Transforming Growth Factor-β Stimulates Collagen VII Expression by Cutaneous Cells In Vitro

Adrian König and Leena Bruckner-Tuderman

Department of Dermatology, University Hospital Zurich, 8091 Zurich, Switzerland

Abstract. Collagen VII, the major component of cutaneous anchoring fibrils is expressed at a low level by normal human keratinocytes and fibroblasts in vitro. In cocultures of these two cell types, signals from fibroblasts enhance expression of collagen VII by keratinocytes and vice versa. In this study, the effects of a possible mediator of such a stimulation, transforming growth factor-β (TGF-β), were investigated. Its effect on the expression and deposition of the highly insoluble collagen VII was assessed in a semiquantitative manner by a newly developed enzyme-linked immunoassay which is based on immunoblotting. In keratinocyte monocultures, 0.5–20 ng/ml of TGF-β_2 induced a dose-dependent stimulation of collagen VII expression as measured per microgram of DNA. The maximal enhancement was about sevenfold compared to controls. The effect of TGF-β_2 was observed already after 12 h, with a steady increase at least up to 3 d. As previous studies have implicated, untreated cocultures of keratinocytes and fibroblasts exhibited a higher basic level of collagen VII expression, which could be further stimulated about twofold by TGF-β_2. Fibroblasts alone synthesized very minor quantities of collagen VII and could be only weakly stimulated by TGF-β_2. This growth factor seems a specific enhancer of collagen VII since the expression of laminin, collagen IV, as well as total protein was increased to a much lesser extent. Our data suggest that TGF-β may be an important mediator of epithelial–mesenchymal interactions and may regulate the synthesis of the anchoring fibrils at the skin basement membrane zone.

The dermo-epidermal junction of the skin represents an epithelial-mesenchymal interface with a unique structure and highly specialized functions (Palade and Farquhar, 1965; Bruns, 1969; Briggaman and Wheeler, 1975*a*; Tidman and Eady, 1984; Burgeson, 1987). One of its main functions is to provide the resistance of the skin against shearing forces which requires strong cohesion of the skin layers. This is achieved by several interconnected macromolecular networks which attach the epidermis to the basement membrane and to the underlying dermal connective tissue (Timpl, 1989; Burgeson et al., 1990; Yurchenko and Schittny, 1990). One of the major structures mediating the attachment is the anchoring fibril network that extends from the basement membrane to the anchoring plaques in the papillary dermis (Sakai et al., 1986; Keene et al., 1987). Abnormalities of the anchoring fibrils lead to separation of the epidermis from the dermis and to clinical blistering of the skin, as seen in dystrophic epidermolysis bullosa, a group of inherited blistering disorders (Briggaman and Wheeler, 1975*b*; Hashimoto et al., 1976; Tidman and Eady, 1985; Heagerty et al., 1986; Bruckner-Tuderman et al., 1989, 1991*a*), or in epidermolysis bullosa acquisita, an inflammatory autoimmune bullous disease (Woodley et al., 1988).

Collagen VII is the major structural component of the anchoring fibrils (Sakai et al., 1986; Burgeson et al., 1990). The structure, biosynthesis and supramolecular assembly of this collagen are known only in part (Lunstrum et al., 1986; Bruckner-Tuderman et al., 1987; Bächinger et al., 1990; Parente et al., 1991), and the regulation of these events remains to be elucidated. Epidermal cells appear the main site of collagen VII production, but their biosynthetic activity is under dermal control (Regauer et al., 1990; König and Bruckner-Tuderman, 1991). In cocultures of keratinocytes and fibroblasts, expression of collagen VII is enhanced as compared to monocultures of either cell type, and in three-dimensional skin equivalent cultures collagen VII can be detected in the epidermal, but not dermal cells (König and Bruckner-Tuderman, 1991).

