intermed	EB20801	*COL17A1*: c.2237delG/c.2237delG	p.Gly746Alafs*53/p.Gly746Alafs*53	Negative (type XVII collagen)	52	47	Yes
6	JEB-intermed	EB08601	*COL17A1*: c.1772-2 A>C/c.3327delT	in-frame exon skipping/p.Pro1110Argfs*21	Negative (type XVII collagen)	14	0	No
7	JEB-loc	EB16801	*COL17A1*: c.4320delT/c.4320delT	p.Gln1442Lysfs*70/p.Gln1442Lysfs*70	Strongly reduced (type XVII collagen)	46	37	No
8	JEB-loc	EB09801	*COL17A1*: c.2251C>T/c.3327delT	p.Gln751*/p.Pro1110Argfs*21	Strongly reduced (type XVII collagen)	47	34	No
LAMB3 patients								
9	JEB-intermed	EB02901	*LAMB3*: c.628G>A/c.628G>A	p.Glu210Lys/p.Glu210Lys	Strongly reduced (lam332)	71	71	Yes
10	JEB-intermed	EB29901	*LAMB3*: c.1106dup/c.1106dup	p.Ala370Serfs*13/p.Ala370Serfs*13	Normal (lam332)	61	57	No
11	JEB- intermed	EB13201	*LAMB3*: c.1063T>C/c.1903C>T	p.Cys355Arg/p.Arg635*	Strongly reduced (lam332)	38	39	No
12	JEB-loc	EB25401	*LAMB3*: c.628G>A/c.1903C>T	p.Glu210Lys/p.Arg635*	Strongly reduced (lam332)	0	0	No
COL7A1 Patients								
13	RDEB-sev	EB26501 (P116)	*COL7A1*: c.887delG/c.6527dup	p.Gly296Valfs*5/p.Gly2177Trpfs*113	Strongly reduced (type XII collagen)	26	25	Yes
14	RDEB-sev	EB29701 (P108)	*COL7A1*: c.2142A>G/c.6527dup	Altered splicing resulting in out-of-frame transcript p.Gly2177Trpfs*113	Negative (type VII collagen)	25	25	Yes
15	RDEB-sev	EB02201	*COL7A1*: c.3G>T/c.4997dup	Start-lost/p.Pro1668fAlafs*4	Negative (type VII collagen)	21	17	No
16	RDEB-sev	EB07701	*COL7A1*: c.925_944dup/c.1264dup	p.Ile315Metfs*12/p.Arg422Profs*19	Negative (type VII collagen)	35	20	No
17	RDEB-sev	EB09001	*COL7A1*: c.4767delA/c.4767delA	p.Asp1590Thrfs*120/p.Asp1590Thrfs*120	Negative (type VII collagen)	16	2	No
18	RDEB-sev	P10	*COL7A1*: c.6527dup/c.6527dup	p.Gly2177Trpfs*113/p.Gly2177Trpfs*113	Strongly reduced (type VII collagen)	9	Not tested	No
19	RDEB-sev	P31	*COL7A1*: c.6527dup/c.6527dup	p.Gly2177Trpfs*113/p.Gly2177Trpfs*113	Strongly reduced (type VII collagen)	21	20	No
20	RDEB-sev	P46	*COL7A1*: c.325_326insCG/c.3277-1G>C	p.Glu109Alafs*39/Altered splicing resulting in out-of-frame transcripts	Strongly reduced (type VII collagen)	48	48	No
21	RDEB-sev	P52	*COL7A1*: c.7756dup/c.7930-1G>C	p.Gln2586Profs*12/In-frame exon skipping	Negative (type VII collagen)	50	45	No
22	RDEB-sev	P104	*COL7A1*: c.6618+1G>A/c.6618+1G>A	p.K2206_G2207insMSL_E220Gfs*86/ p.K2206_ G2207insMSL_E220Gfs*86 Altered splicing resulting in out-of-frame transcripts/Altered splicing resulting in out-of-frame transcripts	Strongly reduced (type VII collagen)	28	28	No
23	RDEB-sev	P14	*COL7A1*: c.6527dup/c.336C>G	p.Gly2177Trpfs*113/p.Tyr112*	Negative (type VII collagen)	7	7	No
24	RDEB-sev	P18	*COL7A1*: c.6527dup/c.2984dup	p.Gly2177Trpfs*113/p.Gly996Trpfs*44	Negative (type VII collagen)	13	Not tested	No

