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Elevated Expression of Type VII Collagen in the Skin of Patients with Systemic Sclerosis

Regulation by Transforming Growth Factor- β

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Abstract

A hallmark of systemic sclerosis (SSc) is the development of tissue fibrosis. Excessive production of several connective tissue components normally present in the dermis, including type I, III, V, and VI collagens as well as fibronectin and proteoglycans, is a consistent finding in the skin of SSc patients. Type VII collagen is a major constituent of anchoring fibrils, present in the skin at the dermal-epidermal basement membrane zone. TGF- β has been shown to upregulate the expression of the type VII collagen gene. In this study, we assessed the expression of type VII collagen and TGF- β in the skin of patients with SSc. Indirect immunofluorescence showed an abundance of type VII collagen in the patients' skin, including the dermis. Ultrastructural analysis of SSc skin revealed an abundance of fibrillar material, possibly representing type VII collagen. The increased expression of type VII collagen epitopes was accompanied by the elevated expression of immunodetectable TGF- β 1 and TGF- β 2. Dermal fibroblasts cultured from the affected individuals showed a statistically significant ($P < 0.02$) increase in the expression of type VII collagen at the mRNA level, as detected by reverse transcription-PCR with a mutated cDNA as an internal standard, and increased deposition of the protein as assessed by indirect immunofluorescence. Thus, type VII collagen is abundantly present in SSc patients' dermis, a location not characteristic of its normal distribution, and its aberrant expression may relate to the presence of TGF- β in the same topographic distribution. The presence of type VII collagen in the dermis may contribute to the tightly bound and indurated appearance of the affected skin in SSc patients. (*J. Clin. Invest.* 1994. 93:1709–1715.) Key words: systemic sclerosis • anchoring fibrils • type VII collagen • tissue fibrosis • transforming growth factor- β

Introduction

Systemic sclerosis (SSc)¹ is a connective tissue disease characterized by fibrosis of the skin, subcutaneous tissue, and various

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1. Abbreviations used in this paper: IIF, indirect immunofluorescence; NC, noncollagenous; RT-PCR, reverse transcription-PCR; SSc, systemic sclerosis.

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internal organs (1). The most prominent pathologic manifestation of the disease is an abnormal accumulation of extracellular matrix components, predominantly collagen types I and III (2–4). More recent data have also shown an increased expression of type VI collagen, mainly in a perivascular location (5). In vitro, fibroblast cultures established from SSc patients' involved skin express increased amounts of collagens type I, III, and V, fibronectin, and proteoglycans (6–11), and contain elevated levels of α 1(I), α 2(I), and α 1(III) collagen mRNAs (12–14). Transient transfection assays have also shown increased activity of the α 2(I) collagen promoter-reporter gene construct in SSc fibroblasts (15), suggesting enhancement of type I collagen gene expression at the transcriptional level.

TGF- β consists of a family of closely related cytokines. Two members of the family, TGF- β 1 and TGF- β 2, have been implicated in the pathogenesis of a variety of fibrotic diseases (16, 17), and several lines of evidence suggest that TGF- β plays a critical role in the development of tissue fibrosis in SSc. For example, TGF- β 1 has been shown to stimulate the synthesis of various connective tissue components by normal fibroblasts in vitro (18, 19), and when injected intradermally TGF- β causes tissue fibrosis (20). Furthermore, an abundance of TGF- β in the skin of SSc patients (21, 22) and the responsiveness of SSc fibroblasts to stimulation by TGF- β (23, 24) make this growth factor a potential candidate molecule in the pathogenesis of SSc.

Type VII collagen consists of three identical α 1(VII) chains, each composed of a long collagenous triple-helix flanked by globular amino- and carboxy-terminal noncollagenous domains (25–27). Type VII collagen is present in human tissues in a restricted distribution, almost exclusively in the basement membrane zone below stratifying squamous epithelia of the skin, mucous membranes, and the cornea of the eye. Type VII collagen is the predominant, if not the exclusive, component of anchoring fibrils, attachment structures which ensure the integrity of the cutaneous basement membrane zone (for reviews see references 27, 28). Under in vitro conditions, type VII collagen is synthesized primarily by epidermal keratinocytes, but dermal fibroblasts have also been shown to express the type VII collagen gene at a very low level (29, 30). The synthesis of type VII collagen can be upregulated by TGF- β 1 and TGF- β 2 both in keratinocyte and fibroblast cultures (30, 31).

