Systemic Collagen VII Replacement Therapy for Advanced Recessive Dystrophic Epidermolysis Bullosa

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Recessive dystrophic epidermolysis bullosa (RDEB) is a genetic skin blistering disease associated with progressive multiorgan fibrosis. RDEB is caused by biallelic mutations in COL7A1 encoding the extracellular matrix protein collagen VII (C7), which is necessary for epidermal–dermal adherence. C7 is not simply a structural protein but also has multiple functions, including the regulation of TGFβ bioavailability and the inhibition of skin scarring. Intravenous (IV) administration of recombinant C7 (rC7) rescues C7-deficient mice from neonatal lethality. However, the effect on established RDEB has not been determined. In this study, we used small and large adult RDEB animal models to investigate the disease-modulating abilities of IV rC7 on established RDEB. In adult RDEB mice, rC7 accumulated at the basement membrane zone in multiple organs after a single infusion. Fortnightly IV injections of rC7 for 7 weeks in adult RDEB mice reduced fibrosis of skin and eye. The fibrosis-delaying effect was associated with a reduction of TGFβ signaling. IV rC7 in adult RDEB dogs incorporated in the dermal–epidermal junction of skin and improved disease by promoting wound healing and reducing dermal–epidermal separation. In both species, IV C7 was well-tolerated. These preclinical studies suggest that repeated IV administration of rC7 is an option for systemic treatment of established adult RDEB.

INTRODUCTION

Recessive dystrophic epidermolysis bullosa (RDEB) is a genetic skin blistering disease caused by biallelic COL7A1 mutations (Bardhan et al., 2020). The gene COL7A1 encodes collagen VII (C7), which is an extracellular matrix (ECM) protein deposited at the dermal–epidermal junction (DEJ) in the skin (Chung and Uitto, 2010). C7 assembles into anchoring fibrils that provide vital support for epidermal–dermal cohesion by firmly anchoring the epidermal basement membrane to ECM macromolecules in the papillary dermis (Chung and Uitto, 2010). In addition to the skin, C7 is present in other epithelia, where it is also essential for stabilizing the basement membrane zone. Consequently, fragility and blistering of extracutaneous organs, including the eye, the oral mucosa, and the esophagus, occur (Fine and Mellerio, 2009).

Although first identified as an anchor protein, C7 also participates in other biological processes, including cell–ECM instruction, the release of ECM-modifying enzymes, and the regulation of GF bioavailability (Akasaka et al., 2021; Arbiser et al., 1998; Atanasova et al., 2019; Küttner et al., 2014; Martins et al., 2015; Nyström et al., 2013; Wang et al., 2013; Watt et al., 2015). To a large extent, the exact mechanistic nature of its involvement in these events remains unknown. However, more diffuse ECM tissue fragility, scarring, and fibrosis in RDEB partially reflect the importance of nonanchoring functions of C7 (Nyström and Bruckner-Tuderman, 2018).

Progressive multiorgan fibrosis is a major complication of RDEB: not only does it reduce tissue functionality, but it also establishes a tissue microenvironment that supports the progression of high-risk carcinomas (Guerra et al., 2017). Evidence points to fibrosis in RDEB being driven by injury-evoked inflammation and facilitated by dysregulation of GF bioavailability, with TGFβs playing a prominent role (Breitenbach et al., 2015; Nyström and Bruckner-Tuderman, 2018).