The nature of the mesenchymal–epithelial signals mediating the stimulation of collagen VII expression in cocultures remains unknown. Direct cell–cell contacts seem less likely because epithelial cells express collagen VII in skin equivalents in which no physical contact between fibroblasts and keratinocytes exists. In contrast, growth factors seem likely candidates as mediators of at least some signals, since their effects on cellular behaviour and production of the extracellular matrix, and their interactions with the matrix have be-

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come known in many in vitro systems (for review, see Nathan and Sporn, 1991). A cytokine with a broad spectrum of effects on extracellular matrix is the transforming growth factor-β (TGF-β)[1] peptide family (for reviews, see Roberts et al., 1988, 1990; Massagué, 1990). In addition to controlling cell growth, differentiation, and function, it has been shown to play an important role in remodelling of tissues by affecting the expression of, e.g., many collagens, fibronectin, elastin, matrix metalloproteinases, or their inhibitors (Roberts et al., 1988, 1990; Massagué, 1990).

In this study we show that TGF-β_2 stimulates in a dose-dependent manner the expression of collagen VII, a major component of the dermo-epidermal junction and a structural protein of the anchoring fibrils. The effect on the expression of collagen VII is significantly larger than on other basement membrane zone proteins such as laminin or collagen IV.

Materials and Methods

TGF-β

Recombinant human TGF-β_2 was a kind gift from Drs. K. Müller and N. Cerletti (Ciba-Geigy AG, Basel, Switzerland). It was solubilized in 10 mM HCl and 10% ethanol at a concentration of 0.1 mg/ml. This stock solution was diluted with PBS containing 0.05% BSA to a final TGF-β_2 concentration of 200 ng/ml.

Cell Cultures

Human skin fibroblasts were initiated from primary explants and grown in DMEM supplemented with 10% FCS, 4 mM L-glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin, and 0.25 μg/ml amphotericin B (Gibco Laboratories, Grand Island, NY). Human keratinocytes were released from skin by trypsinization and cultured in serum-free keratinocyte growth medium (KGM) containing 0.09 mM calcium (Boyce and Ham, 1983), 50 μg/ml bovine pituitary extract (BPE) and 5 ng/ml recombinant human EGF (Gibco Laboratories). Cells from the first or second passage were used in the experiments. For cocultures, fibroblasts were seeded at a density of 8,000 cells/cm^2 in DMEM with 10% FCS. 24 h later, the medium was replaced with KGM, and keratinocytes were added at a density of 40,000–80,000 cells/cm^2. Cylindroma cells were initiated and cultured in KGM as previously described (Bruckner-Tuderman et al., 1991*b*). Before assessing collagen VII expression, serum or BPE were omitted from the media for at least 48 h. For the same time period, 50 μg/ml ascorbate was added to the cultures.

Treatment of Cell Cultures with TGF-β_2

Keratinocytes, fibroblasts, cocultures, or cylindroma cells were cultivated to semi-confluency in complete culture medium. TGF-β_2 treatment was carried out in serum- and BPE-free medium supplemented with 50 μg/ml ascorbate. Appropriate amounts of TGF-β_2 stock solution (see above) were added to the medium once, and the medium was not renewed during the incubation period. The incubation period was 48 h for IF experiments and 72 h for the measurements with the enzyme-linked immunoassay. To control cultures, corresponding amounts of PBS containing 0.05% BSA (TGF-β_2 solvent) were added for the incubation period.

Antibodies

For detection of collagen VII, polyclonal affinity-purified antibodies to the triple helical domain of human collagen VII were used (Bruckner-Tuderman et al., 1987). The mAb to human collagen IV was kindly provided by Dr. B. Odermatt, Department of Pathology, University of Zurich (Odermatt et al., 1984), and the polyclonal antibodies to human laminin were a generous gift from Dr. M. Paulsson, M. E. Müller Institute for Biomechanics, University of Berne (Paulsson et al., 1987). FITC-labeled antirabbit and antimouse antibodies were purchased from Dakopatts (Glostrup, Denmark). Peroxidase-labeled goat antirabbit antibodies were obtained from Kirkegaard & Perry (Gaithersburg, MD).

Indirect Immunofluorescence Staining (IF)

Cells cultured on cover slips or on plastic Lab-Tek[R] chamber slides (Nunc Inc., Naperville, IL) were washed twice with TBS, and permeabilized and fixed with 100% methanol for 15 min at −20°C. Incubation with the first antibody was performed overnight and with the FITC-conjugated second antibody for 1 h.

Protein and DNA Determination

The total cell culture proteins were precipitated with 10% TCA and dissolved in 1 M NaOH. The protein concentration was measured by a modified Lowry-assay (Lowry et al., 1951; Hudson and Hay, 1989) using BSA for calibration. The DNA content of the cultures was measured according to Labarca and Paigen (1980).

