Premium Quality
Primary Stromal/Stem Cells and Medium Reagents

Prices Your Lab Can Afford  Prompt Technical and Research Support

Get 10% off with code “promo10” at https://www.lacell-usa.com

LaCell: Research, Simplified
Concise Review: Transplantation of Human Hematopoietic Cells for Extracellular Matrix Protein Deficiency in Epidermolysis Bullosa

JAKUB TOLAR, BRUCE R. BLAZAR, JOHN E. WAGNER

Division of Blood and Marrow Transplantation, Department of Pediatrics, University of Minnesota, Minneapolis, Minnesota, USA

Key Words. Epidermolysis bullosa • Extracellular matrix • Bone marrow • Cord blood • Stem cell transplantation • Regenerative medicine

ABSTRACT

The skin is constantly exposed to environmental insults and requires effective repair processes to maintain its protective function. Wound healing is severely compromised in people with congenital absence of structural proteins of the skin, such as in dystrophic epidermolysis bullosa, a severe congenital mechanobullous disorder caused by mutations in collagen type VII. Remarkably, stem cell transplantation can ameliorate deficiency of this skin-specific structural protein in both animal models and in children with the disorder. Healthy donor cells from the hematopoietic graft migrate to the injured skin; simultaneously, there is an increase in the production of collagen type VII, increased skin integrity, and reduced tendency to blister formation. How hematogenous stem cells from bone marrow and cord blood can alter skin architecture and wound healing in a robust, clinically meaningful way is unclear. We review the data and the resulting hypotheses that have a potential to illuminate the mechanisms for these effects. Further modifications in the use of stem cell transplantation as a durable source of extracellular matrix proteins may make this regenerative medicine approach effective in other cutaneous and extracutaneous conditions. STEM CELLS 2011;29:900–906

I skate to where the puck is going to be, not to where it has been.
Wayne Gretzky

INTRODUCTION

Wound healing is a highly organized process that integrates the skin cells, skin extracellular matrix, and systemic factors—mainly blood cells and cytokines—into dynamic tissue healing [1–4]. Malfunction of the local arm (e.g., congenital deficiency of a structural skin protein in genodermatoses) or overwhelming of normal skin repair mechanisms (e.g., burn injury) or the systemic arm (e.g., breakdown of regulatory mechanisms that protect the skin from immune-mediated injury) can result in the loss of skin integrity and increased susceptibility to infections. In addition to endogenous repair mechanisms within the skin that might occur through resident skin stem and progenitor cell proliferation, substantial evidence now exists that the bone marrow (BM) contains cells that can persist in mucocutaneous epithelia [5–10].

Although BM-derived cells have been identified in the skin after hematopoietic cell transplantation (HCT) [11, 12], the more important questions are: in which venues do lymphohematopoietic cells substantially contribute to wound healing and how robust must such hematogenous engraftment be to meaningfully contribute to skin function in disease and injury [4, 13, 14]? Recently, we and others have obtained unexpected insights from animal experimentation [15–17] and from our clinical trial of HCT in a prototypic extracellular matrix disorder, epidermolysis bullosa (EB) [18].

SKIN VELCRO

EB is a group of blistering skin disorders resulting from mutations in genes encoding protein components of the cutaneous basement membrane zone. There are four subtypes: EB simplex, dystrophic EB, junctional EB, and Kindler syndrome [19], distinguished by mutations in at least 14 different genes and roughly subdivided based on the plane of cleavage in the dermal-epidermal junction (DEJ). Intraepidermal tissue separation occurs in EB simplex, cleavage through the lamina lucida is characteristic of junctional EB, and blistering below the lamina densa is found in dystrophic forms of EB.