The aim of the present study was to assess the expression and distribution of type VII collagen and TGF- β in the skin of SSc patients and to examine the expression of the type VII collagen gene in SSc fibroblast cultures.

Methods

Patients. This study was performed on skin specimens from seven patients, six of them having a definitive diagnosis of diffuse SSc and one being suggestive of SSc (Raynaud's phenomenon and positive test for

antinuclear antibody). Also, dermal fibroblast cultures were established from eight patients with diffuse SSc of recent onset. In one case, the skin specimens and cultured fibroblasts were obtained from the same patient. The diagnosis of SSc was made according to the criteria of the American College of Rheumatology (formerly American Rheumatism Association) (32), and none of the patients had a limited form of SSc. Clinical features and laboratory findings of the 15 patients (S1–S15) are summarized in Table I. Control fibroblast cultures were established from eight age- and sex-matched healthy individuals, and normal skin specimens were obtained from three healthy individuals.

Full thickness excisional skin biopsies were obtained from the leading edge of the fibrotic process on the dorsal forearm of SSc patients and from site-matched areas from healthy individuals. Each specimen was cut into two pieces. One part was immediately frozen in liquid nitrogen, and 5- μ m-thick frozen sections were used for indirect immunofluorescence (IIF). The second piece was used to establish fibroblast cultures. For IIF, cells were grown on chamber slides (Nunc, Inc., Naperville, IL) to subconfluency. All fibroblast cultures were maintained in DME supplemented with 10% FCS. In experiments testing the effects of TGF- β , the cells were rinsed with PBS and replaced in DME containing 1% FCS 3 h before addition of 10 ng/ml TGF- β 1 or TGF- β 2 (provided by Dr. David R. Olsen, Celtrix Laboratories, Santa Clara, CA).

Reverse transcription-PCR (RT-PCR) amplification of type VII collagen mRNA transcripts. 1 μ g of total RNA isolated from fibroblast cultures (passages 4–6), established from the skin of healthy normal donors or from SSc patients, was used to synthesize the first strand cDNA with avian myeloblastosis virus reverse transcriptase (Bethesda Research Laboratories, Gaithersburg, MD) using oligo-dT_{12–18} primers (33). The total amount of cDNA synthesized served as a template for the subsequent PCR amplification, and the reaction was catalyzed by Taq polymerase (Boehringer Mannheim Corp., Indianapolis, IN) in the presence of 15 μ Ci of [α -³²P]dCTP and 25 pmol of two primers defining a 382-bp segment in the 3'-end of type VII collagen mRNA (34). The primers used were: the upstream primer, 5'-AAT-GATGGCTCTGCTGGTCC-3'; the downstream primer, 5'-CATG-CAGAGGAGGAAGAGGCG-3' (Fig. 1). Amplification was performed for 25 cycles (94°C for 45 s, 61°C for 45 s, and 72°C for 45 s) using a thermal cycler (Coy Laboratory Products Inc., Ann Arbor,

MI). Differences in the efficiency of PCR amplification were corrected for by using a cloned cDNA as internal standard which contained complementary sequences for the same primers and yielded the same size product but contained a single base substitution in the sequence GAGAAG instead of GAGATG found in the normal sequence (35). The mutated PCR product could be cleaved by the endonuclease EarI into 244- and 138-bp fragments (Fig. 1). This homozygous T-to-A substitution has been detected in two siblings with recessive dystrophic epidermolysis bullosa (35). The product of PCR amplification was subcloned into the PCR 1000 vector (Invitrogen, San Diego, CA), amplified, and the plasmid construct was linearized with restriction enzyme digestion using EcoRI (Boehringer Mannheim Corp.). 100 pg of the cDNA containing the mutated sequence (T-to-A substitution) was used as an internal standard mixed with the cDNA template generated by RT-PCR from RNA isolated from fibroblast cultures established from SSc patients or from matched control individuals. 200 pg of cDNA containing the internal standard was used for experiments with cDNA from TGF- β -stimulated fibroblasts.