Enzyme-linked Immunoassay for Collagen VII

Due to an extensive insolubility of the tissue form of collagen VII in physiological solutions, common quantitative immunoassays such as ELISA or RIA were not reproducible. A new solid phase immunoassay based on quantitative extraction of collagen VII with denaturing and reducing agents, immunoblotting and measurement of the antibody-bound enzyme activity with a water soluble chromophore substrate was developed.

Cells were cultured in 25-cm^2 flasks to early confluency and treated with TGF-β_2 according to the experimental protocol. After the incubation period, the medium was harvested and proteinase inhibitors including 1 mM phenylmethane-sulfonyl fluoride, 10 mM EDTA, 20 mM N-ethylmaleimide, and 100 mM ϵ-aminocaproic acid were added. Before further processing, the medium was stored on ice. The cell layer was washed twice with ice-cold TBS, and homogenized in cold distilled water by sonication. After aliquots had been separated for DNA assays, the cells were combined with the medium and precipitated with 75% ethanol on ice for 1 h. After centrifugation with 17,000 *g* for 20 min, the pellet was dissolved in 500 μl of a buffer containing 8 M urea, 2% SDS, 0.1 M 1,4-dithioerythritol and 0.1 M Tris-HCl (pH 6.8). After heating at 100°C for 5 min, the extract was extensively dialyzed against SDS-PAGE sample buffer containing 0.8 M urea, 0.1 M Tris, pH 6.8, 2% SDS, 0.002% bromphenol blue, and 5% glycerine. The volume of all samples was adjusted to the largest volume by adding sample buffer, usually to 750 μl. 50 or 100 μl of each extract was separated by SDS-PAGE (Lämmli, 1970) under reducing conditions, with a 4.5–15% polyacrylamide gradient gel. This was followed by electrotransfer of the proteins onto nitrocellulose (Towbin et al., 1979). To ensure a complete transfer, 0.1% SDS was added to the transfer buffer. The lanes on the filter were marked after staining with 0.2% Ponceau S Red (Sigma Chemical Co., St. Louis, MO) in TBS. After blocking with 2% defatted milk powder in TBS for 30 min, the nitrocellulose sheet was reacted overnight with affinity-purified rabbit antihuman collagen VII antibodies (Bruckner-Tuderman et al., 1987). Washing with TBS was followed by incubation with peroxidase-labeled goat anti-rabbit IgG antibody for 2 h.

After the reaction with the antibodies, lanes with collagen VII standards were cut out and reacted with the water-insoluble substrate 4-chloro-1-naphtol. The migration position of collagen VII on the nonreacted nitrocellulose was marked with help of these standards, and the corresponding bands were excised and transferred to test tubes. They were incubated in 0.5 ml of a substrate solution containing 0.03% *o*-phenylenediamine, 25 mM citric acid, 50 mM Na_2HPO_4, and 0.01% hydrogen peroxide for 15 min. The reaction was stopped by adding 100 μl of 2 M sulfuric acid. The nitrocellulose stripes were transferred into new tubes, and residual bound substrate was eluted with 200 μl of *N,N*-dimethyl formamide. Both chromophore solutions were combined, clarified by short centrifugation in a microfuge, and the absorbance was read at 492 nm.

For calibration, 5–30 μl of collagen VII containing skin extract were run on the same gel and measured in the same assay, parallel with the test samples. Due to the semiquantitative nature of this solid-phase immunoassay, arbitrary units for type VII collagen were defined. One unit corresponded to the amount of collagen VII present in 1 μl of dermis extract that was prepared by using 400 μl of extraction buffer per cm^2 of skin surface, as described (Stanley et al., 1985; Bruckner-Tuderman et al., 1987). Application of 5–30 μl of skin extract as a standard resulted in a linear response in the assay. Fig. 1 (*A*) shows such a standard series stained with a water-insoluble

1. *Abbreviations used in this paper*: BPE, bovine pituitary extract; IF, indirect immunofluorescence staining; KGM, keratinocyte growth medium, substituted with BPE and EGF; TGF-β, transforming growth factor-β.

peroxidase substrate, 4-chloro-l-naphtol, and *B* shows the corresponding absorbances of the reaction with *o*-phenylenediamine as a water-soluble substrate. All samples were measured in the linear range of the assay.

