Intradermal Injection of Bone Marrow Mesenchymal Stromal Cells Corrects Recessive Dystrophic Epidermolysis Bullosa in a Xenograft Model


TO THE EDITOR

Recessive dystrophic epidermolysis bullosa (RDEB) is caused by loss-of-function mutations in COL7A1 encoding type VII collagen (C7) that forms anchoring fibrils (AFs), structures essential for dermal-epidermal adherence (Uitto et al., 2017). Patients with RDEB suffer from skin and mucosal blistering and develop severe complications including invasive squamous cell carcinoma, resulting in a poor prognosis (Guerra et al., 2017). Different therapeutic strategies have been explored, including gene-, protein-, cell-based, and pharmacological therapies that have shown promising preclinical or transitory clinical benefits (Caplan, 1991). hBM-MSCs display properties that could potentially improve wound healing in RDEB: immunomodulation; anti-inflammatory, angiogenic, and antifibrotic properties; secretion of trophic factors; improvement of tissue repair; and the capacity to induce protein expression in the host tissues through a paracrine effect (Nuschke A, Qi et al., 2014).

Herein, we assessed the long-term capacity of hBM-MSCs to survive, produce, and deposit C7 at the dermal-epidermal junction (DEJ) after ID injection into human RDEB skin equivalents (SEs) transplanted onto immune-deficient nude mice that reproduce the skin defect observed in RDEB (Titeux et al., 2010). Human bone marrow—mesenchymal stromal cells (hBM-MSCs) form a heterogeneous cell population that can self-renew or differentiate into mesenchymal lineages (Baldini et al., 2017). We ID injected hBM-MSCs beneath the DEJ up to 6 months in healthy SEs and in hBM-MSC–injected RDEB SEs, whereas vehicle-injected RDEB SEs
showed no detectable C7 staining (Figure 1a). Semiquantitative analysis of C7 fluorescence signal showed that hBM-MSC injection induced a significant increase in C7 deposition (up to 30-40% of the level of healthy control SE) compared with vehicle-injected RDEB SE (Figure 1c), which is considered to be sufficient to prevent loss of dermal-epidermal adhesion (Fritsch et al., 2008). Similar results were observed after injection of hBM-MSCs from donor 5 in RDEB SEs (see Supplementary Figure S5 online).

Transmission electron microscopy showed the presence of AFs up to 6 months with typical loop-shaped structures inserted into the lamina densa in hBM-MSC-injected RDEB SEs and in healthy SEs, but no AF structures were found in vehicle-injected RDEB SEs (Figure 1b). Morphometric analyses showed a significant increase of AF number, especially at 2 months, a time when AF density was similar to healthy SE (95% of healthy control) (Figure 1d, and see Supplementary Figure S6 online). The fate of injected hBM-MSCs in the RDEB SEs was assessed by fluorescent in situ hybridization using probes specific for human X and Y chromosomes (see Supplementary Figure S7 online), because RDEB SEs were made from human female cells and were injected with hBM-MSCs arising from a male donor.

Figure 1. In vivo rescue of C7 expression and AF formation in RDEB SEs injected with hBM-MSCs. (a) Immunofluorescence staining of human C7 in noninjected healthy SEs (6 months after grafting); vehicle-injected RDEB SEs (1 month after injection); and RDEB SEs at 1, 2, 4, and 6 months after hBM-MSCs ID injection. hBM-MSC-injected RDEB SE showed re-expression of C7 at the dermal-epidermal junction compared with vehicle-injected RDEB SE. Staining of the stratum corneum with the C7 antibody is nonspecific. Nuclei are stained blue by DAPI. Scale bar = 20 µm. (b) Ultrastructural analysis of grafted SE by TEM. Pictures display the presence of AF showing typical loop-shaped structures inserted into lamina densa in hBM-MSC-injected RDEB SE up to 6 months. From 2 to 6 months, the lamina densa is continuous, and AFs are structurally similar to healthy SE. AFs are indicated by red arrowheads. Scale bar = 200 nm. (c) Mean average of fluorescence intensity of human C7 protein production over 3 × 5-µm areas in each sample ± standard error of the mean. (d) Mean average of number of AFs ≥ 4 in areas of 10 µm of the basement membrane in each sample ± standard error of the mean. Nonparametric Mann-Whitney test. *P < 0.05, **P < 0.01. n ≥ 3 mice for each time point. AF, anchoring fibril; C7, collagen type VII; hBM-MSC, human bone marrow mesenchymal stromal cell; ID, intradermal; ns, not significant; RDEB, recessive dystrophic epidermolysis bullosa; SE, skin equivalent; TEM, transmission electron microscopy.
human male donor. Fluorescent in situ hybridization analysis showed the presence of human X-Y chromosome—positive cells in the dermis at 1, 2, and 4 months after injection. No X-Y chromosome—positive cells were found in the dermis of RDEB SE 6 months after injection (Figure 2a). Therefore, injected hBM-MSCs were observed in the dermis of RDEB SEs up to 4 months after injection, but their number clearly diminished over time (Figure 2b). Immunofluorescence staining of the active cleaved caspase-3 showed that hBM-MSC—injected RDEB SEs displayed numerous apoptotic cells in the dermis from 2 to 4 months (Figure 2c), whereas no cells were detected in vehicle-injected RDEB SEs. The number of apoptotic cells expanded up to 4 months and decreased at 6 months, suggesting that most of the injected hBM-MSCs underwent apoptosis between 2 and 4 months after injection (Figure 2d).

