Dystrophic epidermolysis bullosa (DEB) is a severe skin fragility disorder associated with trauma-induced blistering, progressive soft tissue scarring, and increased risk of skin cancer. DEB is caused by mutations in the COL7A1 gene which result in reduced, truncated, or absent type VII collagen, and anchoring fibrils at the dermal-epidermal junction (DEJ). Although various topical wound-healing agents have been examined, including autologous cultured keratinocytes and skin bioequivalents, none has shown unequivocal benefits in the treatment of dystrophic forms of EB. Alternative approaches are needed.

**CLASSIFICATION OF RECESSIVE DYSTROPHIC EPIDERMOLYSIS BULLOSA**

RDEB is classified into 2 subtypes, namely generalized severe (RDEB-GS) and generalized, other; the former lacks anchoring fibrils, whereas the latter exhibits reduced or rudimentary-appearing anchoring fibrils. RDEB-GS is easily one of the most devastating, chronic diseases known to humanity. Characterized by mechanical fragility and repeated blister formation within potentially all epithelial-surfaced or lined structures, patients with generalized subtypes of RDEB who survive recurrent bacterial sepsis during early infancy are at high risk of later developing one or more severe complications, including profound growth retardation, multifactorial anemia, esophageal strictures, corneal scarring or blindness, poststreptococcal glomerulonephritis and renal failure, and progressive mutilation and loss (pseudosyndactyly) of the fingers and toes.
**ETIOLOGY AND PATHOGENESIS**

Two possible mechanisms for blister formation have been proposed: (1) destruction of dermal connective tissue by excessive amount of a protease,\(^8\) and (2) a defective structural protein in the dermis that is responsible for the normal integrity of the skin at the epidermal-dermal junction.\(^9\)–\(^11\) Electron microscopy shows destruction of collagen fibrils in association with blister formation, as well as phagocytosis of collagen by macrophages in the skin adjacent to clinical blisters.\(^12\)

The pathogenesis of DEB and structure of collagen VII are reviewed by Bruckner-Tuderman\(^13\) and Uitto.\(^14\) The purpose of “cell therapy” for RDEB is to increase the amount of collagen VII in the basement membrane zone (BMZ) to heal wounds and prevent further wound formation. Many DEB patients have multiple wounds with a large area of involvement, and all of these areas ideally should be treated, along with internal blistering such as may appear in the esophagus.

Compared with gene therapy, cell therapy has many advantages. The most important feature is that fibroblast cell therapy is safe and easy to work with. Several experiments have been performed with allogeneic fibroblasts, autologous fibroblasts, or parent donor fibroblasts in mouse models and in one study in RDEB patients in recent years. Only minor side effects such as temporary erythema, pruritus, mild hypertrophic scarring at the injection sites, and local skin inflammation have been found in some cases. All of these side effects resolved spontaneously.\(^15\) Fibroblasts are more robust and easier to propagate than keratinocytes,\(^16\) are less susceptible to growth arrest and differentiation, and can be frozen, packaged, and stored. Fibroblasts can be used by intradermal injection into skin and no special subsequent wound care is required. Furthermore, unlike stem cell transplantation, in which human leukocyte antigen (HLA) typing is important because the degree of HLA compatibility between donor and recipient will influence the outcome of the transplant, the donor of fibroblasts can be unrelated to the patient. Graft-versus-host disease did not occur when allogeneic fibroblasts were injected in the pilot study of 5 patients.\(^15\) These advantages of fibroblast-based cell therapy give hope to RDEB patients and their families for improving therapy in the near future.

**TARGET CELLS CHANGED FROM KERATINOCYTES TO FIBROBLASTS**

Keratinocytes and dermal fibroblasts both express collagen VII, but keratinocytes are the primary source of collagen VII in the developing skin.\(^17,18\) Many investigators have used keratinocytes as the target cells of RDEB gene therapy. Of note, intradermal injection of normal human fibroblasts or gene-corrected RDEB fibroblasts alone were found capable of synthesizing and secreting stable deposition of type VII collagen in mouse DEJ, thus contributing to the formation of anchoring fibrils.\(^19,20\)

**FIBROBLAST-BASED CELL THERAPY IN MOUSE STUDIES**

*Intradermal Injection of RDEB Fibroblasts Overexpressing Type VII Collagen*

In 2003, RDEB fibroblasts overexpressing collagen VII (RDEB\(^+\) fibroblasts), RDEB fibroblasts, and normal fibroblasts were injected into mouse intact skin and the expression of collagen VII in mouse skin was determined. Compared with normal fibroblasts, which only produce a low level of collagen VII centered around murine dermal hair follicles, and RDEB fibroblasts, which yielded undetectable protein, the results showed that RDEB\(^+\) fibroblasts can produce correctly localized collagen VII at the epidermal-dermal junction, which can be stable for 16 weeks.\(^21\)

*Intradermal Injection of Normal Allogeneic Human Fibroblasts*

Based on the supposition that the administration of actual fibroblasts would result in more sustained type VII collagen deposition, gene-corrected RDEB fibroblasts and normal human fibroblasts alone were administered to immunodeficient mouse skin or transplanted human skin equivalent. The expression of new human collagen VII by gene-corrected fibroblasts were detected stably for at least 4 months, and formation of anchoring fibrils was present in the mouse DEJ after injection. Nevertheless, unlike what had been found by Ortiz-Urda, this study also found that normal human fibroblasts are capable of producing, secreting, and depositing collagen VII at the DEJ as effectively as gene-corrected fibroblasts, which seems to be dependent on the number of cells injected, that is, \(5 \times 10^6\) cells versus \(1 \times 10^6\) cells.\(^19,21\)