Generalized recessive dystrophic EB (RDEB), one of the most severe forms of EB, is an incurable and lethal genodermatosis characterized by reduced or no collagen type VII (C7) [20]. This extracellular matrix protein produced by dermal fibroblasts and basal keratinocytes [20] is the major component of the anchoring fibrils (AFs) that extend from the lamina densa into the papillary dermis, thus providing the adhesion structures (“skin Velcro”) responsible for attachment of the epidermis to dermis [21].
Loss-of-function mutations in the COL7A1 gene in RDEB result in extensive mucocutaneous blistering, esophageal strictures, mutilating scars, life-threatening infections, and aggressive squamous cell carcinomas [19, 22]. Even though suffering is difficult to measure objectively, the painful challenges that individuals with RDEB endure are extraordinary. RDEB skin can blister with a touch, and the resulting wounds heal with mutilating scarring. Their upper alimentary tract can blister just as easily, esophageal strictures develop with aberrant tissue repair, and eating is painful. People with RDEB are separated from many interactions with their peers as most common daily activities are impossible for them. Every moment and aspect of their lives is consumed by this debilitating disorder [23].

Traditionally, palliative measures, such as bandaging, analgesia, surgical debridement, and nutritional support have been used. Faced with the limited impact of the various supportive measures, innovative treatments of wounds such as injecting C7 protein, wild-type cells, or gene-modified cells are being explored [24–30]. As the lesions in RDEB are distributed over large areas of the body both externally (skin) and internally (the proximal and distal gastrointestinal sites), we reasoned that a systemic therapy providing durable correction of C7 deficiency would be preferred.

To assess the efficacy of whole-body correction of RDEB with various stem cell populations [17], we used a murine model of RDEB that recapitulates the human disease [31]. First, we reasoned that skin-derived stem cells might express signals required for skin repair. However, infusion of epidermal stem cells produced no improvement. We then considered a possibility that nonhematopoietic BM stem cells—such as mesenchymal stromal cell (MSC) populations—could migrate to sites of injury and contribute to tissue repair, but again we observed no beneficial effects. Finally, we evaluated whole BM and BM subpopulations [17].

As unmanipulated BM failed to rescue RDEB mice, we infused immature BM cells by depleting hematopoietic lineage-negative cells and positively selecting for Sca1+ or a signaling lymphocyte activating molecule-positive (SLAM+) population (CD150+) known to have significant multipotentiality [32]. Only the infusion of SLAM+ BM cells resulted in survival of RDEB animals [31], presumably because of the differences in proliferation potential and cell lineage commitment among the various stem cell and progenitor cell BM populations. Donor cells were found at the DEJ and produced C7 in the skin of the RDEB mice. Strikingly, paw blisters healed and rudimentary AFs were formed [17]. These data were supported by research of others who demonstrated the positive effect of in utero infusion of BM cells in RDEB and postnatal infusions in junctional EB [15, 16].

An unexpectedly high level of donor chimerism in skin was observed after HCT (Table 1). Thus, the skin of the RDEB recipient of allogeneic HCT is a composite of donor and recipient cells. Although it is likely that the transplanted lymphohematopoietic cells contributed to this result (all the patients in Table 1 achieved donor hematopoietic engraftment), BM and cord blood (CB) contain nonhematopoietic cells as well. These donor nonhematopoietic cells are the likely source of the wild-type C7 protein, as the hematopoietic stem cell and progenitor cells do not express significant amounts of C7. In an effort to define the donor cells in RDEB skin, a combination of fluorescence in situ hybridization and immunofluorescence staining (Fig. 1) was used to identify phenotypes of donor cells in a recipient of sex-mismatched HCT (P5). As expected, some donor cells were hematopoietic (male CD45+ cells) but notably many—both above and below the DEJ—were nonhematopoietic, nonendothelial donor cells (male CD45+CD31− cells) presumably expressing C7, which is clearly visible in their vicinity at the DEJ (Fig. 1).