There are several topical therapies in clinical trials with the intention to treat RDEB (Bardhan et al., 2020). These topical therapies can provide a substantial improvement of QOL by efficiently treating, for example, chronic wounds or frequently injured sites (Marinkovich and Tang, 2019). Nevertheless, because RDEB affects multiple organs, systemic therapies are needed. Toward this end, cellular therapies have provided some transient systemic symptom-relief in patients; it appears that the effect is from both the reduction of inflammation and de novo C7 production (Ebens et al., 2019; Rashidghamat et al., 2020). Challenges remain with
Progression of disease in RDEB is associated with changes in the tissue microenvironment, including stiffening, which can hamper the efficacy and sustainability of potential therapies (Nyström et al., 2016). The progression of RDEB is rapid, so most prospective therapy recipients would, to a certain degree, have somewhat advanced disease (Breitenbach et al., 2015). The potential benefit of C7 in advanced RDEB remains unknown. Because C7 is associated with regulation of the bioavailability of factors involved in inflammation and scarring, replenishment of C7 could by itself reduce these activities and establish a tissue microenvironment that is less prone to developing fibrosis. rC7 protein-replacement therapy would require tolerability of systemic rC7 administration at regular intervals, which has so far not been assessed in vivo in preclinical RDEB models. Toward these ends, we used adult C7 hypomorphic (RDEB) mice and adult Golden Retriever dogs with RDEB, as small and large animal models, respectively, to investigate the disease-modulating abilities of IV rC7 on established RDEB. Our studies show that repeated rC7 injections are well-tolerated. Even in extensively damaged and fibrotic skin, oral mucosa, and eye, systemically administered rC7 will deposit within the basement membrane zone. Importantly, although blister reduction was observed, the major benefit of repeated rC7 treatment in advanced RDEB was by limiting the progression of fibrosis. Thus, this study provides a rationale for using rC7 for patients with not just newly diagnosed RDEB as shown by Hou et al. (2015) but also in those with advanced RDEB.

RESULTS
Systemically delivered rC7 is dose-dependently deposited at the DEJs in the skin and wounds of adult RDEB mice
In skin and other epithelia affected by C7 loss, progressive fibrotic changes occur with age in RDEB. Such changes could alter C7 diffusion through the tissue, affecting the efficacy and localization of its deposition. Therefore, we first analyzed the deposition of systemically administered C7 in the skin of adult RDEB mice. Two rC7 doses—1.5 and 15 mg/kg body weight—were tested (Figure 1a); 15 mg/kg was selected because it was the highest feasible injectable dose. Because injury promotes extravasation of proteins from blood (Nagy et al., 2008), because human RDEB is associated with a high wound burden, and because the production of C7 interaction partners (such as laminin-332 and collagen IV) during tissue repair may facilitate C7 retention, we investigated the deposition of IV administered rC7 in unwounded skin and healing wounds in the adult RDEB mice (Figure 1a). Mice were injected with rC7 the day after wounding, and the wounds were allowed to heal for an additional 6 days. On day 7 after wounding, another dose of rC7 was given 30 minutes before the wounds, and skin was harvested. The presence of human rC7 in the harvested tissues was assessed by staining with the mouse monoclonal (LH7.2) and the rabbit polyclonal (pAb LH7.2) antibodies recognizing human but not murine C7 (Bornert et al., 2019; Kühl et al., 2015).

Linear deposition of human C7 was seen below the epidermis of reepithelialized wounds. This was achieved at both doses: 1.5 and 15 mg rC7/kg body weight (Figure 1b). However, the 15 mg/kg dose of rC7 yielded significantly stronger staining, indicating higher deposition, and was therefore used in all subsequent experiments. Compared with rC7 accumulation in the unwounded skin, rC7 accumulation was notably increased at the wound periphery. The deposition of rC7 from one IV administration was broad in this location (Figure 1c), and rC7 was primarily present in the DEJ under the unwounded epidermis but minimally present in the wound under the regenerating epidermis distal to the original wound border (Figure 1c). Weak but specific deposition of human rC7 at the DEJ was observed in unwounded skin (Figure 1d). In unwounded skin, blood vessels stained strongest for rC7, indicating the presence of rC7 within the circulation when the IV rC7 dose was given 30 minutes before harvesting the tissue. In addition, some granular deposits of rC7 were seen in the upper dermis. Collectively, these data indicate that wound-induced changes in injured and surrounding intact tissue facilitate rC7 deposition and that once deposited at the DEJ, the mobility of rC7 is low.