Results

For initial observation of the effects of TGF-β on collagen VII expression in vitro, small cultures on coverslips were treated with TGF-β_2 and subjected to IF. Semiconfluent keratinocytes, fibroblasts, cocultures, or cylindroma cells were cultivated for 2 d in serum- or BPE-free medium which was supplemented with 20 ng/ml TGF-β_2 and 50 μg/ml ascorbate before processing for IF. In controls, the media were supplemented with ascorbate and PBS containing 0.05% BSA but no TGF-β_2.

In dermal fibroblasts, the basal expression of collagen VII was very low (Fig. 2 *a*), and incubation with TGF-β_2 induced only a discrete increase of immunoreaction (Fig. 2 *b*). In contrast, the expression of collagen VII by keratinocytes could be significantly stimulated by TGF-β_2 (Fig. 2, *c* and *d*). In cocultures, the basal expression of collagen VII was stronger than in either cell type alone (Fig. 2 *e*), but could still be enhanced by TGF-β_2 to some extent (Fig. 2 *f*). As we have shown previously (Bruckner-Tuderman et al., 1991*b*), epithelial cells derived from a benign skin tumor, the cylindroma, expressed collagen VII at a relatively high level (Fig. 2 *g*). Also in these "high producers," an enhancement by TGF-β_2 was observed (Fig. 2 *h*). For preliminary assessment of the degree of stimulation, the response of the cells to different concentrations of TGF-β_2 was estimated by visual scoring of the IF staining (Table I). Addition of 1–30 ng/ml of TGF-β_2 resulted in a very weak stimulation in fibroblasts, whereas a clear dose-dependent response was observed in keratinocytes, cocultures, and cylindroma cells. In control experiments, collagen VII was similarly expressed in keratinocytes and cocultures when BPE was included in the medium (data not shown).

The subjective impression of increased collagen VII expression in TGF-β_2–treated cultures was confirmed with immunoblotting of culture extracts. Small quantities of collagen VII could be extracted from untreated 25-cm^2 subconfluent keratinocyte cultures (Fig. 3, lane *2*). However, significantly higher amounts were found in cultures incubated with 5 ng/ml TGF-β_2 for 3 d (Fig. 3, lane *3*). In all

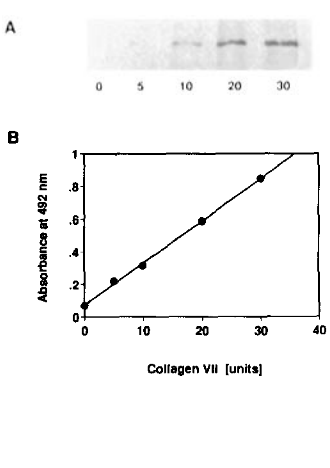

Figure 1. Enzyme-linked solid phase immunoassay for collagen VII. The tissue form of collagen VII was detected in 5–30 μl of dermal extract by conventional immunoblotting (*a*) or by the assay using a soluble chromophore substrate (*b*). For calibration of the assay, the same amounts of dermal extract as in *a* were used as standards. One arbitrary unit of collagen VII corresponds to the amount present in 1 μl of dermal extract, prepared as described in Materials and Methods.

Table I. Comparison of Collagen VII Synthesis by Cutaneous Cells in the Absence or Presence of TGF-β

TGF-β	Fibroblasts	Keratinocytes	Cocultures	Cylindroma Cells
ng/ml				
0	+/−	+	++	++
1	+/−	++		+++
5	+/−	++	+++	+++
10	+/−	+++		++++
20	+	+++	+++	++++
30	+	+++		

Indirect immunofluorescence staining of cells grown on cover slips was scored visually. +/−, questionable staining; +, moderate staining; ++, prominent staining; +++/++++, strong staining.

extracts, the intact tissue form of collagen VII was present, and no degradation products were observed.

