Cell Therapy in Dermatology

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Harnessing the regenerative capacity of keratinocytes and fibroblasts from human skin has created new opportunities to develop cell-based therapies for patients. Cultured cells and bioengineered skin products are being used to treat patients with inherited and acquired skin disorders associated with defective skin, and further clinical trials of new products are in progress. The capacity of extracutaneous sources of cells such as bone marrow is also being investigated for its plasticity in regenerating skin, and new strategies, such as the derivation of inducible pluripotent stem cells, also hold great promise for future cell therapies in dermatology. This article reviews some of the preclinical and clinical studies and future directions relating to cell therapy in dermatology, particularly for inherited skin diseases associated with fragile skin and poor wound healing.

One of the key functions of skin is to provide a mechanical barrier against the external environment. In several inherited and acquired dermatological disorders, however, this resilience is broken. Loss of a functional epidermis can have profound biological and clinical consequences including loss of water and electrolytes, cutaneous and systemic infections, as well as impaired thermoregulation. Epidermal failure can occur from burns, trauma, and adverse drug reactions. Several inherited diseases associated with inherent mechanical weaknesses in epidermal or dermal structural proteins can all be associated with extensive skin wounds and chronic erosions. Ulceration of the skin caused by common pathologies such as venous hypertension, arterial impairment, diabetes mellitus, or neuropathies creates an enormous clinical and health economic burden. Therapeutic in-

terventions to restore an intact epithelium and recover skin function have therefore been an important long-term focus of both traditional and translational medicine, and one in which a number of key advances and clinical benefits have occurred in recent years.

Cell therapy to repair or restore a defective epithelium and possibly deeper skin layers represents an attractive area of translational research that could have significant health benefits for many people. In this review, we discuss the development and application of cell therapy in dermatology, with a special focus on inherited skin disorders in which chronic ulceration has a major impact on quality of life. The main emphasis of the text is on recent clinical studies as well as new and emerging strategies that can exploit and harness the regenerative potential of human cells to restore skin tissue, although

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an overview of the clinical applications of cell therapy across a range of skin diseases is presented in Table 1. With regard to the focus of this review, it is hoped that cell therapy lessons learned from studies on rare skin diseases will also be relevant to improving future healthcare of patients with more common disorders associated with defective skin.

KERATINOCYTE CULTURE AND SKIN GRAFTING

In 1975, the discovery by Rheinwald and Green (1975) that keratinocytes could be cultured in vitro and the subsequent work that showed the enzyme dispase could help create sheets of cells suitable for grafting (Green et al. 1979) led to radical new options for clinical translation, including the treatment of burns patients (O'Connor et al. 1981). Notably, a small piece of skin ($\sim 2 \text{ cm}^2$) could be grown into sheets of epithelium, or cultured keratinocyte grafts that were suitable for treating wounds. Serial subculture enabled rapid expansion of the keratinocytes, such that grafts of a total area equivalent to that of the surface of an adult could be obtained in < 1 mo. Thereafter, the development of composite skin grafts, including a dermal component such as de-epidermized cadaveric dermis, biopolymers, or artificial scaffolds allowed for further refinement of cultured skin for clinical use (Ojeh et al. 2001; Auger et al. 2004). Cultured epidermal grafts have been shown to promote re-epithelialization in some genetic skin diseases associated with chronic wounds (Schofield et al. 1990; Hill et al. 1992; McGrath et al. 1993; Roseeuw et al. 1994), although success has been limited mainly because of the high risk of graft infection.

Newer devices, including cell spray formulations (following digestion of small pieces of skin with trypsin) that can contain keratinocytes, melanocytes, Langerhans cells, and fibroblasts have also been developed (Gravante et al. 2007) and used in clinical trials to augment wound healing (Kirsner et al. 2012). In addition, the use of noncutaneous cells, such as bone marrow (BM) cells, has been explored in skin regeneration, particularly with a view of

treating the entire skin (and possibly mucous membranes) rather than limited areas of damaged skin (Wagner et al. 2010).

INHERITED SKIN DISEASES AND THE NEED FOR INNOVATIVE THERAPIES

Epidermolysis bullosa (EB) refers to a group of inherited skin disorders in which blisters develop after relatively minor trauma to the skin. EB results from mutations in at least 18 different genes, mostly encoding structural components of the dermal–epidermal junction (DEJ) or keratinocytes within the lower epidermis (Fine et al. 2008). Although EB is a rare disease, with an estimated incidence of 1 in 20,000 people, this equates to a worldwide incidence of approximately 500,000 individuals. Collectively, this population has a desperate need for innovative therapies that reduce disease burden, improve quality of life, and make advances toward a cure. In some forms of EB, such as recessive dystrophic epidermolysis bullosa (RDEB), there may be very extensive fragility of the skin and mucous membranes. This profound skin fragility can be associated with delayed wound healing and persistent erosions with longer term complications of scarring and increased incidence of malignancy (Fine and Mellerio 2009a,b). Recently, considerable progress has been made in developing new treatments for EB, including gene, protein, and drug therapy, although cell therapies have perhaps shown the most clinical translation (Uitto et al. 2012). The major challenge is to restore an intact epithelium and provide some protection against mechanical trauma. To that end, therapeutic use of autologous or allogeneic cells, used locally or systemically, is now being explored in clinical trials.

MURINE STUDIES OF FIBROBLAST CELL THERAPY FOR EB

Most murine studies have focused on developing therapies for RDEB in which the underlying pathology involves mutations in the *COL7A1* gene encoding type VII collagen (C7), the major structural component of anchoring fibrils at the DEJ. C7 is synthesized and secreted by basal

Table 1. Summarizing the clinical use of cell-based products to treat defective skin

Cell type	Study type	Indication	Patient numbers	Components	Effect on wound healing	References
<i>Allogeneic epidermal graft</i>						
Keratinocyte	Case report	Burns	3	Cultured epidermal allograft	Healed and remained healthy for 9-mo follow up (f/u)	Hefton et al. 1983
Keratinocyte	Single-center interventional study	Burns	26	Cultured epidermal allograft	Healed in 7 d compared with 14 d (control sites)	Madden et al. 1986
Keratinocyte	Case report	Adult RDEB	1	Cultured allogeneic keratinocyte graft applied to nonhealing wounds (>3-mo duration)	Wounds re-epithelized after 7 d; no site blistering for 4 mo postgraft	McGuire et al. 1987
Keratinocyte	Case report	TEN	1	Cryopreserved epidermal allograft applied to pediatric case of drug-induced TEN	Viable graft; healing epidermis appeared normal	Birchall et al. 1987
Keratinocyte	Prospective study	RDEB	6	Cultured epidermal keratinocytes applied to graft sites	4/6 healed before controls; no difference in C7 levels	Schofield et al. 1990
Keratinocyte	Case report	JEB—non-Herlitz	1	Cultured epidermal allografts applied to chronic central facial erosions	Re-epithelialization over the course of four graft procedures	Hill et al. 1992
Keratinocyte	Multicenter interventional nonblinded prospective study	Chronic venous ulcers	21	Cryopreserved cultured epidermal allografts	20/30 ulcers healed completely within 6 wk	De Luca et al. 1992
Keratinocyte	RCT	Chronic venous ulcers	42	Cryopreserved cultured epidermal allografts compared with hydrocolloid dressings	No difference in healed ulcers between two groups	Teepe et al. 1993