Each PCR reaction product was extracted with phenol-chloroform, digested to completion with the restriction enzyme EarI, and subsequently analyzed by electrophoresis on a 4% polyacrylamide gel (33). The gel was exposed to x-ray film (X-Omat; Eastman Kodak, Rochester, NY) at -70°C for varying time periods, and the autoradiographic signals were quantitated by scanning densitometry in the linear range of the autoradiograms. The intensity of the 382-bp band was normalized by the signal from the 244-bp cleavage product derived from the internal standard. Control experiments indicated that cleavage of the PCR product containing the EarI site was complete (35).

IIF. The slides containing either skin sections or cultured cells were rinsed with PBS and fixed with ice-cold 100% ethanol. For detection of type VII collagen, after a 30-min incubation with 1% BSA in PBS, the samples were exposed to an anti-type VII collagen mAb (36) (L₂D; provided by Dr. Robert Briggaman, University of North Carolina, Chapel Hill, NC) for 45 min at room temperature. Similar procedures were used for the detection of TGF- β 1 or TGF- β 2 epitopes using mAbs (provided by Dr. David R. Olsen, Celtrix Laboratories, Santa Clara, CA). The slides were then rinsed with PBS and exposed to a rhodamine-conjugated monoclonal anti-mouse IgG antibody (Cappel Labo-

Table I. Clinical Characteristics of Patients with Systemic Sclerosis

Patient		Skin involvement		Systemic involvement					Serum antibodies	
Patient number	Age/sex	Duration	Degree*	Pulmonary [†]	Renal	Cardiac [‡]	Gastrointestinal	Raynaud's phenomenon	ANA [§]	Anti-TopoI/ACA
		yr	%							
S1	36/F	<1	25	–	–	+	+	+	2560	+/-
S2	58/M	4	14	+	–	+	+	+	5120	ND/ND [¶]
S3	57/F	<2	54	–	–	+	–	+	500	-/-
S4	45/M	<1	45	–	–	–	+	+	640	-/-
S5	60/F	<1	>60	+	–	–	+	+	640	-/-
S6	35/F	<1	26	–	–	+	–	+	500	ND/-
S7	56/F	<1	20	–	–	+	+	–	5120	-/-
S8	70/F	<1	45	–	–	+	–	+	2560	-/-
S9	54/F	<1	25	ND	ND	–	ND	+	ND	ND/ND
S10	31/F	2	22	–	ND	–	ND	+	>2560	-/-
S11	38/F	2	25	+	–	+	–	+	2560	-/-
S12	37/F	<1	15	+	–	+	–	+	1280	-/-
S13	26/F	3	25	–	–	–	+	+	5120	-/-
S14	34/M	<1	1	+	–	–	+	+	1280	+/-
S15	54/F	<1	0	–	–	–	–	+	80	-/-

* Values indicate semiquantitative estimate of the percentage of body surface area that is affected clinically. [†] The pulmonary and cardiac involvement was determined as described previously (48). [§] ANA, antinuclear antibodies. ^{||} ACA, anticentromere antibodies. [¶] ND, no data available.