Long-term, fortnightly systemic injection of rC7 allows C7 replacement in multiple organs and reduces spontaneous blistering in adult RDEB mice
After determining that rC7 can be delivered by systemic administration to the skin of adult RDEB mice, we assessed its long-term potential in reducing the manifestations associated with advanced RDEB. Toward this end, adult RDEB mice were given fortnightly IV injections of 15 mg rC7/kg body-weight (Figure 2a) or PBS. RDEB mice were treated when they showed prominent signs of development of fibrotic mitten deformities of forepaws. Repeated systemic delivery of rC7 at this high dose was well-tolerated in the adult mice. Compared with the PBS-treated RDEB mice, no significant difference in body weight over the course of the treatment was seen (Supplementary Figure S1). All PBS- and rC7-treated RDEB mice remained active and survived the planned observation period of 7 weeks. Analysis of rC7 in back and forepaw skin after 7 weeks of treatment showed deposition at the DEJ (Figure 2b and c). Consistent with the previous observation, rC7 abundance was increased in areas where macroscopic wounding had been observed (Figure 2c). We also analyzed rC7 in the tongue and eye—two other organs affected by tissue fragility in RDEB. In both tongue and eye, linear deposition of rC7 below the epithelium was seen (Figure 2d and Supplementary Figure S2a).
Next, we investigated whether rC7 deposition promoted tissue stabilization. Fur and adnexa protect skin and other epithelia in adult RDEB mice from blisters and erosions. In such organs, histologically detectable microseparations of the epithelia from the underlying stroma occur. To study the potential improvement of epithelial–stromal cohesion, we performed histological analyses of back skin, forepaw, and tongue; these disclosed no general, obvious improvement on the microscopic level of epithelial–stromal attachment in rC7-treated RDEB mice (Supplementary Figure S3). In RDEB mice, conspicuous blisters form in the anal region; notably, repeated systemic rC7 injections effectively protected adult RDEB mice from spontaneous anal blistering (Figure 2e and f).

These observations indicate that also in advanced RDEB, replenishment of C7 at the DEJ and other epithelial basement membrane zones can be achieved by systemic administration of rC7 and repeated delivery is feasible. rC7 may also functionally improve epithelial–stromal attachment in adult mice with advanced RDEB. However, the benefit and response appear tissue dependent and are likely governed by the level of pre-existing damage and changes in the stromal ECM.

Long-term, fortnightly systemic administration of rC7 slows fibrosis in adult RDEB mice

The progressive fibrotic remodeling of the injured microenvironment that occurs with advanced disease in RDEB is severely debilitating. In an already stiffened and less compliant tissue, the efficacy and benefit of curative therapies may be reduced. Intriguingly, C7 has been suggested to have fibrosis-limiting properties (Akasaka et al., 2021; Ng et al., 2012; Nystro¨m et al., 2013; Wang et al., 2013). Thus, rC7 administration could potentially reduce the activity of fibrotic processes in RDEB skin. Toward this end, we followed the formation of mitten deformities in RDEB mice. Seven weeks after the first injection, RDEB mice that had received rC7 displayed significantly attenuated progression of fibrosis-driven mitten deformities compared with PBS-treated...
mice, as assessed by preservation of toe length (Figure 3a and b).