Continued

Table 1. Continued

Cell type	Study type	Indication	Patient numbers	Components	Effect on wound healing	References
Keratinocyte	Interventional prospective study	Adult RDEB	10	Cultured allogeneic keratinocyte graft applied to dermatome-induced superficial wounds; compared with nongrafted sites	Little benefit detected compared with nongrafted sites	McGrath et al. 1993
Keratinocyte	Case report	Pediatric JEB–Herlitz	1	Cultured epidermal allograft applied to open wounds in a neonate	Significant epithelialization 70% of wounds; new lesions continued to develop	Roseeuw et al. 1994
Keratinocyte	Randomized controlled trial	Burns (deep partial thickness and donor sites)	15	Cultured epidermal allograft compared with control dressings	Improved epithelialization compared with control (6 d vs. 11 d)	Rivas-Torres et al. 1996
Keratinocyte	Prospective interventional study	Giant congenital naevi	5	Cryopreserved epidermal allograft or autologous cultured epithelium applied to lesions excised to adipose tissue of children with giant naevi (10%–52% surface area)	3/5 wounds healed well; 2/5 graft did not take; vigorous rejection reaction	Kumagai et al. 1997
Keratinocyte	Case report	RDEB	1	Autologous meshed split-thickness skin graft + allogeneic cultured keratinocytes	Reduction in pruritus; blister-free at 3 mo f/u	Verplancke et al. 1997
Keratinocyte	Prospective study	RDEB	7	Composite cultured skin allograft used in hand surgery on children with RDEB	Average time to recurrence was increased by approximately twofold	Eisenberg and Llewelyn 1998

Keratinocyte	RCT	Burns (partial thickness)	11	Cryopreserved cultured epidermal allograft	Accelerated re-epithelialization of deep ($n = 9$) and superficial ($n = 2$) wounds	Alvarez-Diaz et al. 2000
Keratinocyte	Single-center interventional study	Burns (deep partial thickness and donor sites)	55	Cryopreserved cultured epidermal allografts applied to wounds in children	Mostly comparable in donor sites, improved epithelialization time in deep partial thickness burns	Yanaga et al. 2001
Keratinocyte	Case report	Cutaneous GvHD following HSCT	1	Cultured epidermal allograft (taken from HSCT donor)	90% of wounds healed by day 21 postoperative	Milner et al. 2011
Keratinocyte	Case report	Pediatric EBS	1	Cultured allogeneic keratinocyte graft applied to nonhealing eroded lesions	Rapid re-epithelialization and wound healing	Shin et al. 2011
Autologous epidermal graft						
Keratinocyte	Case report	Burns	2	Cultured autologous epidermal graft	Grafts survived for the 8 mo f/u	O'Connor et al. 1981
Keratinocyte	Single-center interventional study	Vitiligo	4	Autologous epidermal sheet grafted onto superficially dermabraded vitiliginous area	Incomplete clinical repigmentation in 3/4 patients at 4 wk	Plott et al. 1989
Keratinocyte	Case reports	Burns	2	Cultured autologous epidermal graft applied to freshly excised wound bed or granulation tissue in children >95% full thickness burn	Successful engraftment; 60%–80% of grafts take when applied to healthy granulation tissue	Gallico et al. 1984
Keratinocyte	Case report	JEB–non-Herlitz	3	Cultured epidermal autograft applied with 2% mupirocin ointment to facial wounds	2/3 showed re-epithelialization after 7–10 d	Carter et al. 1987
Keratinocyte	Multicenter retrospective experience	Burns (partial and full thickness)	57	Cultured epidermal autograft and allograft	47% mean take of CEA in children; 28% mean take in adults	De Luca et al. 1989

Continued

Table 1. *Continued*

Cell type	Study type	Indication	Patient numbers	Components	Effect on wound healing	References
Keratinocyte	Single-center interventional study	Burns	17	Cultured epithelial autograft	Improved graft take compared with untreated (47% vs. 15%)	Teepe et al. 1990
Keratinocyte	Single-center retrospective review	Burns	30	Cultured epithelial autograft (Epicel)	Good engraftment of CEA and 90% survival in severely burned	Carsin et al. 2000
Keratinocyte Keratinocyte	Multicenter retrospective analysis	RDEB Burns	240	Cultured epithelial autograft (Biosurface Technology, Cambridge, MA)	64% mean take of CEA in 104 patients for whom complete data were available	Odessey 1992
Keratinocyte	Review of prospectively collected database	Burns	88	Cultured autologous epidermal graft on a cadaver allograft base	72.1% final take rate of graft in burns >50% total surface area; 91% survival rate	Sood et al. 2010
Keratinocyte	Phase I/II clinical trial	JEB—non-Herlitz	1	Genetically corrected cultured epidermal graft transduced using a retroviral vector encoding <i>LAMB3</i> cDNA applied graft site prepared using timed surgery	Stable adherent epidermis at 12 mo f/u; regenerated epidermis contained normal levels of <i>LAM5-β3</i>	Mavilio et al. 2006
Keratinocyte	Retrospective clinical audit	Burns (>50% TBSA)	84 (only 62 treated)	Spray on cultured autologous cell suspension (Cellspray) and autologous sheets	Cell suspension decreased mean number of days to first use of CEA	Wood et al. 2006
Keratinocyte	Single-center retrospective audit	Burns (full thickness)	16	Spray on cultured epithelial autograft (Keraheal) combined with 6:1 mesh graft	90% take rate at 8 wk	Lee 2012
Keratinocyte	Prospective RCT	Skin graft donor sites	45	Autologous keratinocyte suspension + platelet	Reduced healing time with keratinocyte suspension + platelet	Guerid et al. 2013

Keratinocyte	Prospective single-arm study	Vitiligo	27	Autologous epidermal graft using cultured keratinocytes	Good outcome in segmental vitiligo (12/20), not in generalized (0/7)	Matsuzaki and Kumagai 2013
Melanocyte suspension						
Melanocyte	Single-center interventional study	Leucoderma (vitiligo, piebaldism, and others)	26	Autologous noncultured melanocyte enriched cell suspension applied to dermabraded depigmented skin	Patients with piebaldism, halo naevi, and segmental vitiligo healed completely	Olsson and Juhlin 1998
Keratinocyte + melanocyte	Single-center interventional study	Vitiligo	11	Autologous cultured keratinocytes and melanocytes on a biomaterial (Laserskin) grafted onto achromatic skin after de-epithelialization with laser CO ₂	Complete repigmentation in 6/11 patients at 18 mo	Andreassi et al. 1998
Melanocyte + keratinocyte	Single-center interventional study	Vitiligo (segmental and focal)	67	Autologous noncultured epidermal cell suspension applied + collagen dressing to dermabraded skin	Long-term f/u over 5 yr; 41/50 of segmental and 11/16 had excellent repigmentation at 5 yr	Mulekar 2004
Melanocyte + keratinocyte	Single-center study	Genital vitiligo	3	Autologous noncultured epidermal cell suspension	Repigmentation seen in all three cases	Mulekar et al. 2005
Melanocyte + keratinocyte		Vitiligo	11	Autologous noncultured melanocyte and keratinocyte suspension compared with dermabrasion alone	Repigmentation in 4/8 lesions treated with cell therapy compared with 1/6 control group	Toossi et al. 2011