For semiquantitative dose-response measurement, keratinocytes and cocultures were incubated with 0.1–20 ng/ml TGF-β_2 for 3 d. The culture extracts were subjected to the solid-phase immunoassay, and the collagen VII content was assessed per microgram of DNA. In control keratinocytes (Fig. 4 *A*), the collagen VII level corresponded to ~1 U/μg DNA (one arbitrary unit was defined as the amount of collagen VII present in 1 μl of dermal basement membrane zone extract, as described in Materials and Methods). TGF-β_2 induced a dose-dependent stimulation which was about fourfold with 0.5 ng/ml and about sevenfold with 20 ng/ml TGF-β_2. A similar dose-response was found in cocultures (Fig. 4 *B*). As expected from the preliminary experiments with IF staining, the basal expression of collagen VII in these cultures was higher, ~5 U/μg DNA. It could be stimulated ~1.5-fold with 0.5 ng/ml, and ~2-fold with 20 ng/ml TGF-β_2.

In time-course experiments, a slight effect of TGF-β_2 on collagen VII expression was seen after 12 h incubation, and a clear stimulus was measured after 24 h (Fig. 5), with a steady increase up to 72 h.

TGF-β_2 was found to stimulate to a lesser extent the expression of two other basement membrane zone proteins, laminin and collagen IV (Table II). As assessed by IF and visual scoring, the basal expression of laminin in keratinocytes was prominent but could still be stimulated by TGF-β_2 in a dose-dependent way. When stained with antibodies to collagen IV, keratinocytes showed an immunofluorescence signal that was indistinguishable from the negative controls. Incubation with TGF-β_2 induced a barely visible staining for collagen IV.

Total protein content of the keratinocyte cultures treated with up to 5 ng/ml TGF-β_2 for 3 d increased by 20–25%, significantly less than the content of collagen VII. As expected from the well-known inhibitory effect of TGF-β on keratinocyte proliferation (Shipley et al., 1986), the DNA content in cultures treated with 0.5–20 ng/ml TGF-β_2 for 3 d was 25–30% lower than in control cultures.

Discussion

In the present study we demonstrate that TGF-β_2 increases in a dose-dependent manner the expression of collagen VII