Relevant to this, all patients but one (P6, Table 1) had increased C7 deposition at the DEJ, but none had a normal number and structure of AFs. Thus, despite the fact that donor cells in the skin of RDEB patients are capable of expressing wild-type C7, the C7 protein may not organize well into AFs, at least not in the first several years after HCT. It is illuminating that the patient with exon deletions on both maternal and paternal C7 alleles (P6) expressed no C7 and yet had durable, albeit only moderate, clinical response in skin healing and
Marrow in Skin Repair

integrity after HCT. This may suggest that hematogenous donor cells may recruit the cells of the recipient [34, 35] to express additional extracellular matrix proteins relevant to skin fragility after HCT and to increase its reparative and adhesive properties. Thus, the potential mechanisms underlying the changes in wound healing after HCT include effects of wild-type C7 production by donor cells in the epidermis and dermis of the host, as well as induction of host’s skin fibroblasts and keratinocytes by donor cells to secrete mutant C7. C7 is the largest skin collagen. It is synthesized as a pro-collagen molecule composed of three identical alpha chains. Each chain has a long triple-helical collagenous central domain flanked by two globular noncollagenous segments. C7 molecules form antiparallel dimers that polymerize into AF of 785 nm in length. Importantly, C7 binds to other DEJ proteins, such as laminin 332 and to dermal interstitial collagen fibrils [36]. As detailed below, the skin healing can be simultaneously due to expression of fully functional wild-type C7 homotrimer (i.e., a normal AF), trimerization of wild-type C7 and mutant C7 in different ratios (i.e., a partially functional “hybrid” AF) and the adhesive effects of C7 monomers and other extracellular matrix proteins expressed after HCT.

Mutant C7 Stabilizes the Skin

The mutant C7 protein has been detected in all patients who inherited at least one splice site mutation in the C7 gene (Table 1). Moreover, there seems to be a linear relationship between the amount of the mutant C7 at baseline, and the speed and quality of skin repair after HCT. This is relevant as between the amount of the mutant C7 at baseline, and the changes in wound healing after HCT include effects of mutant C7 at baseline, and the speed and quality of skin repair after HCT. This is relevant as the direct effect of pre-HCT chemotherapy on the skin, the nonproductive, tissue repair mechanisms characteristic of RDEB may still provide a scaffold that aids the donor wild-type C7 to assemble into the triple helix of an AF at the cutaneous basement membrane (John McGrath, personal communication, July 19, 2010).

The More C7 the Better

Two of the donors of the six evaluable transplant recipients were heterozygous for one of the parental mutant C7 alleles, and the other four carried homozygous wild-type C7 alleles. While murine studies indicate that C7 expression levels need not be at wild-type, or even at heterozygous, levels for protection from extensive blistering (i.e., 30–40% of wild-type levels of C7 may be sufficient [37, 38]), in our patients, a comparison after HCT between the 100% (homozygous C7) and the 50% (heterozygous C7) gene dose of C7 showed no obvious differences in clinical outcome to date (Table 1).

Stem Cell Niche

Because a high level of donor engraftment is observed in the skin of RDEB recipients of HCT, it is possible that—in a mechanism analogous to the process whereby chemotherapy before HCT “opens up” hematopoietic stem cell niches in the BM—the skin stem cell niches become unoccupied and therefore allow donor BM engraftment. This can result from the direct effect of pre-HCT chemotherapy on the skin, the exhaustion of skin stem cells due to the hyperactive, albeit nonproductive, tissue repair mechanisms characteristic of RDEB [39, 40], or a combination of both of these. Critically, a permanent hematopoietic graft can increase donor BM stem and progenitor cell engraftment in the skin by mobilization of

### Table 1. Donors and recipients before and after hematopoietic cell transplantation

<table>
<thead>
<tr>
<th>Recipient before HCT</th>
<th>Donor</th>
<th>Recipient after HCT</th>
</tr>
</thead>
<tbody>
<tr>
<td>C7 expression*</td>
<td>C7 genotype</td>
<td>C7 expression*</td>
</tr>
<tr>
<td>P1</td>
<td>M c.3472delC (p.Pro1158fsX3); IVS51+1G → A</td>
<td>++</td>
</tr>
<tr>
<td>P2</td>
<td>M c.3472delC (p.Pro1158fsX3); IVS51+1G → A</td>
<td>+</td>
</tr>
<tr>
<td>P3</td>
<td>M IVS85-2delAG; IVS92+5G → A</td>
<td>+</td>
</tr>
<tr>
<td>P4</td>
<td>F c.6176A→G (p.Glu2059Gly); IVS51+1G → A</td>
<td>+</td>
</tr>
<tr>
<td>P5</td>
<td>F c.4919delG (p.Gly1640fsX70); c.8254_8255delAG (p.Arg2751fsX20)</td>
<td>+</td>
</tr>
<tr>
<td>P6</td>
<td>F c.6781C→T (p.Arg2261X); IVS110-1G</td>
<td>+</td>
</tr>
<tr>
<td>P7</td>
<td>F c.6781C→T (p.Arg2261X); IVS110-1G</td>
<td>+</td>
</tr>
</tbody>
</table>