Continued

Table 1. Continued

Cell type	Study type	Indication	Patient numbers	Components	Effect on wound healing	References
Melanocyte + keratinocyte	Pilot study	Vitiligo	11	Melanocyte and keratinocyte cell suspension + dermabrasion compared with dermabrasion alone	Minor improvement in repigmentation compared with dermabrasion alone	Vazquez-Martinez et al. 2011
Fibroblast cell therapy						
Fibroblast	Prospective pilot study	Prominent rhytid or facial scar	10	Intradermal cultured autologous fibroblasts	Size reduction between 10%–85%; histologically increased dermal thickness	Watson et al. 1999
Fibroblast	Prospective placebo-controlled phase III trial	Facial contour deformities	215	Intradermal cultured autologous fibroblasts	Most significant difference evident in acne scars; continued response rates up to 12 mo posttreatment	Weiss et al. 2007
Fibroblast	Interventional nonblinded prospective study	Adult RDEB	5	Intradermal injection allogeneic cultured fibroblasts; intact skin	Increased COL7A1 and C7 for 3 mo; can remain raised for up to 9 mo	Wong et al. 2008; Nagy et al. 2011
Fibroblast	Phase II placebo-controlled double-blind RCT	Adult RDEB	5	Intradermal cultured allogeneic fibroblasts	No significant difference between placebo; improvement in QOL	Venugopal et al. 2013
Fibroblast	Phase II double-blind RCT	Adult RDEB	11	Intradermal cultured allogeneic fibroblasts into wounded skin versus vehicle	Improvement in wound healing noted up to 28 d	Petrof et al. 2013
Fibroblast	Interventional nonblinded study	Aging skin	5	Intradermal cultured autologous fibroblasts	Benefits limited to slight reduction in skin fragility	Eca et al. 2012
Fibroblast	Phase II open label dose escalation pilot study	Aging skin	10	Intradermal cultured allogeneic fibroblasts	Slight reduction in nasolabial crease	Lowe et al. 2010

Fibroblast	Single-center interventional study	Aging skin and scars	20	Intradermal cultured autologous fibroblasts	Variable improvement at 6 mo	Nilforoushadeh et al. 2010
Keratinocyte + fibroblast	Phase II placebo-controlled double-blind RCT	Chronic venous ulcers	205	Spray allogeneic neonatal keratinocyte and fibroblast cell-applied therapy	Greater mean reduction of wound size compared with placebo	Kirsner et al. 2012
Fibroblast	Prospective interventional study	Burns (third degree)	14	Allogeneic fibroblasts on meshed split thickness skin grafts	Improved healing time and hypertrophic scar formation compared with conventional method	Moravvej et al. 2012
Fibroblast	Multicenter double-blind placebo-controlled phase II RCT	Aging skin	372	Intradermal cultured autologous fibroblasts	Moderate improvement in nasolabial fold wrinkles compared to placebo; only 1 point subjective difference	Smith et al. 2012
Cultured dermal substitute						
CDS	Prospective interventional study	RDEB	3	Allogeneic cultured dermal substitute using cryopreserved normal human fibroblasts on matrix of hyaluronic acid and Atelo-collagen	Excessive granulation found on wound surface within 1 wk; epithelialization at margins of ulcer at 4 wk	Hasegawa et al. 2004
CDS	Case report	Digital gangrene associated with hypereosinophilic syndrome	1	Allogeneic cultured dermal substitute using cryopreserved normal human fibroblasts on matrix of hyaluronic acid and Atelo-collagen; replaced every 3 d for 5 wk	Pain reduction at site; healthy granulation tissue during treatment and so replaced with split skin graft	Ohtani et al. 2004
CDS	Prospective interventional study	Full thickness skin defects following tumor resection	12	Allogeneic cultured dermal substitute using cryopreserved normal human fibroblasts on matrix of hyaluronic acid and Atelo-collagen	9/10 patients developed healthy granulation tissue allowing split skin graft; others showed rapid re-epithelialization	Moroi et al. 2004

Continued

Table 1. *Continued*

Cell type	Study type	Indication	Patient numbers	Components	Effect on wound healing	References
CDS	Case report	Chronic venous ulcer	1	Allogeneic cultured dermal substitute using cryopreserved normal human fibroblasts on matrix of hyaluronic acid and Atelo-collagen	Granulation tissue within 2 wk; completely healed by 9 wk	Hasegawa et al. 2007
CDS	Multicenter prospective interventional study	Chronic venous and arterial ulcer	13	Allogeneic cultured dermal substitute using cryopreserved normal human fibroblasts on matrix of hyaluronic acid and Atelo-collagen	9/13 cases (arterial + venous) healed well, one complicated by local infection; larger ulcers still required grafting	Yonezawa et al. 2007
CDS	Case report	Ulcer associated with antiphospholipid syndrome + stasis dermatitis	1	Allogeneic cultured dermal substitute using cryopreserved normal human fibroblasts on matrix of hyaluronic acid and Atelo-collagen; replaced every week	All ulcers healed within 4 wk	Nishimoto et al. 2007
CDS	Prospective interventional study	Intractable ulcers lower leg	5	Allogeneic cultured dermal substitute using cryopreserved normal human fibroblasts on matrix of hyaluronic acid and Atelo-collagen	Wound size decreased in all cases (33% reduction in seven ulcers, complete resurfacing in two cases); no control comparator	Yamada et al. 2008a
CDS	Prospective interventional study	Wound donor site following skin graft	14	Comparative between fresh and cryopreserved human fibroblasts in allogeneic cultured dermal substitute	No difference noted in re-epithelialization between the preparation methods	Yamada et al. 2008b
CDS	Prospective, open label proof-of-concept clinical trial	Diabetic foot ulcer	5	Autologous fibroblast seeded artificial dermis using animal product-free serum	3/5 complete wound healing within 12 wk; no deleterious effects using animal-free serum	Morimoto et al. 2012

Mesenchymal stromal cell therapy

MSC	Case report	Burns (third degree)	1	Allogeneic BM-MSC applied directly to deep thermal burn	Wound regeneration and accelerated rehabilitation of patient	Rasulov et al. 2005
MSC	Case report	Severe radiation burns	1	Intradermal cultured autologous MSC	No recurrence of radiation inflammatory waves during 11 mo f/u	Lataillade et al. 2007
MSC	Interventional nonblinded prospective study	Acute wound Chronic wound (lower leg)	5 8	BM-derived cultured MSC delivered in fibrin spray	No control group but detected correlation between surface density of MSC and wound reduction	Falanga et al. 2007
MSC	Interventional prospective study	Variety nonhealing wounds	20	Cultured autologous BM-derived MSC	Wounds healed in 18/20 patients	Yoshikawa et al. 2008
MSC	Randomized controlled study	Chronic ulcer lower extremity (diabetic and Buerger's)	24	Cultured autologous BM-derived MSC; single IM injection + topical application to wound	Significant improvement in pain-free walking and reduction in wound size by 72% compared with control	Dash et al. 2009
MSC	Interventional nonblinded prospective study	Adult RDEB	2	Intradermal allogeneic BM-derived MSC	Increased epithelialization at wound site; clinical benefit for 4 mo; de novo C7 expression	Conget et al. 2010
MSC	Case report	Severe radiation burns	1	Intradermal cultured autologous MSC	No inflammatory complications during 8 mo f/u	Bey et al. 2010
MSC	Double-blind randomized controlled trial	Diabetic critical limb ischemia and ulcer	41	Autologous cultured BM-MSC versus fresh uncultured BM-mononuclear cells versus control (single IM injection)	Fastest healing time and improvement in pain-free walking seen in MSC-treated group	Lu et al. 2011