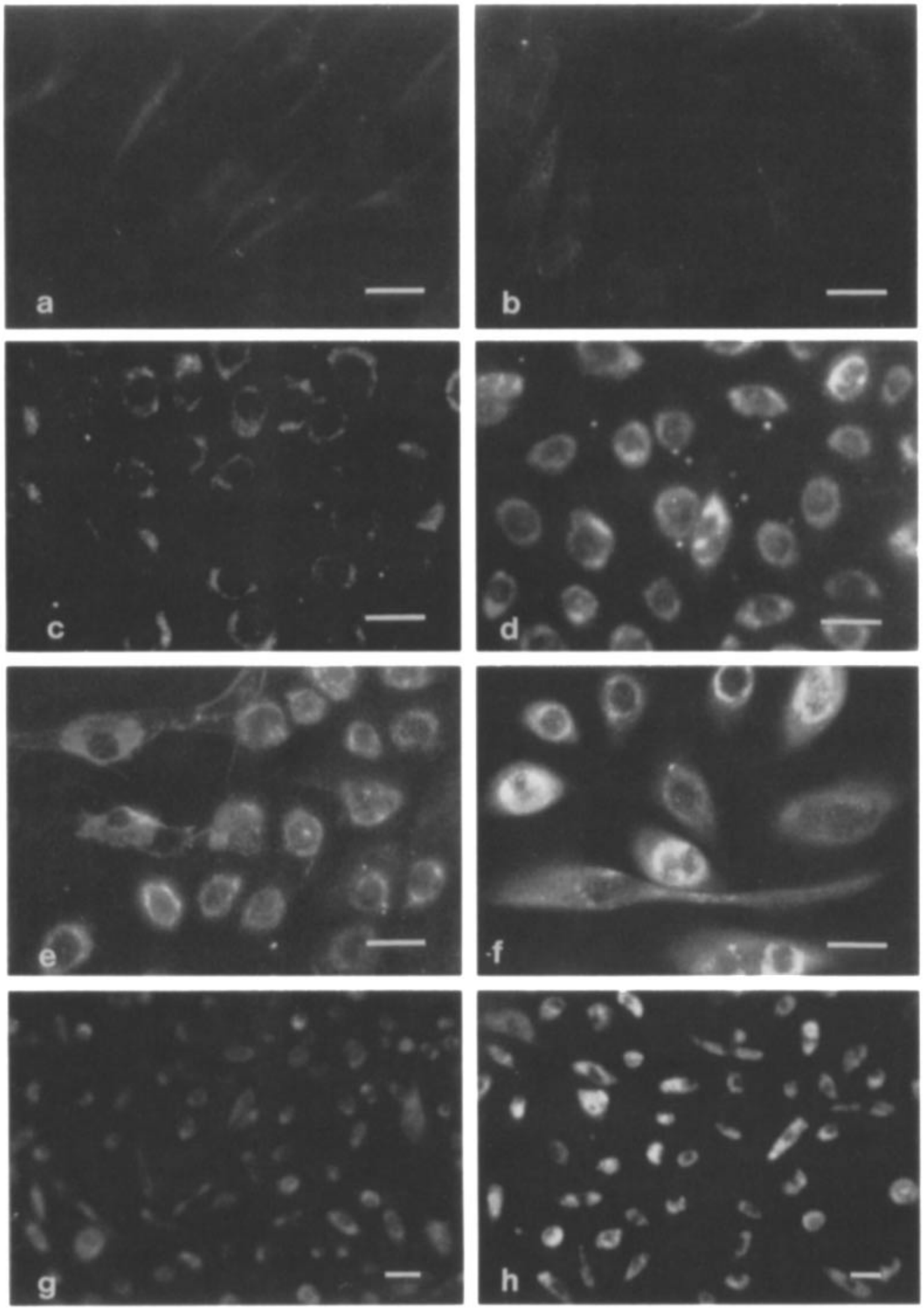

Figure 2. Enhanced collagen VII expression in TGF-β-treated cells. Immunofluorescence staining with antibodies to collagen VII of fibroblasts (*a* and *b*), keratinocytes (*c* and *d*), cocultures (*e* and *f*), and cylindroma cells (*g* and *h*). The cultures were incubated with 20 ng/ml of TGF-β_2 and ascorbate in serum- or BPE-free medium for 3 d (*b*, *d*, *f*, and *h*) as described in Materials and Methods. Control cultures (*a*, *c*, *e*, and *g*) were incubated with ascorbate alone. Bars, 10 μm.

by cutaneous cells. The quantitation of collagen VII in tissues or in cell cultures has been difficult in the past, due to a tendency to aggregation and subsequent insolubility of the protein, a fact that has impaired studies on regulation of this collagen. To circumvent this problem, a new enzyme-linked immunoassay was utilized here. It is based on immunoblotting and the use of a water-soluble chromophore substrate, and allows semiquantitative assessment of collagen VII which can be solubilized under denaturing and reducing conditions. The assay is linear over a wide range of antigen concentration, and shows small interassay variation. This method can be adapted for any other protein which cannot be assessed with ELISA or RIA due to insolubility or tendency to aggregation.

In vitro, collagen VII can be synthesized by both fibroblasts and epithelial cells (Stanley et al., 1985; Lunstrum et al., 1986; Bruckner-Tuderman et al., 1987; König and Bruckner-Tuderman, 1991). It is unclear, however, which cells produce collagen VII and deposit anchoring fibrils in situ, and how these processes are regulated. Recent studies

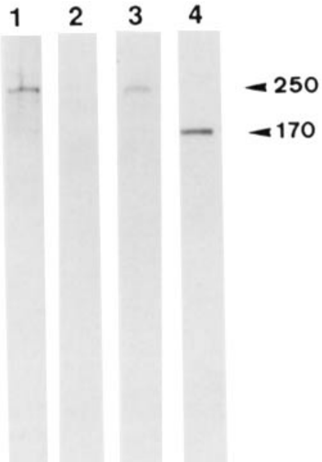

Figure 3. Immunoblotting of collagen VII extracted from TGF-β-treated keratinocyte cultures. 25-cm^2 cultures were solubilized in 750 μl of extraction buffer and immunoblotted with 100 μl per well. (Lane *1*) Standard, tissue form of collagen VII extracted from normal dermis; (lane *2*) collagen VII extracted from control keratinocytes; and (lane *3*) from keratinocytes treated with 5 ng/ml of TGF-β_2 for 3 d as described in Materials and Methods; (lane *4*) standard, triple helical fragment of collagen VII. Molecular weights deduced from collagenous standards are indicated on the right.