*Based on immunofluorescence staining, the skin C7 expression has been estimated as +, 5–25% of wild type; ++, >50% of wild type and +++, >75% of wild type.

Donor chimerism varied over time (between days 24 and 467 after HCT, 4–10 measurements were done for P3, P4, P5, P6, and P7) and with the location of the biopsy site (although all samples were collected approximately 1 mm away from a skin erosion). Therefore, a range of skin chimerism is shown here. Of note, all patients achieved complete or near-complete donor hematopoietic chimerism.

Most recent assessments are shown. At the time of writing (January 15, 2011) P1 is 1,172 days, P4 760 days, P5 641 days, P6 569 days, and P7 503 days after HCT. Before HCT more than 50% of skin was covered with blisters and erosions in all patients. ++, moderate improvement denotes <25% of body surface area affected; +++, dramatic improvement denotes <10% of body surface area affected.

For clarity, the numbering of the patients is the same as in our published report [18]. P2 died before stem cell infusion of heart failure, presumably caused by cyclophosphamide toxicity and pre-existing renal failure. P3 died 183 days after HCT due to infection secondary to graft failure (although he had near-complete hematopoietic engraftment after second HCT).

Abbreviations: HCT, hematopoietic cell transplantation; F, female; M, male; P, patient; WT, wild type.
multiple waves of BM progenitor cells—presumably guided by the stress signals released from the injured skin [41, 42]—and by the recirculation of cells between BM and skin.

**Conclusion**

Regardless of the mechanisms responsible for clinical improvement, taken collectively these data suggest that HCT has changed the natural history of RDEB into a phenotype reminiscent of a milder form of RDEB. Inevitably, many questions remain. Chief among them is that the donor hematopoietic cell population capable of both C7 production and homing into the skin of recipients is unknown. It is possible that several hematopoietic and nonhematopoietic populations, perhaps functioning together, are involved. Such cells contained in the BM or CB graft may not express C7 when collected but rather become “what they are” [43] only after homing into the activated niche microenvironment in the skin.
injured skin. Stem cell niches in RDEB skin depleted of its own stem cells by repeated and futile attempts at skin regeneration, may serve as permissive “docking stations” for donor cells. Such cells may in turn release factors that operate on the host or that may differentiate into cells that directly participate in the wound healing process via release of C7 or other tissue repair proteins [39, 40]. For example, both BM and CB grafts used in the clinical trial contain MSCs that are known to migrate to sites of injury including skin wounds [4, 42, 44, 45]. There the MSCs engage in tissue healing, predominantly by secretion of paracrine factors, including appropriate extracellular matrix molecules, and by the recruitment of reparative cells from the recipient [34].

HCT has the promise of being a durable, systemic therapy for RDEB. This is true especially for patients with particularly severe disease where the risk:benefit ratio may be sufficiently favorable to warrant consideration of this therapeutic approach despite the side effects associated with HCT. Furthermore, HCT also opens up an elegant possibility for the local treatment [28–30] of remaining lesions. Local injections of donor cells might maximize the efficacy of direct injections of allogeneic cells while extending the half-life and usefulness of these cells because they are not expected to be recognized as foreign by a recipient with a fully engrafted lymphohematopoietic system from the same donor.

In this fashion, improvements in HCT (e.g., coinfusion of MSCs and changes in pre-HCT conditioning regimens) will coincide with the development of alternative experimental therapies (e.g., injections of C7 gene or protein, cell progeny of personalized RDEB pluripotent stem cells, or local injection of allogeneic skin or mesenchymal stem cells) [24–26, 28–30, 40, 46–48], each of which is now being simultaneously explored.