Continued

Table 1. Continued

Cell type	Study type	Indication	Patient numbers	Components	Effect on wound healing	References
MSC	Case report	Hypertrophic scar following burn	1	Intradermal BM-MSD + split skin graft	Reduction of skin graft contracture compared with control site (graft only) at 2 yr f/u	Xu et al. 2012
MSC	Prospective interventional study	Sclerodermatous GvHD	4	Intra-BM injection of BM-MSD derived from unrelated donor; multiple infusions	Overall cutaneous improvement including healing of ulcers, skin lesions, less desquamation and edema, with greater joint mobility	Zhou et al. 2010
Adipose-derived stem cells						
ADSD	Multicenter phase I/IIa clinical trial	Complex perianal fistula–Crohn’s disease	24	Cultured allogeneic adipose-derived stem cells applied intralesionally	56% achieved closure of fistula at week 24; no adverse effects	de la Portilla et al. 2012
Bone marrow transplant						
BM	Prospective interventional study	Chronic wounds > 1 yr	3	Unfractionated BM, derived cells directly applied to wound	Complete wound closure in all patients; dermal rebuilding evident on histology	Badiavas and Falanga 2003a,b
BMT	Interventional study	Pediatric Herlitz JEB	1	Intravenous whole BMT (matched related donor) with conditioning and skin graft from matched donor	80% graft uptake; patient death caused by infection and severe disease phenotype	Kopp et al. 2005
BMT	Phase I clinical trial	Pediatric RDEB	6	Intravenous whole BMT (matched related donor) with myeloablative conditioning	All show clinical improvement; 5/6 patients show increased levels C7 at DEJ	Wagner et al. 2010

BM	Prospective interventional study	Chronic diabetic wounds	8	BM-derived cells (TNC fractionated BM) applied to wound edge + platelets, fibrin glue and BM impregnated collagen matrix	Complete wound closure in 3/8 patients; reduction in wound size in remaining	Ravari et al. 2011
BMT + MSC	Phase I nonrandomized interventional study	Pediatric RDEB	9 (7 underwent conditioning)	Intravenous whole BMT and allogeneic MSC	4/9 survived 12 mo posttransplant; all showed clinical improvement; increased levels of C7 in three patients	Tolar et al. 2012c
		Pediatric JEB–Herlitz	3 (2 underwent conditioning)		1/3 patient survived 12 mo posttransplant; increased levels of L332 in one patient	
BMT + MSC	Phase I nonrandomized interventional study	RDEB (0.7–20 yr)	18 (13 myeloablative conditioning/5 nonmyeloablative conditioning)	Intravenous whole BMT and adjuvant allogeneic MSC therapy	Nonmyeloablative conditioning well tolerated; BM preferred to UC transplant; 11 patients demonstrating partial to marked biochemical and clinical improvement in mucocutaneous disease on the basis of C7 expression, body surface area affected, and resistance to blistering	Tolar et al. 2013

TEN, toxic epidermal necrolysis; ADSC, adipose-derived stem cells; CDS, cultured dermal substitute; GvHD, graft versus host disease; HSCT, hematopoietic stem cell transplantation; MSC, mesenchymal stromal cell; BM, bone marrow; RDEB, recessive dystrophic epidermolysis bullosa; C7, type VII collagen; BMT, bone marrow transplant; JEB, junctional epidermolysis bullosa; QOL, quality of life; RCT, randomized control trial; EBS, epidermolysis bullosa simplex; IM, intramuscular; TBSA, total body surface area; TNC, total nucleated cells; DEJ, dermal–epidermal junction; CEA, cultured epithelial autograft; f/u, follow-up.

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keratinocytes and dermal fibroblasts (Stanley et al. 1985; Regauer et al. 1990; Woodley et al. 2003; Goto et al. 2006; Ito et al. 2009). Given that fibroblasts are much easier to isolate and maintain in culture than keratinocytes, fibroblasts present an attractive target for cell-based therapies for RDEB. Normal and *COL7A1*-gene-corrected human RDEB fibroblasts overexpressing C7 have been injected intradermally into immunodeficient mouse skin or transplanted human RDEB skin equivalents (Ortiz-Urda et al. 2003; Woodley et al. 2003). Both these interventions led to sustained human C7 deposition and new anchoring fibril formation at the DEJ. It was also shown that intradermal injections of wild-type fibroblasts, that is, without *COL7A1*-gene correction, could correct the RDEB skin pathology, but only when injected at sufficient cell density (Woodley et al. 2003). Increase in C7 at the DEJ after intradermal injections of wild-type human fibroblasts was also confirmed in studies using a hypomorphic C7 mouse model of RDEB that expresses C7 at ~10% of normal levels (Fritsch et al. 2008; Kern et al. 2009). Moreover, intravenously injected wild-type or *COL7A1*-gene-corrected human RDEB fibroblasts could home to wounded mouse skin and improve wound healing (Woodley et al. 2007). Collectively, these studies provide a rationale for the use of allogeneic wild-type or *COL7A1*-gene corrected fibroblasts in human RDEB studies.

FIRST-IN-MAN USE OF FIBROBLASTS IN DYSTROPHIC EB

Based on the mouse data, a proof-of-concept study in five RDEB subjects showed that a single intradermal injection of allogeneic fibroblasts (5×10^6 cells in 25- μ l volume injected into the superficial dermis over an ~1 cm² area) increased *COL7A1* gene expression for at least 3 mo (the end point of that study) in most individuals (Wong et al. 2008). The study also showed the low immunogenicity of allogeneic fibroblasts and lack of host response at an immunological and histological level. The injected cells were not detectable at 2 wk postinjection, the time point at which an increase in C7 protein at the DEJ was seen. Of note, the increase in C7

was most apparent in RDEB subjects who had some baseline expression of C7 compared to those who had a complete absence of the protein. However, the increase in C7 was not accompanied by the formation of new, normal-appearing anchoring fibrils; there was an increase in the number of fibrillar structures below the lamina densa of the basement membrane, but these were somewhat wispy and lacked characteristic ultrastructural features of mature anchoring fibrils. Nevertheless, there was some subjective evidence for increased epidermal–dermal adhesion following the allogeneic fibroblast injections. Given the increase in *COL7A1* gene and C7 protein expression beyond the physical presence of the injected allogeneic cells, however, the changes were thought to be caused by a paracrine effect induced by the fibroblasts. A subsequent study was able to show that a single injection of allogeneic fibroblasts could increase *COL7A1* gene expression for 3–6 mo and C7 protein for 9–12 mo (Nagy et al. 2011).