Molecular analysis of proteins associated with fibrotic changes supported the macroscopic observation. Significantly reduced deposition of fibrosis-associated tenascin C was seen in the paws of RDEB mice that had received rC7 for 7 weeks (Figure 3c and d). Western blot analysis confirmed that this change was indeed due to lower tissue abundance of tenascin C (Figure 3e and f). It also confirmed the presence of full-length rC7 in the tissue (Figure 3e). Importantly, rC7 administration significantly reduced TGFβ activity in the forepaw skin of RDEB mice with advanced disease, as evidenced by significantly lower phosphorylated SMAD2/3 levels (Figure 3e and f). Accordingly, it also reduced the levels of the TGFβ-induced myofibroblast marker α-smooth muscle actin (Hinz and Lagares, 2020) in the forepaws (Figure 3e and f). In contrast, rC7 had no obvious effect on tissue inflammation as assessed by the number of myeloid cells and T cells and the level of tissue-bound IgG in the paws (Supplementary Figure S4a). This indicates that the tissue-protective mechanism was independent of a reduction in general tissue inflammation. Analysis of the eye in which fibrotic changes occur in adult RDEB mice (Chen et al., 2018) disclosed that rC7 also evoked a fibrosis-delaying response in extracutaneous organs (Supplementary Figure S2b). Thus, fibrosis-limiting activities could be a major benefit of systemic rC7 protein-replacement therapy.

**Systemically delivered rC7 is deposited at the DEJs in skin and wounds and reduces spontaneous blistering in an adult RDEB dog model**

To provide more support of the feasibility and effectiveness of treating advanced RDEB with rC7, we evaluated an rC7 IV infusion in a large animal model with spontaneous RDEB. For this purpose, we treated Golden Retriever dogs with mild RDEB caused by G1906S substitution in the collagenous P2 domain close to the hinge region of C7 (Gache et al., 2011; Palazzi et al., 2000). Dogs homozygous for the G1906S mutation are notably smaller. Similar to the skin of RDEB mice, fur protects the skin of the RDEB dogs from injury and blistering. Therefore, scarring in these dogs is restricted to the oral mucosa, ears, and paws (Figure 4a). Two adult RDEB dogs were infused once with 1 mg/kg body weight rC7 (in total, 23 mg rC7 in one dog and 28 mg rC7 in the second dog), which was the maximal feasible dose. Before the infusion, selected skin areas were rubbed to induce wounds. Skin biopsies were collected 1 or 4 weeks after infusion. To assess for changes in tissue stabilization, some biopsies were taken from the skin that had been rubbed in one direction with an eraser with force multiple times to induce epidermal–dermal separation in untreated RDEB dogs (Figure 4b). Histological analysis revealed that the rC7 infusions stabilized epidermal–dermal attachment and protected from friction-induced blistering 1 and 4 weeks after injection (Figure 4b). To detect deposition of rC7, we screened monoclonal C7 antibodies for reactivity of human but not...
The mouse mAb NP185 strongly stained C7 at the DEJ in healthy human skin but not in canine skin (Figure 4c). Thus NP185 staining could be used to specifically detect rC7 in canine skin. Our analyses showed that rC7 was present at the DEJs 1 and 4 weeks after injection (Figure 4d and e). In accordance with the data from the RDEB mouse, deposition of rC7 at the DEJs was enhanced in wounded tissue (Figure 4d and e).

Finally, we assessed the clinical effect of rC7 infusions on the naturally occurring RDEB blisters and wounds in these dogs. Toward this end, we followed wounds in the oral mucosa. Notably, rC7 promoted sustained healing of oral wounds (Figure 4f); the wounds remained closed 7 weeks after the rC7 infusion (Figure 4f). Collectively, these data suggest that IV rC7, through its deposition at the DEJs, promotes tissue stabilization in adult dogs with RDEB.

**DISCUSSION**

In this study, we show that systemic rC7 in animals with advanced RDEB reduces the burden of fibrosis while at the same time displays therapeutic activity such as reduced new blister formation and durable closure of skin wounds. IV rC7 was effective in treating small and large animal models of advanced RDEB, which should encourage and facilitate translation into clinical testing because repeated rC7 IV infusions may provide meaningful systemic benefits in teenagers and adults with RDEB.

