*Cytotherapy*, 2010; 12: 429–431

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healthcare

**ORIGINAL ARTICLE**

# Replenishment of type VII collagen and re-epithelialization of chronically ulcerated skin after intradermal administration of allogeneic mesenchymal stromal cells in two patients with recessive dystrophic epidermolysis bullosa

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**Abstract**

In animal models it has been shown that mesenchymal stromal cells (MSC) contribute to skin regeneration and accelerate wound healing. We evaluated whether allogeneic MSC administration resulted in an improvement in the skin of two patients with recessive dystrophic epidermolysis bullosa (RDEB; OMIM 226600). Patients had absent type VII collagen immunohistofluorescence and since birth had suffered severe blistering and wounds that heal with scarring. Vehicle or $0.5 \times 10^6$ MSC were infused intradermally in intact and chronic ulcerated sites. One week after intervention, in MSC-treated skin type VII collagen was detected along the basement membrane zone and the dermal–epidermal junction was continuous. Re-epithelialization of chronic ulcerated skin was observed only near MSC administration sites. In both patients the observed clinical benefit lasted for 4 months. Thus intradermal administration of allogeneic MSC associates with type VII collagen replenishment at the dermal–epidermal junction, prevents blistering and improves wound healing in unconditioned patients with RDEB.

**Key Words:** *chronic wound healing, dystrophic epidermolysis bullosa, mesenchymal stromal cell*

Type VII collagen recessive dystrophic epidermolysis bullosa (RDEB; OMIM 226600) is a genodermatosis characterized by tense blisters and erosions that heal with extensive scarring (1). RDEB is caused by mutations in *COL7A1,* the gene coding for type VII collagen (2). Patients with RDEB present altered or no expression of type VII collagen, resulting in defective anchoring fibrils and dermal–epidermal separation (3). At present, there is no cure for RDEB. Therapeutic strategies under development are gene- (4), protein- (5) and cell-based (6). They have proven beneficial effects but still present complications that limit their clinical use: cancer risk inherent to genetic manipulation, rapid degradation of recombinant type VII collagen because of the generation of antibodies against exogenous proteins, administration of a large number of cells (e.g. $5\times10^6$ fibroblasts/site), very short permanence of differentiated cells (e.g. 2 weeks) and scarcity of histocompatible donors.

The population of adherent cells derived from adult bone marrow known as mesenchymal stromal cells (MSC), also referred as mesenchymal stem cells, migth be an ideal tool for treating patients with RDEB. *In vitro* and *in vivo,* MSC give rise to mesodermic cell lineages and secrete extracellular matrix proteins (7). Together, MSC produce trophic factors that promote endogenous progenitor migration, proliferation and differentiation, but also tissue neovascularization (8). In addition, MSC are hypo-immunogenic cells that allow safe allogeneic transplantation without the requirement of patient conditioning (9). Data from animal models show that locally implanted MSC engraft in the skin of recipients, contribute to the regeneration of normal and injured areas, and accelerate wound healing (10).

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*(Received 20 January 2009; accepted 29 December 2009)*

ISSN 1465-3249 print/ISSN 1477-2566 online 
DOI: 10.3109/14653241003587637

We evaluated whether the local administration of adult unrelated allogeneic MSC results in an improvement in the skin of patients with severe generalized RDEB. Case 1 was a 25-year-old female homozygous for the *COL7A1*:c.7708delG mutation. When enrolled in the study, the patient presented with large hard-to-heal lesions on her extremities. Since birth, her skin biopsies showed dermal–epidermal separation below the lamina densa (Figure 1a) and negative immunohistofluorescence (IHF) for type VII collagen. Zones of intact skin on the back (data not shown) and ulcerated skin around a chronic wound (Figure 1b) were selected for transplantation. At each site, vehicle (control) or $0.5 \times 10^6$ cells positive for CD73, CD90, CD105 and ASMA, isolated from the bone marrow of a healthy female volunteer unrelated to the patient (MSC-treated), were administered intradermally.

One week after intervention, on control sites blister formation was evident (Figure 1c). In contrast, MSC-treated skin showed a continuous dermal–epidermal junction (Figure 1d). On control sites, type VII collagen immunohistochemistry (IHC) showed faint and diffuse staining at the cytoplasm of keratinocytes and fibroblasts (Figure 1c), while in MSC-treated skin the immunoreactivity was confined to the basement membrane zone (Figure 1d). At week 12, IHF for type VII collagen was positive in MSC-treated skin (Figure 1g) but negative at control sites (Figure 1f). Asymmetric re-epithelialization of the treated wound was evident 1 week after intervention (Figure 1e). Regeneration initiated from the sites where MSC were administered but not from the control site. At week 12 the wound was almost healed, except for the region where vehicle was infused (Figure 1h). The regenerated epidermis remained firmly adhered to the dermis, did not itch and did not blister even after mechanical stress.