MODE OF ACTION OF ALLOGENEIC FIBROBLAST CELL THERAPY IN DYSTROPHIC EB

Transcriptomic analysis of serial skin biopsies following injection of allogeneic fibroblasts in one subject with RDEB revealed that expression of heparin-binding epidermal growth factor-like growth factor (*HB-EGF*) was up-regulated and that expression levels mirrored those seen for the *COL7A1* gene (Nagy et al. 2011). In vitro studies were able to show that HB-EGF could up-regulate *COL7A1* gene expression in both normal-control and RDEB keratinocytes and fibroblasts, and this was preceded by increased expression of *JUN* and *FOS*, components of the AP1 transcription factor that are known to bind to and up-regulate *COL7A1*. The interpretation of the in vivo situation was that allogeneic fibroblasts were able to induce up-regulation of HB-EGF, predominantly in neighboring keratinocytes. The HB-EGF could then lead to a sustained increase in C7 by acting in an autocrine, paracrine, and juxtacrine manner to up-regulate *COL7A1*. The new C7 was thought to be predominantly recipient-derived, that is, mutant

but partially functional. A slight increase in *HB-EGF* and *COL7A1* gene expression was also noted after injection of saline into skin, although up-regulation was less marked than following fibroblast injection and was for a shorter duration. It is also plausible that the donor fibroblasts could release wild-type full length C7 for incorporation into the DEJ during the time (few days) the cells are present in the RDEB skin. Evidence for this “direct release” theory is supported by studies in mice (Kern et al. 2009), although both mechanisms are not mutually exclusive. Although often thought as differentiated somatic cells, cultured skin fibroblasts may also contain a subpopulation of cells with stem cell properties, capable of differentiation into mesoderm, endoderm, and ectoderm. Termed MUSE (multi-lineage-differentiating stress-enduring) cells, these cells are also found in the skin and BM (Wakao et al. 2011).

FIBROBLAST CELL THERAPY AND WOUND HEALING IN DYSTROPHIC EB

The initial studies of allogeneic fibroblast injections in RDEB focused on responses at the DEJ in intact skin. However, the major clinical burden in affected individuals is poor wound healing. One randomized double-blind study that assessed the impact of allogeneic fibroblasts in wound healing in RDEB showed no differences in the extent or rate of re-epithelialization of chronic erosions following injection of fibroblasts or inert carrier solution (Venugopal et al. 2013). In contrast, another clinical trial found that a single injection of allogeneic fibroblasts can speed up wound healing for 28 d compared to vehicle (Petrof et al. 2013). The putative up-regulation of HB-EGF following allogeneic fibroblast injection (Nagy et al. 2011) may have benefits for wound healing beyond up-regulation of C7, given that HB-EGF has been shown to promote keratinocyte migration (Shirakata et al. 2005). However, one caveat may be the effects of HB-EGF on epidermal growth factor receptor signaling and risk of malignancy (Zenz et al. 2003). Given that individuals with RDEB have an increased incidence of squamous cell carcinoma, especially in chronic wounds (Fine

and Mellerio 2009a), further safety monitoring of allogeneic fibroblasts injected into RDEB wounds is required. At the bedside, allogeneic fibroblasts have been used off licence to treat a number of patients with RDEB (Fig. 1). The clinical impression and experience indicates that for a subgroup of individuals with RDEB, notably those with mild to moderate disease severity and some baseline expression of C7 at the DEJ, allogeneic fibroblast therapy may be useful, in contrast to those individuals with more severe disease that lack C7 expression. For the latter individuals, alternative cell therapy or other strategic approaches may be more effective.

BM STEM CELLS AND THE SKIN

BM stem cells have a fundamental role in generating erythrocytes, leukocytes, and platelets, but also show plasticity in being able to show lineage differentiation into tissues of mesodermal, endodermal, and ectodermal origin, including skin (Grove et al. 2004). Some subpopulations of BM cells can differentiate into keratinocytes (Badiavas et al. 2003a; Borue et al. 2004; Fathke et al. 2004), and in humans who have undergone bone marrow transplanta-

Figure 1. Intradermal injections of allogeneic fibroblasts promote wound healing in RDEB. A stubborn wound on the side of the neck in this 23-yr-old man with RDEB showed limited improvement and persisting erosions after skin grafting. However, 8 d after fibroblast injection there was significant healing of erosions and improved quality of life; he was able to wear a shirt without discomfort. The clinical benefits of the fibroblast injections lasted for 5–6 mo.

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tion (BMT), donor cells that have differentiated into keratinocytes can be detected in the epidermis for at least 3 yr (Korbling et al. 2002). Further studies have shown that the BM is also a source of fibroblast-like cells in the dermis (of hematopoietic and mesenchymal lineages) and that the number of these cells increases after skin wounding (Fathke et al. 2004; Ishii et al. 2005; Sasaki et al. 2008). BM cells also contribute to skin development; infusion of BM cells in utero in mice leads to accumulation of a subpopulation of these cells in the dermis, particularly around developing hair follicles (Chino et al. 2008). Overall, however, BM-derived keratinocytes appear to be an extremely rare finding, perhaps contributing only $\sim 0.0001\%$ – 0.0003% of all cells in the new epidermis (Tamai et al. 2011). The physiological role of BM cells in epithelial regeneration therefore has been questioned, although recent studies focusing on RDEB have started to provide new insight into key BM cells and mechanisms germane to skin repair and regeneration.

MURINE STUDIES OF BM CELLS AND SKIN REPAIR

Initial attempts to use BM cells to correct the inherent skin fragility of RDEB were made in the *Col7a1* knockout mouse that recapitulates some of the features of the human disease (Heinonen et al. 1999; Tolar et al. 2009). Nonmanipulated BM failed to rescue the RDEB mice, but alternative use of the signaling lymphocyte activating molecule (SLAM)-positive subpopulation of BM cells resulted in new C7 and presence of donor cells at the DEJ. Following infusion of SLAM-positive BM cells, there was improved healing of blisters on the mouth and paws, and histological evidence of rudimentary anchoring fibril formation (Tolar et al. 2009). Other studies have also shown correction of RDEB skin defects with BM cells (Fig. 2), that repair can occur after in utero infusion of BM cells (Chino et al. 2008), and BM-derived mesenchymal stromal cells (MSCs) can stimulate secretion of C7 with partial restoration of the damaged basement membrane zone and less blister formation (Alexeev et al. 2011). In a different mouse that

Figure 2. BM cells can correct lack of C7 in RDEB mouse skin. Grafting of mouse skin lacking C7 onto a mouse with green fluorescent BM leads to some BM cells entering the epidermis and follicular epithelium. Moreover, new basement membrane C7 (red) is noted in proximity to these green cells. Scale bar, 25 μ m. (This figure is based on data reported by Tamai et al. 2011.)

lacks type XVII collagen, a model for non-Herlitz junctional EB, both hematopoietic and non-hematopoietic BM stem cells have been shown to correct basement membrane protein expression and lead to phenotypic rescue (Fujita et al. 2010). Collectively, these murine studies provide a scientific rationale for translation of BM cell therapies into human clinical trials. Moreover, several additional mouse models for different forms of EB have been developed recently that will also be useful for assessing BM cell therapy, including the hypomorphic C7 mouse (Fritsch et al. 2008) and a hypomorphic model for non-Herlitz junctional EB with a spontaneous mutation in the *Lamc2* gene (Bubier et al. 2010).