have suggested that keratinocytes represent the main site of collagen VII production in vitro (Goldsmith et al., 1991; König and Bruckner-Tuderman, 1991) and in vivo (Smith and Sybert, 1990; Regauer et al., 1991). However, fibroblasts possess an inherent capacity to produce this protein, and they can be stimulated to do so under certain conditions, such as in cocultures with keratinocytes. This stimulation of collagen VII expression in cocultures is mutual as the expression by keratinocytes was enhanced as well (König and Bruckner-Tuderman, 1991). The nature of the signals mediating the stimulatory effects remains elusive, but it is likely that autocrine and paracrine factors are important because direct cell–cell contact is not required for enhanced collagen VII expression in cocultures.

A cytokine with a central role in the development and maintenance of the extracellular matrix in general, is the TGF-β peptide family. It upregulates the expression of multiple genes coding for extracellular matrix proteins, such as fibronectin, laminin (Ignotz et al., 1987, Wikner et al.,

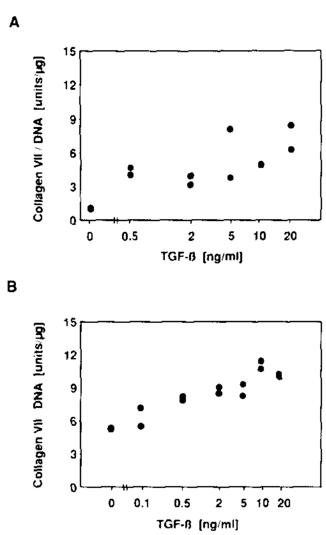

Figure 4. Response of collagen VII expression to increasing concentrations of TGF-β. Duplicate cultures of subconfluent keratinocytes (*a*) or cocultures (*b*) were treated with TGF-β_2 for 3 d, and collagen VII content of the cultures was assessed by the enzyme-linked immunoassay as described in Materials and Methods. Results are expressed as arbitrary units of collagen VII per microgram of DNA and plotted against the concentration of TGF-β_2 on a semilogarithmic scale. Each dot represents the value obtained from one culture flask. At both 0 and 10 ng/ml TGF-β_2 in *a*, and at 0 ng/ml in *b*, two dots are superimposed.

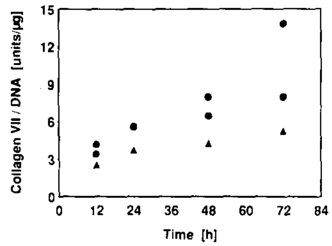

Figure 5. Time course of the effect of TGF-β on collagen VII expression by keratinocytes. Subconfluent cultures were incubated with 5 ng/ml TGF-β_2 for 12–72 h in the presence of ascorbate (●). The growth factor was omitted from parallel control cultures (▲). A clear stimulation of collagen VII expression was visible after 24 h, with a steady increase up to 72 h. Collagen VII content of the cultures was assessed by the enzyme-linked immunoassay as described in Materials and Methods. Results are expressed as arbitrary units of collagen VII per microgram of DNA. Each dot represents the value obtained from one culture flask. At 24 h, two dots are superimposed.

1988; Vollberg et al., 1991), and collagens I, III, V (Rossi et al., 1988), II (Varga et al., 1987), or IV (Vollberg et al., 1991). In the present study we demonstrated that TGF-β_2 also stimulates the production of collagen VII by cutaneous cells in a dose- and time-dependent manner. Time course experiments showed an effect that was visible after 12 h, with a steady increase up to 48–72 h. Therefore, the cells were treated with TGF-β_2 for this period of time before assessing changes in collagen VII production.

Keratinocytes and cocultures were very responsive to 0.1–20 ng/ml TGF-β_2, showing a two- to sevenfold increase of collagen VII production, whereas fibroblasts in monoculture were poor responders. The stimulation was stronger in keratinocytes than in cocultures, most likely due to the already enhanced expression in untreated cocultures. The effect of TGF-β_2 did not depend on the presence of EGF in the culture medium, since control experiments in the absence of EGF exhibited similar results (data not shown). The effect of TGF-β_2 on collagen VII expression was clearly greater than on the production of other basement membrane components such as laminin and collagen IV. In addition, TGF-β_2 increased the total protein synthesis only by 25–30%, implicating that the increase in collagen VII production was not a mere reflection of a generally induced protein synthesis.

