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Transglutaminase 1 Delivery to Lamellar Ichthyosis Keratinocytes

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ABSTRACT

Therapeutic gene delivery in severe genetic skin disease may require production of a uniformly corrected population of cells capable of regeneration of normal skin elements when returned to the host. To achieve this, we have used lamellar ichthyosis (LI), a disorder of epidermal differentiation recently associated with defects in keratinocyte transglutaminase (TGase1), as a prototype. We have used a high-efficiency retroviral delivery approach to uniformly restore normal levels of TGase1 expression to primary keratinocytes from severely affected LI patients previously lacking TGase1. Delivered TGase1 was correctly targeted to membrane association and restored patient cell transglutaminase activity levels to normal. Corrected primary LI patient keratinocytes also demonstrated restoration of previously defective involucrin cross-linking and *in vitro* measures of cornification to levels found in normal cells. These results indicate that efficient TGase1 delivery to early passage keratinocytes can produce a population of corrected LI patient cells. The capability to produce such cells may provide a basis for future efforts at gene therapy for genetic skin disease.

OVERVIEW SUMMARY

Production and characterization of uniformly corrected primary cells *in vitro* suitable for therapeutic return to patients with monogenic diseases can pose a significant challenge. The recently characterized transglutaminase 1 (TGase1)-deficient disorder, lamellar ichthyosis (LI), offers a prototype for development of this capability using keratinocytes from patients with this severe genetic skin disease. We show here that primary cells from LI patient skin can be rapidly engineered at high efficiency and without subsequent selection *via* a retroviral expression vector for TGase1. It is shown that this gene delivery restores measures of cellular functions to normal. This rapid and high-efficiency approach for corrective gene delivery to primary patient cells *in vitro* may serve as a model for efforts in the skin and other tissues.

INTRODUCTION

THE ICHTHYOSES ARE A HETEROGENEOUS GROUP OF DISEASES characterized by abnormal keratinocyte differentiation leading to excessive scaling of the skin (Williams and Elias, 1987; Bale and Doyle, 1994). Their severity can range from the perpetually flaking "dry skin" seen in mild cases of ichthyosis vulgaris to the severely disfiguring and occasionally life-threatening complications found in cases of lamellar ichthyosis (LI). In LI, the patients are born encased in a "collodian" membrane that is later shed and followed by development of large, thick scales with varying degrees of erythema. Subsequent complications include episodes of sepsis, fluid and electrolyte imbalances due to impaired skin barrier function, and failure to thrive. Current treatments are largely palliative (Williams and Elias, 1987; Bale and Doyle, 1994).

The recent identification of defects in the gene encoding keratinocyte transglutaminase (TGase1) in a number of patients with LI (Huber *et al.,* 1995; Russell *et al.,* 1995) has offered insights into the pathophysiology of this disease. On the basis of genetic linkage and mutation analysis, TGase1 loss in this setting is believed to be causal. TGase1 is expressed in differentiating keratinocytes and catalyzes the formation of N^{ϵ}(γ-glutamyl) lysine bonds involved in cross-linking cornified envelope precursor molecules such as involucrin, loricrin, and small

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proline-rich proteins (SPRs) essential for normal cutaneous barrier function (Rice *et al.*, 1992; Kim *et al.*, 1994). With loss of TGase1 function in the formation of the insoluble cornified envelope, it is believed that the normal skin barrier to environmental irritants and pathogens is disrupted (Lavrijsen *et al.*, 1993; Roop, 1995). Impaired cutaneous barrier function leads to increased transepidermal water loss in this disorder (Lavrijsen *et al.*, 1993), itself capable of triggering cytokine release and epidermal hyperplasia (Williams and Elias, 1993). Defective cornified envelope formation with resulting ineffective skin barrier function, then, may play a vital role in the pathogenesis of LI. Therefore, restoring the capability for normal cornification to LI patient keratinocytes may constitute an important therapeutic goal.

We have characterized primary keratinocytes from severely affected LI patients who lack expression of functional TGase1 protein [TGase1(−)] and are defective in cornified envelope formation. We have then used high-efficiency retroviral gene transfer to express wild-type human TGase1 in >98% of these cells in correct membrane association and demonstrate that this intervention restored patient cell capabilities for cornified envelope formation to levels found in cells from normal skin.