Previous RDEB studies using hBM-MSCs showed restoration of C7 at the DEJ for at least 12 weeks, although C7 production decreased over time (Conget et al., 2010; Kuhl et al., 2015). We show a longer effect of hBM-MSCs on C7 production and AF formation (up to 6 months). This is likely due to the extended survival time of injected hBM-MSCs (up to 4 months) compared with the 28 days previously reported (Kuhl et al., 2015), attributable to the lack of immune rejection in our immune-deficient model.

Localized ID injections and the survival of injected hBM-MSCs may represent limitations to treating all symptoms of RDEB. However, ID injections could be repeated over time to sustain clinical benefit in RDEB patients. In addition, systemic delivery of hBM-MSCs could be envisaged, although the extent to which systemic delivery of hBM-MSCs allows engraftment of injected cells in the skin and restoration of C7 is not known (Webber et al., 2017). Therefore, if the treatment has to be repeated for a long period, the risk of allo-reactions to allogeneic hBM-MSCs through the semidirect allore cognition pathway (Alegre et al., 2016) may impair the efficacy of these approaches. An alternative would be to use gene-corrected autologous hBM-MSCs to improve persistence of injected cells and to allow repeated injections.

This study was conducted in accordance with ethical principles stated in the Declaration of Helsinki. Patient consent for experiments was not required, because the French law considers human tissue left over from surgery as discarded material. The mice experiments were performed in compliance with guidelines for animal experiments in France and were approved by the local animal research ethics committee.

Figure 2. Injected hBM-MSCs are detectable until 4 months in RDEB human SEs. (a) FISH analysis of female RDEB SEs at 1, 2, 4, and 6 months after injection of male healthy donor hBM-MSCs, and 1 month after vehicle injection. Because nude mice do not reject human cells, we could follow hBM-MSCs maintenance over time after a single ID injection. Fluorescent X (red) and Y (green) probes in situ hybridization showed detectable X and Y chromosome-positive hBM-MSCs (yellow arrowheads) in the dermis. Nuclei were DAPI stained and considered positive if they contained two nuclear fluorescent spots. The X-probe (red) is the internal positive control. Scale bar = 10 μm. (b) Mean average of positive cells for the active form of caspase 3 over 3 × 5-μm areas in each sample ± standard error of the mean. Nonparametric Mann-Whitney test, *P < 0.05. n ≥ 3 mice for each time point. FISH, fluorescent in situ hybridization; hBM-MSC, human bone marrow mesenchymal stromal cell; ID, intradermal; ns, not significant; RDEB, recessive dystrophic epidermolysis bullosa; SE, skin equivalent.

CONFLICT OF INTEREST
The authors state no conflict of interest.

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Purinergic Molecules in the Epidermis


TO THE EDITOR

In the epidermis, adenosine triphosphate (ATP) is released from keratinocytes (kCs) by various environmental stimuli via nonlytic mechanisms, cell damage, or acute cell death (Burnstock et al., 2012). Because ATP is a potent inducer of skin inflammation, it has to be promptly hydrolyzed for the skin to achieve homeostasis (Mizumoto et al., 2003). All experiments that use human and murine materials were performed under institutional approval, and written informed consent was obtained from all subjects. Intradermal injection of ATP induced skin inflammation accompanied by significant neutrophil infiltration (see Supplementary Figure S1a and b online) (Takahashi et al., 2013). Five-week-old BALB/c mice were fed a zinc-deficient (ZD) or zinc-adequate (ZA) diet. As we previously showed (Kawamura et al., 2012), ATP release from the skin in response to an irritant (1% N,N’-Bis(acryloyl)cystamine) was significantly increased in ZD mice compared with ZA mice (see Supplementary Figure S1c). However, the amount of ATP contained in the epidermis was comparable between ZD and ZA mice (see Supplementary Figure S1d). This suggests that ATP hydrolysis is attenuated in ZD mice. In the epidermis, ENTPD-1 (CD39), which potently hydrolyzes ATP into AMP, is expressed in Langerhans cells (LCs) in both mice and humans but not in kCs (Georgiou et al., 2005; Ho et al., 2013; Mizumoto et al., 2002). CD39-expressing LCs disappeared from the epidermis of ZD mice (Kawamura et al., 2012). Therefore, the major...