Although TGF-β_1 which is also expressed in normal skin and keratinocytes in vitro (Gruschwitz et al., 1990) was not assessed in this study, a similar effect on collagen VII expression can be expected. In other cell systems, the two subtypes exert similar effects mediated by shared receptors which bind

Table II. Effect of TGF-β on Expression of Collagen IV and Laminin by Keratinocytes In Vitro

TGF-β	Laminin	Collagen IV
ng/ml		
0	+++	–
1	+++	–
5	++++	+/–
10	++++	+/–
20	++++	+/–

Indirect immunofluorescence staining of cells grown on cover slips was scored visually. –, no staining; +/–, questionable staining; ++, prominent staining; +++/++++, strong staining.

both subtypes (Massagué, 1990), and fibronectin production is stimulated by both subtypes but stronger by TGF-β_1 (Hashiro et al., 1991).

The response of normal, low passage skin cells to TGF-β_2 in the present study was comparable to observations made in other cell culture systems where 0.5–12.5 ng/ml of TGF-β stimulated ∼6-10-fold the expression of fibronectin, laminin (Ignotz and Massagué, 1986, Vollberg et al., 1991, Hashiro et al., 1991) and other collagens (Varga et al., 1987, Rossi et al., 1988, Madri et al., 1988, Vollberg et al., 1991). This suggests that TGF-β in picomolar concentrations regulates the expression of extracellular matrix components, in particular collagen VII, by keratinocytes and fibroblasts along the cutaneous basement membrane zone. Both human fibroblasts and keratinocytes are capable of synthesizing and secreting TGF-β (Lawrence et al., 1984; Partridge et al., 1989) and they possess specific receptors for this factor (Tucker et al., 1984). It thus seems likely that TGF-β is responsible for at least part of the stimulation of collagen VII observed in cocultures of keratinocytes and fibroblasts.

However, the fact that fibroblasts in monoculture can be stimulated with TGF-β_2 to express collagen VII to a lesser extent than fibroblasts that are cocultured with keratinocytes, points to the existence of additional keratinocyte-derived mediators that regulate mesenchymal–epithelial interactions in vitro. Potential candidate factors include FGF, PDGF, and EGF which modulate connective tissue metabolism, e.g., during reparatory processes and wound healing (Nanney, 1990; Mustoe et al., 1991). The role of such factors in the regulation of mesenchymal–epithelial interactions in skin and of biogenesis of the anchoring fibrils is being presently investigated in our laboratory.

The precise mechanisms of TGF-β action on expression of collagen VII remain unknown. In other instances, stimulation of collagen gene transcription via nuclear factor 1 binding (Rossi et al., 1988), or preferential stabilization of collagen mRNA (Raghow et al., 1987) have been reported. On the other hand, TGF-β represses matrix metalloproteinase expression and activity (Woessner 1991), an effect which could lead to accumulation of a protein in the matrix.

In many dermatologic disorders with blistering tendency, abnormal structure and function of the anchoring fibrils can be accompanied by secondary proteolysis. In genetic diseases such as dystrophic epidermolysis bullosa, synthesis of collagen VII is impaired, the dermo–epidermal coherence inadequate, and destruction of the dermal connective tissue often evident (Hashimoto et al., 1976; Bruckner-Tuderman et al., 1989, 1990). Another example is found in healing burn wounds, where blisters tend to form very easily in spite of complete re-epithelialization of the wound. In such wounds, the globular domains of the anchoring fibrils are present but the collagenous domains are missing, probably due to excessive collagenolytic activity in the dermal granulation tissue (D. Woodley, E. Bauer, and L. Bruckner-Tuderman, manuscript in preparation). In pathologic situations like the above, TGF-β may exhibit therapeutic potential due to its multiple regulatory effects on the extracellular matrix, i.e., stimulation of collagen VII but inhibition of collagenase and stromelysin expression, as well as stimulation of specific matrix metalloproteinase inhibitors (Werb, 1989; Massagué, 1990; Woessner, 1991). Profound understanding of the effects of growth factors and cytokines on the biology of the skin basement membrane zone is essential for planning therapeutic regimens utilizing TGF-β or other biologically active factors.

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