MATERIALS AND METHODS

Cells and cell culture

In preparation for cell culture, 6-mm punch biopsies were taken from patients who showed the severe lamellar ichthyosis phenotype under approval of human subjects protocols at Stanford and UCSF medical centers. Biopsies were stored in SFM media (GIBCO/BRL) with 5 μg/ml amphotericin and 200 μg/ml penicillin/streptomycin until culture. Each biopsy was bisected and a portion used for immunofluorescence with the remainder placed in 1× sterile dispase with 2.5 μg/ml amphotericin 500 units/ml of penicillin/streptomycin. Biopsies were incubated in dispase solution for 1 hr at 37°C and then placed in sterile phosphate-buffered saline (PBS) where epidermis was removed from the dermis using a sterile forceps and placed into 0.05% trypsin and 2% EDTA for incubation at 37°C for 20 min with frequent pipetting to release cells. Remaining dermis was scraped at the epidermal interface to release basal keratinocytes. Trypsin was neutralized with DMEM-10% FCS and cells were then centrifuged at 300× *g* for 5 min. Cell pellets were resuspended in keratinocyte primary culture medium consisting of DMEM, 10% fetal calf serum (FCS), 0.05 μg/ml hydrocortisone, 10 μg/ml epidermal growth factor (EGF), 10^{-10} *M* cholera toxin, 5 μg/ml insulin, 500 units penicillin/streptomycin per ml, and 2.5 μg amphotericin/ml. The cell resuspension was divided and plated on two 100-mm tissue culture dishes that had been preseeded with lethally irradiated 3T3 cells at 30% confluence (Rheinwald and Green, 1975). Cells were left untouched for 5 days, after which they were grown in 50% SFM/50% medium 154 (Cascade Biologics) with supplements and then used for gene transfer studies.

mRNA analysis

Keratinocytes from severely affected LI patients, along with normal control, were grown to confluence; then, calcium was raised to 1.2 m*M* in the culture media for 48 hr. mRNA was prepared and reverse transcriptase polymerase chain reaction (RT-PCR) was performed (Chang *et al.*, 1989; Kutty *et al.*, 1993) using the following primers for human TGase1: 5′-GTGCCTCCACCTGC ATGGCCACATCCTCCG-3′ and 5′-AACGGCGGGGGATGCCATATGG AGGCCGTGGAGAC-3′. All RT-PCR reactions were internally controlled for GAPDH expression using primers for human GAPDH within the same PCR reaction; the sequences of the human GAPDH primers were 5′-GGGGA GCGAGATCCCTCCAAAATCAAGTGGGG-3′ and 5′-GGTCATGAGTCCTTCCACGATACCAAAGTTG-3′.

Retroviral expression vector and gene transfer

The full-length TGase1 cDNA (Phillips *et al.*, 1990) was subcloned as an *Eco* RI fragment into MFG-based LZRS vector and infectious retrovirus prepared with this vector, along with a β-galactosidase (β-Gal) control marker vector, in human BING 293 packaging cells as described (Kinsella and Nolan, 1996). Then, 10 μg of plasmid was transfected into 2×10^6 BING cells *via* calcium-phosphate co-precipitation and cells were selected in 1 μg/ml puromycin for 10 days (Kinsella and Nolan, 1996). Cells were shifted to puromycin-free media for 24 hr and retroviral supernatants were collected as described (Kinsella and Nolan, 1996). Retroviral supernatants were titered and utilized at estimated titers of 5×10^6. Titer estimates were obtained using the LZRS β-Gal marker vector (Kinsella and Nolan, 1996); X-Gal staining of NIH-3T3 cells was used as a reference standard with TGase1 vector compared to marker vectors by semiquantitative RT-PCR in filtered retroviral supernatants. In addition, these data were confirmed by semiquantitative PCR of the isolated genomic DNA of transduced cells, using GAPDH genomic sequences as an internal control. Patient cells were dissociated from culture dishes with trypsin-EDTA and counted with a hemocytometer. A total of 100,000 cells were plated in each 35-mm well of a six-well cluster plate and incubated for 24 hr. Prior to infection, medium was removed and cells washed with sterile PBS. Then, 3 ml SFM/154 with 5 μg/ml Polybrene was added to each well for 5 min and then aspirated from wells prior to infection. Viral supernatant was diluted to 50% with SFM/154 and supplemented with 5 μg/ml Polybrene. Plates were then centrifuged at 300 *g* for 1 hr at 32°C. They were then placed in a 37°C incubator for 24 hr, after which media was changed to 3 ml of fresh SFM/154.