**FUTURE APPROACH: STEM CELL GENE THERAPY**

Stem cell and gene therapy efforts in the treatment of EB will likely merge over time by genetically correcting the patient’s own cells, thereby avoiding the complications of allogeneic cell therapies as has been done for congenital immunodeiciencies (X-linked severe combined immunodeficiency, adenosine deaminase deficiency, chronic granulomatous disease, and Wiskott-Aldrich syndrome) and a hemoglobinopathy (β-thalassemia) [49]. That such stem cell gene therapy will be effective in a nonhematopoietic disorder, and specifically in EB, is supported by the observations that a minority of individuals with EB develop self-correcting mutations in one of the genes underlying the EB pathology and that when such somatic mutations occur, the gene self-corrected skin is resistant to trauma [50–52].

In contrast to other conditions with prominent mosaicism, such as BM failure syndrome Fanconi anemia, where a self-correcting somatic mutation occurring in a single stem cell is capable of restoring normal hematopoiesis [53], the mosaicism in EB remains confined to small area of skin. Therefore, expansion of such naturally corrected cells, for example, using induced pluripotency technology followed by parallel differentiation into both hematopoietic (for HCT) and skin cells (for local wound therapy) [46], may obviate the need for gene correction of the patient-specific cells altogether. For now, however, C7 gene augmentation, gene editing via homologous recombination, or C7 RNA transsplicing [27] using viral-mediated transgenesis of hematopoietic stem cells harvested from individuals with RDEB are more realistic means of providing autologous grafts and systemic treatment, whereby in principle the efficacy of HCT is preserved while the complications of allogeneic HCT are avoided.

**OUTLOOK**

No treatment is devoid of risks, and a view that that any of the strategies in practice or envisioned will be free of side effects is not realistic [54]. Patients with RDEB define acceptable risk differently than a healthy person: to a person with RDEB simply living life is inherently unsafe [23]. The goals of therapy in RDEB are safe treatment of mucocutaneous lesions and prevention of squamous cell carcinoma. As every blister brings the patient closer to cancer, it seems likely that the risk of cancer will be diminished after successful HCT, but years or decades of observation will be needed for such confirmation. Recent data from clinical observations, animal models, and modeling RDEB in a patient-specific fashion with induced pluripotent stem cells [46] (“ex vivo clinical trials”) offer the possibility of translating new therapeutic modalities into the clinic. Thus, there is hope for new curative approaches for RDEB and other extracellular matrix disorders.

**ACKNOWLEDGMENTS**

We thank Drs. John McGrath and Walter Burgdorf for comments on the manuscript. For superb papers that have expanded our understanding of skin biology (even though not all are quoted here because of the formatting constraints), we wish to acknowledge Drs. Leena Bruckner-Tuderman, Jo-David Fine, Dennis Roop, Thomas Kupper, Jouni Uitto, Gerard Socie, David Woodley, Mei Chen, Peter Marinkovich, Helmut Hintner, Angela Christiano, Marcel Jonkman, and Robin Eady. For generosity with their time and for discussions, we are grateful to Drs. Anne Lucky, Amy Paller, Elena Pope, Dedee Murrell, Alan Arbuckle, Hiroshi Shimizu, Katsuji Tamai, Alain Hovnanian, Maria Horodinsky, Mark Osborn, Troy Lund, and Doug Keene. We are indebted to all the patients and their families for their persistence, smarts and survivorship—the traits we strive to reflect and translate with both basic and clinical science into clinically meaningful changes in the lives of people with EB. This research was supported by DebRA International; Epidermolysis Bullosa Medical Research Foundation; Pioneering Unique Cures for Kids; and Children’s Cancer Research Fund, MN (to J.T.), Children’s Cancer Research Fund, MN (B.R.B.), Epidermolysis Bullosa Medical Research Foundation; Children’s Cancer Research Fund, MN (J.E.W.).

**DISCLOSURE OF POTENTIAL CONFLICTS OF INTEREST**

The authors indicate no potential conflicts of interest.

**STEM CELLS**