(*continued*)

| Patient | | | | | | Age | | Revertant |
NR	EB type	Database NR	Mutation (DNA)	Mutation (Protein)	Protein Expression in Nonblistered Skin	Serum Sampling	Biopsy Sampling	Mosaicism Identified
25	RDEB-sev	P61	*COL7A1*: c.6527dup/c.6527dup	p.Gly2177Trpfs*113/p.Gly2177Trpfs*113	Strongly reduced (type VII collagen)	6	0	No
26	RDEB-sev	P104.I	*COL7A1*: c.6618+1G>A/c.6618+1G>A	p.K2206_G2207insMSL_E220Gfs*86/ p.K2206_ G2207insMSL_E220Gfs*86 Altered splicing resulting in out-of-frame transcripts/Altered splicing resulting in out-of-frame transcripts?	Strongly reduced (type VII collagen)	35	32	No
27	RDEB-sev	P78	*COL7A1*: c.6527dup/ c.5130_5131insCTCAC	p.Gly2177Trpfs*113/p.Thr1711Leufs*132	Negative (type VII collagen)	5	0	No
28	RDEB-sev	EB26001 (P17)	*COL7A1*: c.6527dup/c.6527dup	p.Gly2177Trpfs*113/p.Gly2177Trpfs*113	Strongly reduced (type VII collagen)	44	43	Yes
29	RDEB-sev	EB02401	*COL7A1*: c.6508C>T/c.6508C>T	p.Gln2170*/p.Gln2170*	Negative (type VII collagen)	25	27	Yes
30	RDEB-sev	EB06401	*COL7A1*: c.1573C>T/c.6508C>T	p.Arg525*/p.Gln2170*	Negative (type VII collagen)	29	27	No
31	RDEB-intermed	EB09001	*COL7A1*: c.2699G>A/c.7237G>A	p.Trp900*/p.Gly2413Arg	Minimally reduced (type VII collagen)	30	26	No
32	RDEB-intermed	EB34701	*COL7A1*: c.5272G>A/(unknown)	p.Gly1758Arg/unknown	Minimally reduced (type VII collagen)	43	43	No
33	RDEB-intermed	P36	*COL7A1*: c.6527dup/c.7300G>A	p.Gly2177Trpfs*113/p.Gly2434Arg	Reduced (type VII collagen)	14	8	No
34	RDEB-intermed	P204	*COL7A1*: c.2722_2723delCA/c.5188 C>T	p.Gln908Valfs*45/p.Arg1730*	Reduced (type VII collagen)	22	21	No
35	RDEB-intermed	P120	*COL7A1*: c.5576_5577delAA/ c.4012G>A	p.Lys1858Argfs*12/p.Gly1338Arg	Strongly reduced (type VII collagen)	4	0	No
36	RDEB-inversa	EB04701	*COL7A1*: c.8083G>A/c.8083G>A	p.Gly2695Ser/p.Gly2695Ser	Minimally reduced (type VII collagen)	27	9	No
37	DDEB	P50	*COL7A1*: c.6182G>T/c.=	p.Gly2061Val/p.=	Reduced (type VII collagen)	17	17	No

Abbreviations: DDEB, dominant dystrophic epidermolysis bullosa; DEB, dystrophic epidermolysis bullosa; EB, epidermolysis bullosa; JEB, junctional epidermolysis bullosa; JEB-intermed, junctional epidermolysis bullosa intermediate; JEB-loc, junctional epidermolysis bullosa localized; NR, number; RDEB-intermed, recessive dystrophic epidermolysis bullosa intermediate; RDEB-inversa, recessive dystrophic epidermolysis bullosa inversa; RDEB-sev, recessive dystrophic epidermolysis bullosa severe.

The age at serum and biopsy sampling is indicated per patient with EB. In 10 patients, revertant mosaicism was identified (6 JEB and 4 DEB). In patients in which revertant mosaicism was not identified, it cannot be completely excluded that these patients may have a revertant area that has remained unnoticed until now.

Supplementary Table 2. The Age at Serum Sampling Per Patient with Burn Wounds

Burn Wound Patient NR	Percentage of Body Surface Affected	Age Serum Sampling
38	65	70
39	80	unknown
40	24	67
41	Unknown	20
42	80	76
43	Unknown	unknown
44	50	38
45	62	6
46	70	41
47	90	45
48	90	53
49	90	86
50	95	46

Abbreviation: NR, number.

In addition, the percentage of affected skin area is indicated.