Transglutaminase assay

Transduced cells were grown for 48–72 hr post-transduction prior to addition of calcium to induce keratinocyte differentiation and transglutaminase expression. Forty-eight hours before assay, control and infected normal and patient cells were plated in 1.2 m*M* $CaCl_2$ to induce differentiation. Cells were harvested with 100 μl of 20 m*M* Tris-EDTA 1% Tween 20 and homogenized in a sonicator for 25 sec. A 10-μl amount of the resulting cell extract homogenate was added to achieve a final concentration of 100 m*M* Tris-HCl pH 8.8, 0.5 m*M* dithiothreitol (DTT), 400 μ*M* EDTA, 15 μ*M* putrescine, 400 μ*M* $CaCl_2$, and 0.5 mg/ml dimethylcasein in the presence of 0.5 μCi ^{3}H-labeled putrescine in a final volume of 0.25 ml as described (Rice,

1994). This solution was incubated for 30 min at 37°C. The reaction was stopped with 2.5 ml 12% trichloroacetic acid (TCA) and 1 m*M* putrescine, and the resulting precipitate collected by centrifugation and decanting of the supernatant. Precipitate pellets were resuspended in 5% TCA and 1 m*M* putrescine and suction-filtered onto GF/A Whatman filters. Filters were rinsed three times with 5% TCA and 1 m*M* putrescine wash solution and dried with 3 ml of 95% ethanol. Radioactivity was assessed by liquid scintillation counting; data was normalized for protein concentration. Preparation of membrane-bound particulate and soluble fractions was performed as previously described (Phillips *et al.*, 1993) with the following modifications: after sonication and centrifugation at 2,100 × *g*, membrane pellets were rinsed with ice-cold 2 m*M* HEPES pH 7.2, 2 m*M* EDTA prior to resuspension in 2 m*M* HEPES pH 7.2, 2 m*M* EDTA, 2 m*M* DTT, and 1% Triton-X for transglutaminase assay (Phillips *et al.*, 1993).

Immunofluorescence and laser confocal microscopy

Frozen tissue skin sections were fixed and incubated with the mouse monoclonal antibody to human TGase1, BC.1 (Thacher and Rice, 1985), or polyclonal antisera to the amino and carboxyl termini of human TGase1 (generous gift of R. Rice) and then washed with PBS containing bovine serum albumin (BSA), and treated with fluoroisothiocyanate (FITC)-conjugated fluorescent secondary antibody. Slides were then mounted with DABCO or DABCO/propidium iodide mounting solution and viewed *via* immunofluorescent or confocal microscopy.

Involucrin cross-linking assay

Untreated and engineered LI patient keratinocytes along with normal controls were grown to confluence in tissue culture and then subjected to the involucrin cross-linking assay as previously described (Rorke and Eckert, 1991). Briefly, cells were triggered with calcium to activate transglutaminase activity; cystamine, a potent inhibitor of transglutaminase activity, was included as a negative control along with untriggered cells (Rorke and Eckert, 1991). Extracts prepared from test and control cells were then subjected to denaturing gel electrophoresis and Western analysis utilizing polyclonal antisera to human involucrin (Murphy *et al.*, 1984).