Our studies disclosed two tissue-preservative actions of rC7: in some recipients and tissues, it stabilized epidermal–dermal cohesion and reduced activities linked to fibrosis. The latter finding is intriguing and could suggest that longer treatment with rC7 may yield dermal stabilization and restoration of skin compliance in RDEB skin and tissue with
established fibrosis. Owing to ethical reasons, we were unfortunately not able to follow RDEB mice treated with rC7 for a longer period than 7 weeks.

Injury- and inflammation-induced remodeling of the dermal ECM negatively affects the efficacy of therapies for RDEB (Nystrom and Bruckner-Tuderman, 2016). This is illustrated by data showing that rC7 improved epidermal–dermal cohesion in dogs with milder disease and low fibrosis, whereas rC7 yielded no clear improvement of this in RDEB mice with severe, advanced disease and high level of fibrosis.

The changes of the papillary dermal matrix that occurs during RDEB progression likely render the dermis less elastic and more breakable. Alterations of the collagen matrix could impede the ability of anchoring fibrils to properly interact with collagen fibrils. In addition, key C7 interaction partners, laminin-332 and collagen type IV, are reduced in the epidermal basement membrane of RDEB skin (Küttner et al., 2013; Thriene et al., 2018). Furthermore, meprins, which mature pro-C7—enabling stable anchoring fibril formation, are reduced in advanced RDEB skin (Kruppa et al., 2021).
Collectively, these deficiencies would reduce the ability of rC7 to improve epidermal–dermal cohesion in advanced RDEB skin.

The efficacy of rC7 to establish epidermal–dermal attachment could be increased with time of treatment because rC7 also limits fibrotic activities of RDEB skin. Maintenance of healthy, functional skin is dependent on replacement of its proteome at regular intervals (Nauroy and Nystro¨m, 2019; Zigrino et al., 2016). The length of these intervals differs between the different compartments of the skin; the murine and human epidermis has a turnover of around 28 days (Epstein and Maibach, 1965; Hoath and Leahy, 2003); the DEJ appears to have a half-life of ~1 month (Kühl et al., 2016). The renewal rate of fibroblasts is low in homeostatic adult dermis (Rognoni et al., 2018) and that of the dermal ECM is component dependent, but for more inert proteins such as elastins or fibrillar collagens, it is nonexistent or extremely extended; in human dermis, the half-life of the collagen matrix can be estimated to 15 years (Verzijl et al., 2000). Inflammation and injury boost degradation and synthesis, which change the turnover rate, but restoration of dermis after transiently induced fibrosis still takes many months in mice (Zigrino et al., 2016). Thus, the pre-existing ECM destabilization and remodeling in the skin with advanced RDEB may take a long time to reverse. This is indeed what we observed in this study; over 7 weeks, repeated systemic rC7 provided little stabilization of epidermal–dermal cohesion. Given that rC7 slows down the profibrotic activities in RDEB skin and improves wound healing (Wang et al., 2013), it may create a dermal microenvironment that over time would promote the restoration of the dermal ECM. Consequently, the benefit of rC7 on promoting epidermal–dermal cohesion could be expected to improve with longer durations of treatment.

rC7 appeared to have little influence on skin inflammation. Our analyses of inflammation were limited to the number of immune cells and tissue-bound antibodies. It is possible that rC7 could confer more subtle inflammation-modulating activities and affect immune cell lineage composition, their phenotype, or their activity. Nevertheless, our data would indicate that the absence or presence of C7 is not by itself sufficient to limit general tissue inflammation. Interestingly, rC7 reduced TGFβ signaling and deposition of tenascin C, which is associated with dermal fibrosis (Bhattacharyya et al., 2016). These observations support the previously noted profibrotic activity-limiting properties of C7 (Akasaka et al., 2021; Martins et al., 2015; Ng et al., 2012; Nystro¨m et al., 2013; Wang et al., 2013). Importantly, our study discloses an in vivo support of the nonstructural functions of C7 that are key to maintaining tissue homeostasis.

To conclude, our study provides evidence in small and large adult RDEB animal models that repeated rC7 IV infusions could provide systemic therapeutic benefit for advanced RDEB.