Bruckner-Tuderman’s group succeeded in creating a mouse model for RDEB that expressed collagen VII at about 10% normal level, and their phenotype closely resembled characteristics of RDEB.\(^22\) Intradermal injection of wild-type (WT) fibroblasts resulted in restoration of the DEJ, and increased deposition of collagen VII and resistance to induced stress compared with untreated areas. Recently, a long-term study of
fibroblast-based cell therapy has been performed on a mouse model for RDEB. Collagen VII expression at the DEJ was increased by 3.5- to 4.7-fold for at least 100 days after intradermal injection of WT fibroblasts, and injected fibroblasts were the major source of newly deposited collagen VII, although injected fibroblasts gradually become apoptotic within 28 days. Skin integrity and resistance to mechanical forces also improved for at least 100 days. This preclinical test paves the way for human clinical trials.23

**Intravenous Injection of Normal Human Fibroblasts**

Severe RDEB patients usually have widespread lesions and multiple wounds spanning large areas as well as internal esophageal erosions; hence, a systemic delivery mode for cell therapy would be ideal. One problem with therapy involving intradermal injection of fibroblasts is that many intradermal injections need to be performed into numerous wound sites for each patient. An alternative strategy might be to inject allogeneic fibroblasts into the patient’s circulation that home to the skin wounds and deposit the transgene product. A recent study showed that intravenously injected normal human fibroblasts home to the sites of wounded human RDEB skin engrafted onto immunosuppressed mice, and continually synthesize and secrete collagen VII to the BMZ of the human skin, forming anchoring fibril structures.24,25 Thus, the mouse had a heterogeneous population of anchoring fibrils made up of both mouse collagen VII α chains and human collagen VII α chains. Of note, the investigators found that collagen VII delivered to the wound sites significantly enhanced wound healing. This study provides the first demonstration of the potential use of intravenously injected normal fibroblasts to restore collagen VII in DEB patients who have multiple open wounds.

**FIBROBLAST-BASED CELL THERAPY FOR RDEB PATIENTS**

Among the current cell therapies for RDEB using skin fibroblasts are the use of allogeneic fibroblasts (cultured from the patients’ parents or unrelated individuals) or autologous fibroblasts (cultured from the patients themselves). Specifically, investigators evaluated the clinical benefits that may accrue from a single intradermal injection of these cells in subjects with RDEB. To assess potential clinical benefits in humans, Wong and colleagues15 gave single intradermal injections of allogeneic fibroblasts to nonwounded skin on the backs of 5 subjects with collagen VII positive RDEB. These investigators noted a 1.5- to 2-fold increase of type VII collagen at the DEJ at 2 weeks and at 3 months following injection, and a 1.5-fold increase of anchoring fibrils. Molecular analysis suggested that the major effect of allogeneic fibroblasts is to increase the recipients’ own COL7A1 mRNA levels, with greater deposition of mutant type VII collagen at the DEJ and formation of additional rudimentary anchoring fibrils. No significant immune reactions were observed in skin biopsies, and none of these patients developed autoantibodies to collagen VII.

In a clinical trial of allogeneic cultured dermal substitute for the treatment of skin wounds in patients with RDEB, Hasegawa and colleagues26,27 employed an allogeneic cultured dermal substitute (CDS) prepared by plating normal human fibroblasts on a double-layer sponge matrix of hyaluronic acid (HA) and Atelo-collagen (Col) to treat RDEB patients. During a 6-week treatment, abundant granulation was found on the wound surface within a week and epithelialization began from the margins of the ulcer after 4 weeks. This study indicated that cryopreserved human fibroblasts in the CDS are able to release vascular endothelial growth factor and fibronectin, which in turn induce prompt granulation in the wound beds. These data demonstrated the feasibility of fibroblast-based therapeutic approaches in a preclinical setting, and lay a basis for further dissection of quantitative and qualitative details associated with development of a clinically applicable therapy regimen.

McGrath’s group have been conducting an open-label study of cultured foreskin fibroblasts obtained from Intercytex to RDEB wounds with encouraging results (EB 2009, Vienna, oral communication). The authors’ group has conducted a double-blind randomized placebo-controlled trial of allogeneic fibroblasts versus their transport media in paired symmetric wounds in RDEB, demonstrating efficacy and safety of the technique.28

**SUMMARY AND FUTURE OVERVIEW**

RDEB is caused by recessive mutations in the human type VII collagen gene. The current lack of specific treatment for RDEB is the impetus to develop fibroblast-based cell therapy strategies that have many advantages including technical ease of use, wide application, and minor side effects. Over the past 10 years, tremendous progress has been made in fibroblast-based cell therapy for RDEB. Fibroblast-based cell therapy can dramatically restore stable collagen VII at the DEJ and normalize the substructure changes of DEB for at least a few months. Even though the mechanism and the duration of newly produced
collagen VII at the DEJ are still unknown, cell therapy provides a new effective approach to therapy for RDEB.

REFERENCES