Cornified envelope assay

Untreated and engineered LI keratinocytes from 3 separate LI patients along with cells from patients with normal skin were grown to confluence then calcium added to a final concentration of 1.2 m*M* for 24 hr. Normal keratinocytes grown under such conditions form cornified envelope structures *in vitro* with consistent kinetics (Michel *et al.*, 1988; Hough-Monroe and Milstone, 1991). Cells were grown and differentiated under identical conditions of cell density, calcium concentration, and duration of differentiating stimuli. Triplicate cultures from each patient were then used for cornified envelope assay. Cornified envelopes were prepared under denaturing conditions and counted using phase-contrast microscopy as previously described (Michel *et al.*, 1988; Hough-Monroe and Milstone, 1991).

RESULTS

Recent data suggest that there may be other defects aside from TGase1 loss in a subgroup of LI patients displaying normal levels of functional TGase1 protein in their keratinocytes (Huber *et al.*, 1995). We wished to determine if our LI patients displayed the loss of functional TGase1 expression previously identified in LI as a basis for further studies with gene replacement. Severely affected LI patients diagnosed by clinical and histologic criteria (Williams and Elias, 1987) underwent skin biopsy in accordance with institutionally approved human subjects protocols. Monoclonal and polyclonal antibodies specific for human TGase1 were used in standard immuofluorescence to test for an absence of expression in the outer epidermis when compared to normal (Fig. 1*a*). Laser confocal studies further confirmed the plasma membrane localization of TGase1 in normal skin and its complete absence in this location in our affected patients (Fig. 1*b*). Lack of TGase1 mRNA expression in purified primary keratinocytes from severely afflicted LI patients was found when compared to keratinocytes from normal patients that were isolated and grown under identical conditions (Fig. 1*c*). These findings confirmed a lack of TGase1 expression in the epidermal keratinocytes in our LI patients.

Although a loss of functional TGase1 has been associated with LI, restoration of the ability to cross-link cornified envelope precursors and form cornified envelope structures by TGase1 replacement to TGase1(−) keratinocytes will clarify its role in the pathogenesis of the disease. To accomplish this, we generated retroviral vehicles at high titer using a replicating, episomal approach in human 293 packaging cells (Kinsella and Nolan, 1996) and low-speed centrifugation for high-efficiency TGase1 gene transfer into LI patient keratinocytes. The TGase1 retroviral vector was designed to transcribe full-length human TGase1 from the retroviral long terminal repeat (LTR) to facilitate TGase1 protein expression in the setting of 1.2 mM calcium *in vitro* (Fig. 2*a*). Gene transfer into primary LI cells, as measured by proportion of keratinocytes positive by immunofluorescence for the transferred gene, compared with the total number of cells determined by propidium iodide counterstaining, was achieved with these vectors at efficiencies >98% (Fig. 2*b*). TGase1 transfer was due to transfer of the TGase1 gene, as confirmed by genomic DNA analysis of transduced cells for delivered TGase1 and vector sequences (data not shown). The possibility of direct TGase1 protein transfer to TGase1[−] keratinocytes *via* retroviral supernatant was excluded by Western analysis of vector preparations with monoclonal antibody to TGase1, demonstrating a complete absence of TGase1 protein in the vector preparation (data not shown).

The enzymatic function of TGase1 is believed to serve a key role in cornified envelope formation (Kim *et al.*, 1994) and restoration of its normal biologic effects in TGase1(−) patient epidermis centers on meeting two requirements—the restoration of TGase1 enzymatic activity to physiologic levels and the targeting of this expression to the appropriate subcellular location at the plasma membrane. We expressed wild-type TGase1 in TGase1(−) LI patient cells using the vector above and confirmed restoration of enzymatic activity from low background levels to ±5% of normal levels in unfractionated cell extracts (Fig. 3*a*). Careful standardization of protein concentration, cell