MATERIALS AND METHODS

Additional descriptions of materials and methods are found in the Supplementary Materials and Methods.

Ethics statement

Experiments with the C7 hypomorphic (RDEB) mouse were approved by the regional ethics review board (Regierungspra¨sidium Freiburg) (approval number G11/70). Experiments on RDEB dogs were approved by the Animal Care and Use Committee of VetAgro Sup Campus Vétérinaire de Lyon (Marcy-l’Étoile, France) (approval number 1848).

Production and purification of rC7

rC7 was purified from RDEB fibroblasts stably transduced with a lentiviral vector coding for full-length C7 as previously described (Woodley et al., 2004). The purified rC7 used for injections was formulated in PBS with 0.5 mM EDTA. rC7 was provided by Lotus Tissue Repair (currently Phoenix Tissue Repair, Boston, MA).

Mouse studies

The C7 hypomorphic (RDEB) mouse model has previously been described (Fritsch et al., 2008). RDEB mice were on a mixed 129s/C57 BL/6 background, which increases the survival of the mice while mitten deformation of forepaws still occurs (Nystro¨m et al., 2015).

To test rC7 distribution and dosing in RDEB mice aged 4 weeks, mice carrying 1-day-old wounds were injected by the tail vein with two doses of rC7, that is, 1.5 mg/kg body weight or 15 mg/kg body weight. Six days after the first injection, mice were given a second injection with 1.5 mg/kg body weight or 15 mg/kg body weight rC7. Skin and wounds were harvested 30 minutes after the second injection and analyzed for rC7.

On first signs of mitten deformities, mice were injected through the tail vein with 15 mg/kg body weight rC7 or vehicle (PBS + 0.5 mM EDTA). At the start of treatment, the mice in the PBS group were on average aged 32 ± 7 days, and those in the rC7 group were on average aged 35 ± 8 days. The injection was repeated every 2 weeks for a total number of four injections. Mice were photographed weekly. Seven weeks after the first injection, paws, skin, eyes, and tongues were collected and analyzed for rC7 and fibrosis. In total, six mice received rC7, and eight mice received PBS.

Toe length was measured with ImageJ software (National Institutes of Health, Bethesda, MD) from photos. The two most prominent toes on the mouse forepaw were measured. Toe length was expressed as a percentage of the length of the toe length at the start of treatment.

Anal blisters were scored from photos taken at the end of the treatment. The scoring was performed by providing photos of PBS- and rC7-treated mice to a blinded observer.

Dog studies

Before the start of the study, to record baseline data, punch biopsies of the skin of the trunk, pinnae, and oral mucosa for immunostaining and histopathology were collected under general anesthesia. In addition, photos of the lesions of the oral cavity and ears were taken. At the same time, wounding was induced in both dogs by deep skin rubbing of the left lip, the dorsal muzzle, and the back. Next, the dogs were infused with rC7 (rC7 was in a concentration of 0.46 mg/ml; 2.1 ml rC7/kg body weight was injected for a total dose of 23 or 28 mg per dog); this did not require general anesthesia, and the dogs were infused in the front leg using a catheter. Vital signs (blood pressure, pulse, and others) were monitored. Skin biopsies from wounded and unwounded sites for immunostaining and histopathology were taken 1 and 4 weeks after infusion as well as photos of the oral cavity and ears under general anesthesia.
Statistical analyses
The GraphPad Prism 9 (GraphPad Software, San Diego, CA) was used for statistical testing. The datasets were tested for normality and variance using Shapiro–Wilk and F tests. For analysis of statistical significance, Fisher’s exact test and unpaired or paired two-tailed Student’s t-tests were used as stated in the figure legends. P < 0.05 was considered statistically significant.

Data availability statement
No large datasets were generated or analyzed in the study.