FIRST-IN-MAN STUDIES OF MSC THERAPY IN DYSTROPHIC EB

The use of intradermal MSC therapy was first reported in two patients with RDEB in 2010 (Conget et al. 2010), although successful topical use of BM cells to heal chronic wounds in other diseases has been reported previously (Badiavas and Falanga 2003b; Humpert et al. 2005; Falanga et al. 2007). The MSCs used in the RDEB study were derived from the BM of a healthy, unrelated individual. The RDEB subjects had a complete

absence of C7 expression at the DEJ on immunofluorescence microscopy and clinically had severe blistering and wounds that healed slowly with scarring. The investigators injected 0.5×10^6 MSCs or vehicle into both intact and chronically ulcerated sites (Fig. 3). Skin biopsy was performed 1 wk after intervention and showed that the MSC-treated skin expressed C7 at the DEJ. Moreover, there was re-epithelialization of chronically ulcerated skin near the MSC injection site, but not near the vehicle injected site. Both patients in the study showed clinical benefits that lasted for 4 mo. Thereafter, the skin fragility resembled the pretreatment state. No adverse effects were noted in the injected skin or systemically. This pilot study showed that the intradermal administration of allogeneic MSCs can lead to de novo C7 expression at the DEJ as well as preventing blistering and improving

wound healing in patients with RDEB. A clinical trial using intravenous unrelated donor BM-derived MSCs in subjects with RDEB has recently been performed (M El Darouti, pers. comm., 2012). Although no skin biopsies were performed in that study, clinical evaluation reported a reduction in the number of blisters, less skin inflammation, better wound healing, and improved quality of life, which lasted for 4–6 mo following a single infusion of MSCs. Further evaluation of systemic MSCs in RDEB is anticipated.

BMT IN DYSTROPHIC EB

Following the effectiveness of BM stem cells in murine RDEB, a clinical trial of whole BMT was performed in children with RDEB (Wagner et al. 2010). The approach used high-dose chemotherapy to immunoablate individuals with RDEB to permit more reliable lymphohematopoietic engraftment. The BM was not filtered so that all the hematogenous populations could be preserved, allowing for the possibility that non-hematopoietic MSC populations might also be needed for clinical benefits. Seven patients entered the trial and six underwent BMT. All individuals had some clinical improvement and five of the six showed increased C7 at the DEJ (Fig. 4). The first patient in the trial is now 5 yr since transplantation. Before the BMT, each individual had more than 50% of their skin covered with blisters and erosions. Three of the six individuals showed a dramatic clinical improvement, such that <10% of the body surface area remained affected. In the other three subjects, there was a moderate improvement with less than 25% of the body surface area affected. Despite the clinical improvement, some toxicity was noted. One patient died before the BMT because of heart failure, possibly related to cyclophosphamide toxicity and pre-existing renal failure. Another individual died 6 mo after transplantation because of infection secondary to graft failure. Translational research is now attempting to refine BMT protocols to consider use of reduced intensity conditioning and perhaps subpopulations of BM cells once mechanisms of action in repairing defective EB skin

Figure 3. Healing of a chronic erosion in RDEB is enhanced by intradermal injection of allogeneic MSCs. Injecting third-party BM-derived MSCs, as shown in the day 0 image (top left), leads to re-epithelialization and clinical improvement seen at day 7 and day 64. The clinical benefits were sustained for 4–6 mo. (The original images were kindly provided by Dr. Francis Palisson, based on data reported by Conget et al. 2010.)

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Figure 4. Whole BMT results in clinicopathological correction of the skin pathology in RDEB. This 15-mo-old child shows a marked improvement with healing of chronic wounds and fewer blisters 100 d after BMT. There is also increased C7 at the DEJ (arrows). Scale bar, 25 μ m. (The original images were kindly provided by Dr. Jakub Tolar, based on data reported by Wagner et al. 2010.)

are better known. Clinical application of BMT is also being expanded to Herlitz and non-Herlitz junctional EB, although RDEB remains the predominant clinical indication for now.

MODE OF ACTION OF SKIN REPAIR IN DYSTROPHIC EB FOLLOWING BMT

The precise mode of action of BMT leading to clinicopathological improvement in RDEB is not known. High levels of chimerism (donor and recipient cells) were noted in skin after BMT, with donor nonhematopoietic cells being the probable source of new C7 at the DEJ. Notably, although there were some hematopoietic cells in the skin ($CD45^{+}$), the majority were nonhematopoietic, nonendothelial cells ($CD45^{-}$, $CD31^{-}$). In spite of the increased C7, however, there was a lack of normal anchoring fibrils on transmission electron microscopy. Although the lack of these adhesion structures may relate to gradual maturation of new anchoring fibrils (as has been observed for several months following burns), it is plausible that some of the increased C7 may reflect an up-regulation of mutant but partially functional en-

dogenous C7, similar to the proposed mode of action for allogeneic fibroblast therapy. Clearly, however, there is more to the reparative story in that the transplanted RDEB patient who did not show any increase in C7 also showed a sustained clinical response in skin healing and integrity at the DEJ. This may indicate the incorporation or availability of additional extracellular matrix proteins that can contribute to better skin repair and adhesion, although such a possibility remains speculative. Functional improvement in skin adhesion in patients following BMT was shown by progressive increases in suction blister times (the time it takes to form a blister following placing a negative-pressure suction cup on the skin surface). Although mode of action of BMT in skin repair in EB continues to be investigated, current clinical data provide evidence for a sustained functional improvement in the majority of patients.

EPITHELIAL PROGENITORS WITHIN SUBPOPULATIONS OF BM STEM CELLS

A recent study has shown that BM contains a specific population of epithelial progenitors and

that these are mobilized and recruited by certain key factors. Tamai et al. (2011) used an irradiated mouse model in which green fluorescent protein-labeled BMT was performed. Subsequent skin grafting in this mouse was able to identify that BM-derived keratinocytes are not of hematopoietic origin, but instead are derived from a specific subpopulation of lineage-negative, platelet-derived growth factor receptor α -positive ($\text{Lin}^-/\text{PDGFR}\alpha^+$) BM cells. This is still a somewhat heterogeneous cell population that represents approximately 1 in 450 BM cells. Indeed, these cells may show lineage overlap with other defined subpopulations of MSCs, including multipotent adult progenitor cells, $\text{Lin}^-/\text{SCAR1}^+/\text{CD45}^-$ very small embryonic-like stem cells in BM, CD45^- unrestricted somatic cell stems in cord blood, and nestin-positive cells that maintain the stem cell niche with hematopoietic stem cells in BM. The study by Tamai et al. (2011) proposed that the skin graft acted as a hypoxic bioreactor, rapidly releasing the nonhistone nuclear protein high-mobility group box-1 (HMGB1). Following skin grafting, HMGB1 levels in serum increased and HMGB1 was shown to mobilize these cells along an HMGB1 concentration gradient to the area of hypoxic keratinocytes. Differentiation of these cells into keratinocytes was clearly shown. The study also showed that in RDEB the main source of HMGB1 was hypoxic keratinocytes in blister roofs. The implication of this study is that there is a subpopulation of nonhematopoietic BM cells comprising $\sim 0.22\%$ of the BM population that has the capacity to restore defective epithelium, including targeting and correcting the pathology of RDEB.

BMT FOR OTHER INHERITED SKIN DISEASES

BMT has been used as a form of stem cell therapy for more than 40 years and is really the only stem cell therapy with a definite therapeutic benefit. Although the initial applications were for leukemia, lymphoma, aplastic anemia, and hemoglobinopathies, the clinical indications for BMT have expanded and approximately 50,000 people worldwide have received this form of therapy. As well as clinical trials in

RDEB, BMT is being investigated in other forms of EB including Herlitz junctional EB (Tolar et al. 2012b). One further consideration in Herlitz junctional EB, however, is that the defective protein (laminin-332) is not synthesized by fibroblasts (unlike C7); it is only generated by keratinocytes. Thus, whether corrective donor cells in the dermis can secrete the defective basement membrane protein is uncertain. Perhaps correction of epithelial cells is essential in this form of EB. Nevertheless, early studies have indicated that BMT may increase laminin-332 expression at the DEJ in some individuals. It is still too early, however, to reflect on the clinical indications and possible benefits from this treatment. The same caveats exist for epithelial synthesized proteins germane to non-Herlitz junctional EB in which either laminin-332 or type XVII collagen may be defective, although the clinicopathological improvement seen in type XVII collagen-deficient mice after BM cell therapy provides some justification for human studies (Fujita et al. 2010). Gradually, therefore, it seems likely that other forms of EB associated with severe morbidity may be evaluated in clinical trials of BMT. Conceptually, this form of cell therapy should still be regarded as a highly experimental procedure.