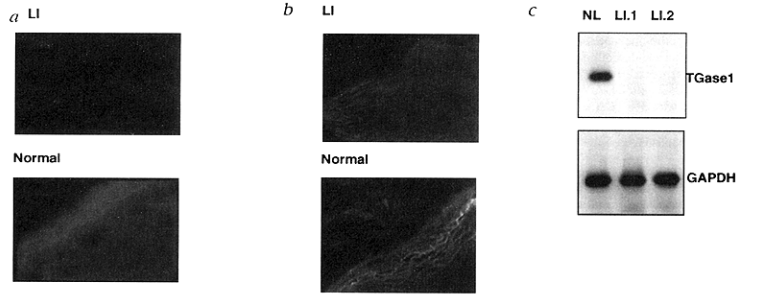


FIG. 1. TGase1 is absent from severely afflicted LI patients. *a.* Skin biopsies obtained from severely afflicted LI patients were analyzed by immunofluorescence microscopy using a monoclonal antibody (Thacher and Rice, 1985) to human TGase1. LI skin (left) demonstrates markedly reduced staining in the outer layers of the epidermis compared with the normal control (right). *b.* Laser confocal immunofluorescence microscopy was performed to detect any TGase1 expression at the plasma membrane or in other subcellular locations. LI skin exhibits an absence of plasma membrane TGase1 staining while normal skin demonstrates prominent staining around cellular borders. *c.* Internally controlled RT-PCR was performed using mRNA isolated from keratinocytes of normal (NL) and severely affected LI patients (LI.1, LI.2). Using primers for human TGase1 and GAPDH, LI patient keratinocytes grown in 1.2 m*M* calcium demonstrate a lack of TGase1 mRNA expression, compared with normal control.

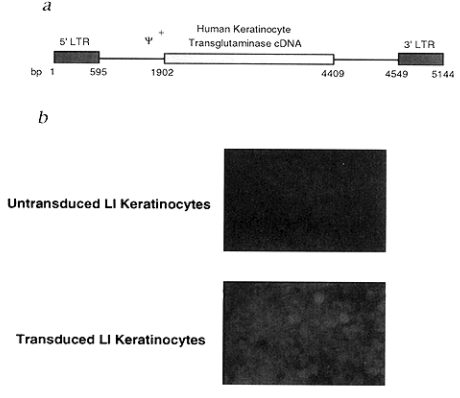
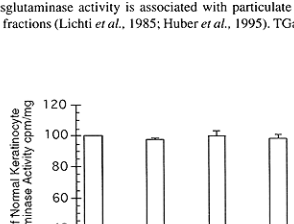


FIG. 2. High-efficiency restoration of TGase1 protein expression in LI patient cells. *a.* A TGase1 expression vector was constructed using the full-length human TGase1 cDNA minus 3′ polyadenylation sequences in an MFG-based retroviral construct (Riviere *et al.*, 1995). This construct, along with the LZRS β-Gal marker construct (Kinsella and Nolan, 1996) was utilized to produce amphotropic retrovirus for TGase1 expression. *b.* Gene transfer into primary keratinocytes from LI patients was accomplished using retroviral vehicles followed by immunofluorescence staining to ascertain gene transfer efficiency. Shown is representative immunofluorescence staining for the expressed gene. Efficiency of gene transfer was calculated by determining the number of cells positive by immunofluorescence as a percentage of total cells present was determined by propidium iodide counterstaining.

density, and calcium treatment was used to produce uniform cell populations and these experimental factors, plus the fact that TGase1 expression may be regulated at the post-transcriptional level, may account for the tight clustering of enzymatic activity around normal levels. To confirm targeting of expressed TGase1, extracts of membrane bound and soluble protein were made (Lichti *et al.,* 1985) and TGase1 enzymatic activity assayed in each protein fraction. In normal keratinocytes, 75–95% of transglutaminase activity is associated with particulate cell extract fractions (Lichti *et al.,* 1985; Huber *et al.,* 1995). TGase1

a



b

	% of Total TGase1 Enzymatic Activity	
	Membrane Fraction	Soluble Fraction
Normal	75±3	25±1
LI+	81±1	19±1

c

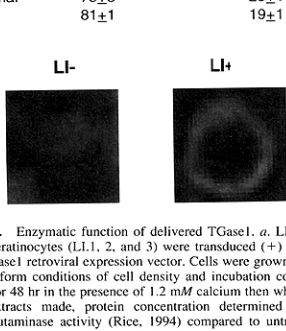
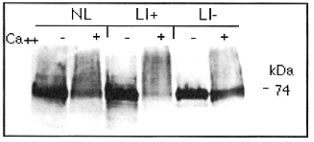