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CONFLICT OF INTEREST
DTW holds patents on type VII collagen with the University of Southern California and is part owner of Phoenix Tissue Repair. MPdS is a paid consultant and shareholder in Phoenix Tissue Repair. AN is an advisor and consultant for Phoenix Tissue repair. The remaining authors state no conflict of interest.

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AUTHOR CONTRIBUTIONS
Conceptualization: MC, DTW, MPdS, LBT, AN; Formal Analysis: CG, DP, MC, DTW, LBT, MPdS, AN; Investigation: CG, DP, AN; Methodology: MC, DTW, DP, JSK, AN; Supervision: AN, DP; Writing - Original Draft Preparation: AN; Writing - Review and Editing: CG, DP, JSK, MC, DTW, LBT, MPdS, AN

SUPPLEMENTARY MATERIAL
Supplementary material is linked to the online version of the paper at www.jidonline.org, and at https://doi.org/10.1016/j.jid.2021.09.008.

REFERENCES


SUPPLEMENTAL MATERIALS AND METHODS

Western blotting
Protein lysates were prepared by boiling crushed tissue in 4× blue sample buffer (50 mM Tris-hydrochloride, pH 6.8, 2% SDS, 10% glycerol, 1% β-mercaptoethanol, 12.5 mM EDTA, and 0.02% bromophenol blue [all Sigma-Aldrich, St. Louis, MO]) with 8 M Urea as previously described (Kühl et al., 2016). Western blots were performed under standard conditions (Kühl et al., 2016). The blots were probed with rat anti-mouse tenascin C clone 578 from R&D Systems (Minneapolis, MN); rabbit anti-phosphorylated SMAD2 (Ser 465/467) (138D4) from Cell Signaling Technology (Danvers, MA); rabbit anti-phosphorylated SMAD3 (S423/425) (ab52903) from Abcam (Cambridge, United Kingdom); mouse anti-α-smooth muscle actin (clone 1A4) from Sigma-Aldrich; rabbit anti-human collagen VII NC1 domain (pAb LH7.2) (this antibody recognizes human but not murine C7 [Bornert et al., 2019; Kühl et al., 2013]); rabbit anti-β-tubulin (ab6046) from Abcam; and horseradish peroxidase–conjugated mouse anti-β-actin (collagen 4) (sc-47778) (Santa Cruz Biotechnology, Dallas, TX), horseradish peroxidase–conjugated goat anti-rabbit (catalog number 474-1506, Seracare Life sciences, Gaithersburg, MD), horseradish peroxidase–conjugated goat anti-rat (catalog number 112-035-167, Jackson ImmunoResearch, West Grove, PA), and horseradish peroxidase–conjugated goat anti-mouse IgG (catalog number 401253; Merck Millipore, Darmstadt, Germany).

Western blots were developed with ECL Prime (GE Healthcare, Chicago, IL), and images were captured with Amersham Imager 600 (GE Healthcare). For quantification, ImageJ software (National Institutes of Health, Bethesda, MD) was used.

Histology and immunostaining
Tissue was embedded in optimal cutting temperature medium (Sakura, Alphen aan den Rijn, Netherlands) after collection and was snap frozen on liquid nitrogen. Cryosections were analyzed by H&E staining using standard protocols or by immunostaining. For immunostaining, sections were fixed in ice-cold acetone, blocked in 10% normal goat serum or 4% bovine serum albumin (Sigma-Aldrich), and stained with primary antibodies. Primary antibodies used were rat anti-mouse tenascin C clone 578 from R&D Systems, rabbit anti–C7 (detecting both mouse and human C7) (reference 234192, Calbiochem; Merck Millipore), mouse anti-human C7 NC1 domain (clone LH7.2, Sigma-Aldrich) (this antibody recognizes human but not mouse C7), mouse anti-human C7 NC1 domain NP185 (MAB2501 from Merck Millipore) (this antibody recognizes human but not canine C7), and rabbit anti-human C7 NC1 (pAb LH7.2, this antibody recognizes human but not murine C7 [Bornert et al., 2019; Kühl et al., 2013]). For analysis of tissue inflammation, forepaw skin sections were stained with rat anti-mouse CD11b clone M1/70 from BD Biosciences (Heidelberg, Germany) and hamster anti-mouse CD3e clone 145-2C11 (BD Biosciences).