UMBILICAL CORD BLOOD TRANSPLANTATION AND THE SKIN

Cord blood (CB) and other parts of the umbilical cord, such as the Wharton's jelly or tissues associated with the placenta, are rich sources of stem cells. Like hematopoietic stem cells, CB is an important source of other progenitor cells, as well as MSCs, very small embryonic-/epiblast-like stem cells and unrestricted somatic stem cells, which may have individual or collective value in regenerative medicine. Using some stem cell populations, notably the MSCs, a number of clinical studies have been initiated, mostly to repair tissue, including some studies on skin. The first transplant in humans using CB was reported in 1989 with the use of human leukocyte antigen-matched sibling CB to treat Fanconi anemia (Gluckman et al. 1989). Subsequently, CB banks were established for the

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collection and cryopreservation of cells and more than 20,000 CB transplants have been performed worldwide. MSCs from CB are similar to BM-derived MSCs in having low immunogenicity and are already being used in several studies of regenerative medicine. That said, comparison of umbilical cord cells versus BM stem cells in individuals with RDEB has shown better skin engraftment with a BM-derived population (Tolar et al. 2012a) and, therefore, the clinical utility of cord cells in EB or other skin disorders remains to be determined in future clinical trials. Recent, preclinical data, however, have shown that unrestricted somatic stem cells can express C7 and accelerate wound healing, including improving the quality of wound healing, as evidenced by formation of new skin appendages (Liao et al. 2013).

REVERTANT MOSAICISM AND SPONTANEOUS NATURAL CELL THERAPY

One striking observation in recent years has been that some patches of skin in patients with various inherited skin diseases can under-

go spontaneous correction of the genetic defect underlying the skin pathology, a concept known as revertant mosaicism or “natural gene therapy.” This was first noticed in the late 1990s in a patient with non-Herlitz junctional EB resulting from autosomal recessive mutations in type XVII collagen (Jonkman et al. 1997). Revertant mosaicism is a naturally occurring process characterized by spontaneous genetic repair that can lead to a partial or complete reversal of an affected to a wild-type phenotype (Fig. 5). In addition to non-Herlitz junctional EB, other variants of EB including EB simplex, RDEB, and Kindler syndrome have all shown evidence of revertant mosaicism (Lai-Cheong et al. 2011). Likewise, skin diseases such as ichthyosis with confetti and dyskeratosis congenita have also been shown to display this phenomenon. Indeed, a recent study has suggested that in vivo reversion may occur in all patients with generalized non-Herlitz junctional EB (Pasmooij et al. 2012). The predominant mechanisms of gene correction include back mutation, gene conversion, intragenic recombination, and second-site mutation. The genetic correction appears to

Figure 5. Revertant mosaicism represents a form of natural gene therapy in RDEB skin. This 40-yr-old man with RDEB has a patch of skin on his right shin that does not blister (unlike the surrounding skin that blisters after minor trauma). Skin from the reverted area shows near-normal intensity C7 immunoreactivity at the DEJ in contrast to the complete absence of C7 in the unreverted skin. Scale bar, 25 μ m. (Images are based on data reported by Almaani et al. 2010.)

be limited to keratinocytes rather than fibroblasts or other cell populations, perhaps reflecting higher proliferation rates in keratinocytes (Jonkman et al. 1997). The main clinical consequence of revertant mosaicism is the phenotypic improvement of disease, although the timing of the reversion may influence the extent of revert mosaicism and the severity of the condition, that is, early reversion of events during embryonic development is more likely to result in larger revertant patches.

REVERTANT CELL THERAPY FOR EB

The opportunity to expand keratinocytes derived from a patch of revertant mosaicism creates a translational opportunity for revertant cell therapy that is personalized and patient specific. The key advantage of this approach is that the cells are naturally corrected and, therefore, additional genetic manipulation should not be necessary. The first attempt at revertant cell therapy was reported in an individual with revertant mosaicism in non-Herlitz junctional EB (Gostynski et al. 2009). Revertant keratinocytes from this patient were isolated, expanded into epidermal sheets, and grafted onto a de-epidermized area of mutant skin. In culture, ~30% of keratinocytes displayed reversion, but, for reasons unknown, fewer than 3% of the corrected cells persisted in the graft and thus clinically, the graft remained fragile and skin integrity did not improve. This case provided proof-of-principle about the feasibility of revertant cell therapy, but showed that further optimization would be required for successful transplantation. Future improvements in graft production and maintenance of reversion in culture might lead to better function in future attempts at skin transplantation. An alternative approach might be to use pinch/punch grafting using skin from the revertant patches and transplanting these pieces of “normal” skin into affected areas within the same individual. For example, this may be clinically useful if reverted skin was seeded adjacent or within chronic erosions to promote wound healing. Whether creating minor injury to the edge of a patch of revertant mosaicism would lead to expansion of area of reverted ke-

ratinocytes is not known; most patches of reversion tend to enlarge initially, but then remain static (Lai-Cheong and McGrath 2013). Although fibroblast culture is considerably easier than keratinocyte culture (especially in patients with EB), the lack of demonstrable revertant mosaicism in fibroblasts means that revertant cell therapy is not possible with this cell population.

FUTURE CELL THERAPY STRATEGIES: I. REFINEMENT OF EXISTING BM CELL PROTOCOLS

Although the majority of RDEB children who participated in the initial BMT trial showed remarkable clinical benefit, two of seven children died (one before BMT) (Wagner et al. 2010). To try to reduce morbidity and mortality, additional protocols have been developed to use reduced intensity conditioning rather than full myeloablation. Modifications to the original protocol, such as the additional subsequent infusion of MSCs from the original donor, have also been adopted. A further issue is whether to use a subpopulation of cells that can specifically repair skin and mucous membranes. For example, use of the $\text{Lin}^-/\text{PDGFR}\alpha^+$ MSCs that were previously shown to harbor epithelial progenitors might be suitable (Tamai et al. 2011). Another consideration may be just to use allogeneic MSCs, as has been trialed in several other diseases because of their anti-inflammatory properties. MSCs can home to damaged tissue and release various immunomodulatory factors that influence the behavior of dendritic cells, T cells, and natural killer cells. MSCs can reduce B-cell proliferation, monocyte maturation, secretion of $\text{IFN-}\gamma$ and $\text{TNF-}\alpha$ while promoting induction of T-regulatory cells and secretion of anti-inflammatory IL-10 from macrophages in direct contact with MSCs. Although the precise microenvironmental contributions to tissue repair are not fully known, a more detailed understanding of the trophic mechanisms associated with MSCs in tissue regeneration is likely to lead to further exploration of clinical utility. In general, whatever immune conditioning or cell population is being planned for use as cell

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therapy, for diseases such as EB, it is evident that treatment that is both systemic and given at an early age is likely to have the most clinical benefit, certainly for patients with widespread disease.