FIG. 3. Enzymatic function of delivered TGase1. *a.* LI patient keratinocytes (LI.1, 2, and 3) were transduced (+) with the TGase1 retroviral expression vector. Cells were grown under uniform conditions of cell density and incubation conditions for 48 hr in the presence of 1.2 m*M* calcium then whole-cell extracts made, protein concentration determined and transglutaminase activity (Rice, 1994) compared to untransduced (−) and to normal cells (NL). The results shown are the result of five separate gene transfer experiments for each. *b.* Membrane and cytosolic extracts were made of the cell populations noted in *a* and TGase1 activity determined for each protein fraction. *c.* Laser confocal immunofluorescence microscopy was performed using antibodies to human TGase1 to confirm further the subcellular localization of transduced TGase1 in infected LI keratinocytes (LI+) versus untreated (LI−) when both were grown *in vitro* in the presence of 1.2 m*M* calcium.

membrane localization, is mediated by fatty acid thioesterification dependent on a cluster of 5 cysteine residues at the amino-terminal portion of TGase1 (Phillips *et al.,* 1993), with subsequent processing of TGase1 into smaller subunits (Steinert and Marekov, 1995). Corrected LI patient cells demonstrated TGase1 activity in the particulate membrane fraction in a proportion similar to normal keratinocytes (Fig. 3*b*). This biochemical result suggesting plasma membrane localization of delivered TGase1 was confirmed by laser confocal microscopy with antibodies to human TGase1. LI cells receiving the TGase1 vector demonstrated a ring of staining around the cell border while untreated cells showed only the background staining seen with secondary antibody alone (Fig. 3*c*); the corrected cell pattern was similar to that seen in normal cells (data not shown). These results indicate that physiologic amounts of enzymatically active TGase1 are expressed in correct subcellular localization by this approach in LI patient cells and raise the possibility that this intervention may restore capability for cross-linking the substrates necessary for normal cornified envelope formation.

After obtaining evidence of restoration of TGase1 expression to formerly TGase1(−) LI patient primary keratinocytes, we wished to determine if these restored patient cells had regained the ability to perform features of cornified envelope formation. Cornified envelope formation involves cross-linking of precursor molecules, such as involucrin, into the specialized envelope structure that replaces the plasma membrane of terminally differentiating keratinocytes (Roop, 1995; Steinert and Marekov, 1995). Involucrin exists as a soluble 68-kD protein in suprabasal keratinocytes and its cross-linking by TGase1 into the insoluble, higher-molecular-weight constituents of the cornified envelope in keratinocytes is associated with its disappearance from the 68-kD position on Western analysis (Rorke and Eckert, 1991). We compared the ability of TGase1(−) *versus* transduced LI patient keratinocytes from the same patient with normal human cells in achieving involucrin cross-linking by this method. Transduced LI keratinocytes regained the ability to cross-link involucrin into insoluble higher-molecular-weight complexes at levels comparable to normal whereas untransduced LI cells retained the majority of the non-cross-linked involucrin band (Fig. 4*a*). Formerly TGase1(−) LI patient keratinocytes, then, regain the ability to cross-link a physiologic TGase1 substrate important in cornified envelope formation after TGase1 gene transfer.

Cornified envelope formation is markedly defective in LI (Hohl *et al.,* 1993). After establishing restoration of cross-linking activity on a specific TGase1 substrate in previously TGase1(−) LI cells, we wished to extend the studies to determine if a more global measure of cornification *in vitro,* cornified envelope formation, had also been restored to normal. Keratinocytes grown to confluence *in vitro* and then incubated in media with 1.2 m*M* calcium form cornified envelope structures with predictable kinetics. These cornified envelopes resemble envelopes purified from biopsy specimens of intact skin *in vivo,* both biochemically and microscopically (Michel *et al.,*1988; Hough-Monroe and Milstone, 1991). Therefore, we examined this *in vitro* measure of cornification in TGase1(−), transduced and normal cells *via* standard assay previously correlated with direct quantitation of insoluble cross-linked protein (Fig. 4*b*) (Michel *et al.,* 1988; Hough-Monroe and