Secondary antibodies and detection reagents were Alexa Fluor 488 goat anti-rabbit IgG (reference A32731, Invitrogen, Carlsbad, CA), Alexa Fluor 594 goat anti-rat IgG (reference A11007, Invitrogen), Alexa Fluor 594 goat anti-mouse IgG (reference A32742, Invitrogen), FITC-conjugated goat anti-mouse IgG (Sigma-Aldrich), and Streptavidin Alexa Fluor 488 (S32354, Invitrogen). Nuclei were counterstained with DAPI (Thermo Fisher Scientific, Waltham, MA).

Sections were mounted in PermaFluor (Thermo Fisher Scientific) and analyzed with an Axioplan 2 fluorescence microscope (Zeiss, Jena, Germany). Images were captured using a monochrome AxioCam camera (Zeiss). For comparative analyses, images were captured in one session and using identical settings.

SUPPLEMENTARY REFERENCES
Supplementary Figure S2. Systemic rC7 is deposited in the eye of adult RDEB mice and limits fibrosis. (a) Staining of sections of eyes from PBS-treated or rC7-treated adult RDEB mice after 7 weeks of treatment. Corneas are shown. The eyes were stained with a rabbit polyclonal antibody detecting human but not mouse C7 (Kühl et al., 2015) (rC7, green). Nuclei were counterstained with DAPI (blue). Bar = 50 μm. Arrows indicate rC7 deposition at the corneal epithelial basement membrane. (b) Western blot analysis of eyes as in a for full-length human C7, fibrosis-associated markers tenascin C, and pSMAD2/3. β-Tubulin was used as the loading control. C7, collagen VII; pSMAD, phosphorylated SMAD; rC7, recombinant collagen VII; RDEB, recessive dystrophic epidermolysis bullosa.

Supplementary Figure S1. Bodyweight curve of PBS- or rC7-treated adult RDEB mice. Plotted are weekly body weights of adult RDEB mice during their 7-week treatment with PBS or rC7. The body weights are expressed as the percentage of the bodyweight at the start of treatment (day 0). Values represent mean ± SEM, n = 6 mice. C7, collagen VII; rC7, recombinant collagen VII; RDEB, recessive dystrophic epidermolysis bullosa.
Supplementary Figure S3. No evident stabilization of epithelial–stromal cohesion in adult RDEB mice after rC7 treatment. (a–c) H&E staining of sections of back skin, forepaws, and tongues from adult RDEB mice treated with PBS or rC7 for 7 weeks. Epithelial–stromal separation is visible for all samples. Bar = 100 μm. IV, intravenous; rC7, recombinant collagen VII; RDEB, recessive dystrophic epidermolysis bullosa.

Supplementary Figure S4. Limited effect of rC7 on general tissue inflammation in adult RDEB mouse forepaw skin. (a) Sections of forepaws from RDEB mice treated for 7 weeks with PBS or rC7 stained for CD11b (red), CD3e (green), and IgG (red) to detect myeloid cells, T cells (indicated with white arrows), and tissue-bound antibodies, respectively. Nuclei were counterstained with DAPI (blue). Bar = 100 μm. (b) Top: western blots of forepaws as in a for IgG and β-actin as the loading control. Bottom: graph showing the results of densitometric quantification of blot as above; the values are expressed as the percentage of abundance in forepaws from PBS-treated mice. The graph shows the mean values for each analyzed mouse (dots) and mean ± SEM. Data were tested with Student’s paired t-test. n.s., not significant; rC7, recombinant collagen VII; RDEB, recessive dystrophic epidermolysis bullosa.