FUTURE CELL THERAPY STRATEGIES: II. TARGETING OF SUBPOPULATIONS OF BM CELLS TO DAMAGED SKIN

The murine wound healing studies performed by Tamai et al. (2011) provide some insight into how skin grafting may work. Grafting a split thickness or full thickness piece of skin to ulcers and burns is widely used in clinical practice and the benefits may be derived from direct restoration of a covering skin barrier. A further reparative mechanism, however, might be through local release of HMGB1 into the circulation with recruitment of $\text{Lin}^-/\text{PDGFR}\alpha^+$ cells from the BM. Large skin grafts might release sufficient HMGB1 into the sera to reach a threshold that is sufficient to mobilize this subpopulation of MSCs from BM. Smaller skin grafts that do not release sufficient HMGB1 to reach threshold would not be expected to have the capacity to mobilize these cells unless additional local or systemic HMGB1 was given. The recruitment of $\text{Lin}^-/\text{PDGFR}\alpha^+$ cells to a skin graft may be dependent on the gradient of HMGB1 (i.e., the local skin levels initially being greater than those in the serum). Although patients with RDEB have been shown to have increased HMGB1 in their blister fluid (released from the damaged keratinocytes in the blister roof) and serum (Tamai et al. 2011), it is not known whether this elevated HMGB1 can mobilize the $\text{Lin}^-/\text{PDGFR}\alpha^+$ cells from the patient's BM, or whether there may be some partial or complete exhaustion of these cells because of the chronic subepidermal skin blistering. BMT strategies aimed at restoring this cell population or promoting targeting of these particular MSCs in patients with RDEB may be helpful in tissue repair, including mobilization of these MSCs from BM or recruitment to damaged skin being augmented by use of recombinant HMGB1. These MSCs may also represent an additional attractive target for *COL7A1* gene therapy.

FUTURE CELL THERAPY STRATEGIES: III. COMPLEMENTARY APPROACHES WITH COMBINATION GENE THERAPY

Although phase I/II clinical trials of cell therapy in EB have been initiated, it is clear that the ideal therapy for EB is unlikely to involve just allogeneic cells. More likely to be clinically useful is a combination of gene, protein, drug, and cell therapies. To that end, some progress is being made, although so far, only one proof-of-principle study has been published using somatic gene therapy in EB (Mavilio et al. 2006). In that study, a retroviral vector was used to insert the *LAMB3* transgene into autologous keratinocytes that were then grafted back as a confluent sheet onto the thigh of an adult male with non-Herlitz junctional EB. One key practical problem was the lack of holoclone stem cells in the patient, which was probably a consequence of long-term skin blistering. Over the subsequent 6 yr, however, the graft has continued to express laminin-332 at the DEJ with functional improvement and lack of blistering. Other studies of gene therapy, notably for RDEB, are at preclinical or early clinical stages mostly involving ex vivo keratinocyte grafting following introduction of a transgene or correction of both keratinocytes and fibroblasts and construction of a skin equivalent. Whether keratinocytes are the ideal cell to target, however, is debatable. Local gene therapy might involve genetic correction of autologous fibroblasts with the reparative transgene, an approach that might avoid the probable pitfalls of wound infection and graft loss using a topical approach in RDEB. Alternatively, if systemic gene therapy is used, it may be more appropriate to target BM cells that include epithelial progenitors or cells that can contribute to skin repair.

FUTURE CELL THERAPY STRATEGIES: IV. COMPLEMENTARY APPROACHES WITH COMBINATION PROTEIN AND DRUG THERAPIES

With regard to protein therapy, this has also been tested with recombinant C7 in small and large animal models of RDEB (Palazzi et al. 2000;

Woodley et al. 2013). Initial studies used C7-null mice showed that intradermally injected recombinant C7 resulted in linear organization of C7 at the DEJ and formation of anchoring fibrils (Remington et al. 2009). Subsequent intravenous infusion of recombinant C7 was shown to home to wounds in murine RDEB models and restore C7 expression and function. Subsequently, a naturally occurring RDEB dog model has been used to test intravenous protein therapy (Palazzi et al. 2000). Although not yet at a clinical stage, it is likely that intradermal, and possibly intravenous, delivery of C7 will transfer into clinical trials shortly. It is also likely that some individuals with EB will benefit from a new group of compounds that have the capability of inducing readthrough of nonsense mutations (Arakawa et al. 2003; Cardno et al. 2009). Several recessive forms of EB involve loss-of-function mutations. Notably, in RDEB, ~15% of all pathogenic mutations involve nonsense mutations (Dang and Murrell 2008) and these are likely to be a focus for readthrough therapeutics. Such drugs can increase mRNA expression by ~5%–10%, leading to increased protein synthesis (Rowe and Clancy 2009). Given that mouse models of RDEB have indicated that it is necessary to have 30%–40% of normal levels of C7 at the DEJ to prevent blistering (Fritsch et al. 2008; Kern et al. 2009), the use of a systemic readthrough drug in appropriate patients may help contribute toward this target and complement other therapies.

FUTURE CELL THERAPY STRATEGIES: V. COMBINING REVERTANT MOSAICISM WITH INDUCIBLE PLURIPOTENT STEM CELL TECHNOLOGIES

One potentially exciting future translation that exploits the therapeutic potential of revertant keratinocytes in different forms of EB and other diseases may be to combine the natural phenomenon of revertant mosaicism with recent stem cell biology techniques, specifically in the creation of inducible pluripotent stem cells (iPSCs). This could be an extremely attractive option because it would avoid the potential genotoxic risk of retroviral vectors needed for gene

therapy. Spontaneously corrected cells for iPSC generation that are derived from revertant keratinocytes avoid the need for further genetic correction. With regard to the skin, iPSCs have recently been generated from keratinocytes isolated from patients with RDEB and fibroblasts derived from individuals with Herlitz junctional EB (Bilousova et al. 2011; Itoh et al. 2011; Tolar et al. 2011, 2013). One translational vision might be to create a bank of iPSCs to differentiate into skin cells for repairing skin wounds, but there are other options. Specifically, one possibility might be to differentiate these cells into the MSCs that contain the population of epithelial progenitors recently identified in BM. These $\text{Lin}^-/\text{PDGFR}\alpha^+$ cells could be given locally to wounded skin to augment wound healing responses. Alternatively, the cells could be given systemically and might be expected to home to areas of hypoxic skin (i.e., wounds) or reside in BM until a future episode of skin trauma occurs, at which time they would be mobilized and recruited to that damaged, hypoxic site. Clinical use of iPSCs in dermatology has not yet been achieved, but remains a future option for consideration.

CONCLUDING REMARKS

Having the capacity and technical ability to culture and expand human keratinocytes and fibroblasts has led to the widespread use of skin grafts to treat chronic wounds and ulcers in both inherited and acquired diseases. Although further refinements in bioengineering and construction of skin grafts are likely to lead to additional clinical benefits for patients, the concept and application of cell therapy for skin diseases recently has expanded to encompass other aspects of regenerative medicine. For inherited skin diseases, the potential to harness and exploit the potential of natural phenomena such as revertant mosaicism (revertant cell therapy), or the plasticity of BM cells to regenerate skin, provide exciting new opportunities to further develop cell therapy in dermatology. Moreover, emerging technologies such as the creation of iPSCs offer an even broader horizon to help deliver clinical benefits from regenerative medicine in dermatology.

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