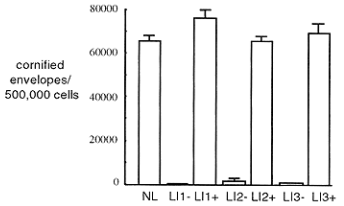


FIG. 4. Restoration of features of normal cornification to LI cells. *a.* An involucrin cross-linking assay (Rorke and Eckert, 1991) was performed using normal (NL), uncorrected (−) and corrected (+) LI keratinocytes treated or untreated with calcium and sodium chloride. Cellular extracts were subjected to Western analysis using an antiserum specific for human involucrin (Murphy *et al.,* 1984); involucrin cross-linking is demonstrated by incorporation of the involucrin band into a higher-molecular-weight smear. *b.* A total of 5×10^5 uncorrected LI (−), corrected LI (+) or normal keratinocytes were incubated in 35-mm plates under uniform conditions of cell density with media containing 1.2 m*M* calcium to promote differentiation and cornification. Insoluble cornified envelopes were prepared under denaturing conditions as described (Michel *et al.,* 1988; Hough-Monroe *et al.,* 1991) and quantitated by phase-contrast microscopy; data are expressed as the number of cornified envelopes per 500,000 cells ± SEM.

Milstone, 1991). Restoration of TGase1 expression to formerly TGase1(−) LI keratinocytes was associated with reversion in the numbers of cornified envelopes from low levels in untreated LI cells to values in the normal range. This finding provides additional confirmation for the role of TGase1 in cornified envelope formation and extends the utility of this established assay in examining functional correction of defects in cornification *in vitro.* These data indicate that the high-efficiency restoration of physiologic levels of TGase1 to formerly TGase1(−) LI patient keratinocytes restores these *in vitro* indicators of cornified envelope substrate cross-linking and formation to levels seen in keratinocytes from individuals with normal skin.

DISCUSSION

In this report we provide evidence for high-efficiency expression of human TGase1 at the correct subcellular location in formerly TGase1(−) LI patient keratinocytes and demonstrate that this expression restores cornified envelope formation *in vitro* to normal. These results provide additional support for a central role for TGase1 in both the etiology and potential future therapy of LI. They also indicate that other transglutaminases expressed in keratinocytes, such as epidermal transglutaminase, fail to compensate for the biologic activity restored by re-expression of TGase1. Although introduction of wild-type TGase1 into a transformed LI patient keratinocyte line may be of interest for future biochemical studies, we have focused on accomplishing reversion of biochemical phenotype in primary patient cells. We have done this because primary keratinocytes may be expanded form a 1-cm^2 biopsy to an equivalent of greater than the entire body surface area in less than 10 weeks and because these cells have been widely used in grafting of burn and cutaneous ulcer patients (Gallico *et al.,* 1984; Limova and Mauro, 1995). With successful correction of these cells *in vitro,* the foundation is laid for efforts to graft them directly *in vivo* by established protocols. The physiologic expression of TGase1 in formerly TGase1(−) LI patient keratinocytes by the approach described above, then, restores features of keratinocyte cornification to normal and suggests this approach may be useful in future efforts at therapeutic gene delivery in the treatment of this disease.

A number of features contribute to the future potential for cutaneous gene therapy efforts *via ex vivo* gene transfer in humans. These include ready biopsy excision and growth of cells from the skin in culture, successful grafting of keratinocytes and fibroblasts onto human burn patients, and achievement of varying degrees of efficiency in gene transfer to these cells (Gallico *et al.,* 1984; Greenhalgh *et al.,*1994; Taichman, 1994; Limova and Mauro, 1995). Major challenges, however, remain. Several of these include full characterization and high-efficiency gene transfer to tissue stem cells, avoidance of immunologic reactions, prevention of gene inactivation, and regulation of the magnitude of therapeutic gene expression. Here, we have provided evidence for high-efficiency corrective gene delivery in early passage keratinocytes from patients with a severe genetic skin disease *in vitro* as a foundation for future work in developing approaches to therapeutic gene delivery to the skin.

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