In case 2, a 13-year-old male homozygous for the *COL7A1:c.*6527insC mutation, similar results were observed (data not shown). With both patients, 4 months after MSC transplantation treated lesions started to reulcerate. The restricted duration of the observed therapeutic effect migth be related to donor MSC exhaustion. If all transplanted cells had survived and formed a monolayer they would have covered 4 $cm^2$. The regenerated area (wound extension)

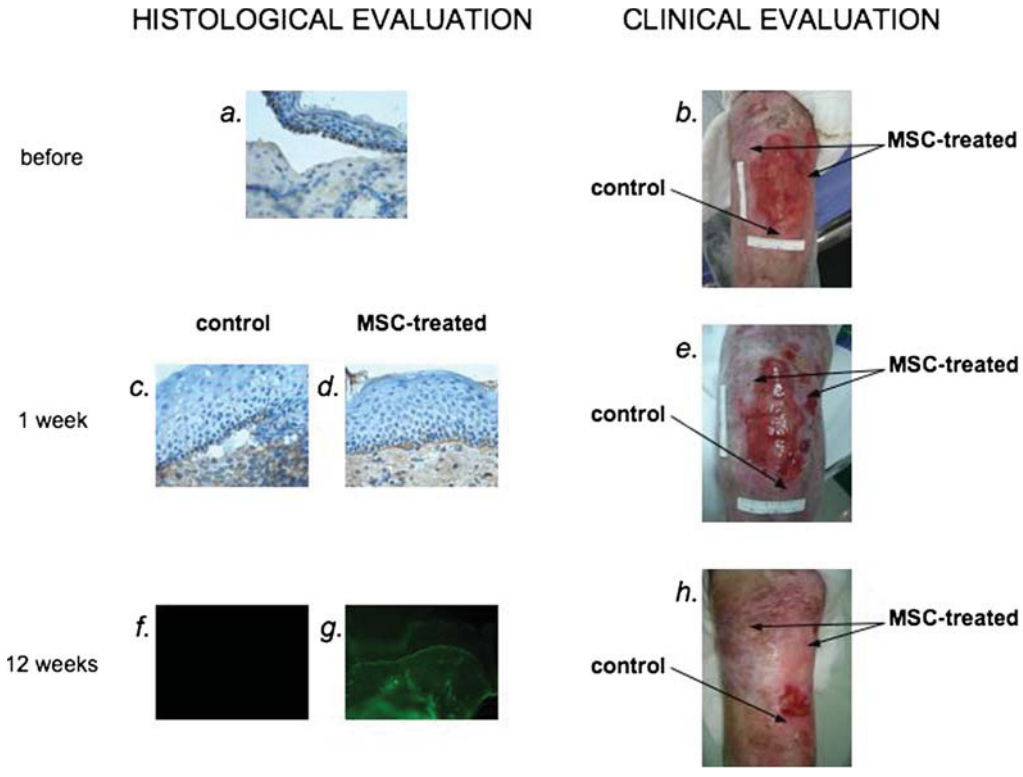


Figure 1. Replenishment of type VII collagen at the dermal–epidermal junction and re-epithelialization after intradermal administration of allogeneic MSC in a patient with severe generalized RDEB (case 1). IHC (a, c, d) and IHF (f and g) analysis of punch biopsies taken from the skin around a chronic wound before, 1 week and 12 weeks after intradermal administration of vehicle (control) or $0.5 \times 10^6$ MSC (MSC-treated). Type VII collagen was stained with monoclonal antibody LH7:2. Macroscopic analysis (b, e, h) of the chronic wound before, 1 week and 12 weeks after intradermal administration of vehicle (control) or $0.5 \times 10^6$ MSC (MSC-treated). Rulers of 5 cm were placed at 90° at the border of the area under study and clinical photographs were obtained using a SONYCyber–Shot 5.1 digital camera.

was approximately 100 $cm^2$ and, during the observed therapeutic window, the skin may have undergone at least four renewing cycles.

Macroscopical changes and inflammatory cell infiltration (Figure 1d) were not observed in intact or ulcerated MSC-treated skin. As the patients were homozygous for  mutations predicted to preclude normal assembly of anchoring fibrils, and independent biopsies obtained throughout their lives were all negative for type VII collagen, the positiveness of IHF observed in MSC-treated skin suggests that donor cells lodged into the recipient skin and produced locally normal type VII collagen. Because of the scarcity of biopsies obtained after the intervention, we were unable to confirm it by chimerism study.

The data reported here show that the intradermal administration of adult, unrelated allogeneic MSC into two unconditioned patients with severe generalized RDEB does not produce acute adverse effects, associates with type VII collagen replenishment at the dermal–epidermal junction, prevents blistering and improves wound healing. Further studies are necessary to determine the origin of the detected type VII collagen, the mechanism associated with skin improvement and the proper dose to prolong the therapeutic effect observed after adult, unrelated allogeneic MSC transplantation into patients with RDEB.

***Declaration of interest:*** The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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