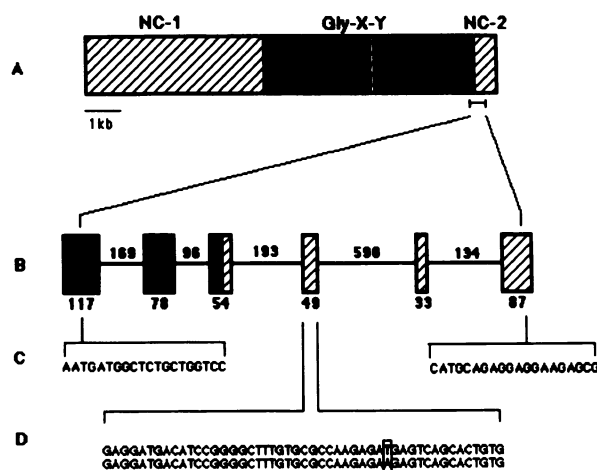


Figure 1. Schematic representation of type VII collagen domain organization, as deduced from cloned cDNAs, and the genomic positions of primer sequences used for RT-PCR amplification of type VII collagen mRNA transcripts. (A) Type VII collagen consists of a central collagenous region depicting characteristic Gly-X-Y amino acid sequence (filled boxes). This collagenous domain is flanked by a large amino-terminal NC domain (NC-1) and a smaller carboxy-terminal NC domain (NC-2) (hatched boxes). (B) The intron-exon organization of the genomic segment corresponding to the collagenous/NC-2 junction in the mRNA (35), as delineated by a bar in A. The sizes (basepair) of the introns (horizontal line) and exons either in the collagenous (filled boxes) or NC domain (hatched boxes) are indicated. (C) The positions and nucleotide sequences of the primers used for PCR amplification of a 382-bp segment from fibroblast mRNA. (D) The nucleotide sequence of a 49-bp exon within the NC-2 domain. The upper line depicts a conserved normal sequence (34) while the lower line depicts a mutated sequence detected in two siblings with recessive dystrophic epidermolysis bullosa (35). The mutated sequence was used as a template to develop a cloned cDNA which was used as an internal standard for RT-PCR. As indicated in the text, the T-to-A substitution creates a restriction enzyme site for the endonuclease *EarI*, resulting in the cleavage of the 382-bp PCR amplicon into 244- and 138-bp fragments.

ratories, Malvern, PA) for 45 min. Nonadherent secondary antibodies were then rinsed off with PBS. Nonspecific staining was excluded by performing staining reactions with the secondary antibody only.

Electron microscopy. For ultrastructural analysis, punch biopsies from two affected patients were fixed in 2.5% glutaraldehyde, 0.1 M sodium phosphate, pH 7.2, postfixed in OsO_4 , dehydrated in ethanol, and embedded in Spurr. 1- μm -thick sections were stained in toluidine blue, while thin sections were stained with uranyl acetate and lead citrate, and the samples were examined in a transmission electron microscope (100CX; JEOL U.S.A. Inc., Peabody, MA) operated at 80 kV.

Results

RT-PCR demonstrates elevated expression of type VII collagen mRNA transcripts in cultured SSc fibroblasts. To determine the levels of type VII collagen mRNAs in cultured SSc and control fibroblasts, RT-PCR was performed with total RNA isolated from cultures established from seven healthy donors and seven SSc patients. An internal standard consisting of a cDNA template with the identical sequence, with the exception of a single T-to-A nucleotide substitution which created a new restriction site for *EarI* endonuclease (see Fig. 1), was included in each reaction. Scanning densitometry of the RT-PCR products, after correction by the value from the internal standard (see

Methods), demonstrated on the average an ~ 3.4 -fold higher level of type VII collagen mRNA transcripts in fibroblast cultures from SSc patients, as compared with those from healthy donors ($P < 0.02$) (Fig. 2). Incubation with TGF- $\beta 1$ and TGF- $\beta 2$ increased the expression of type VII collagen in cultured fibroblasts established from normal controls (5.3- and 6.7-fold, respectively) and from SSc patients (1.5- and 1.3-fold, respectively) (Fig. 3).

Demonstration of elevated type VII collagen expression at the protein level by IIF. To evaluate the expression of type VII collagen at the protein level, IIF with an mAb recognizing an epitope in the amino-terminal noncollagenous (NC) domain (NC-1) (37) was performed on cultured SSc and control cells. As reported previously (29), unstimulated dermal fibroblasts from healthy controls display a faint, yet clearly detectable, staining reaction (+) for type VII collagen epitopes (Fig. 4A). Fibroblasts from eight SSc patients were stained in parallel with the same monoclonal anti-type VII collagen antibody (36). In five cases, an intense (++) to very intense (+++) staining reaction, as illustrated in Fig. 4B, could be observed. In the remaining three cases, no apparent difference between the control and SSc fibroblasts could be noted (not shown). Exposure of control fibroblasts to either TGF- $\beta 1$ or TGF- $\beta 2$ resulted in a marked increase in the immunofluorescence, in accordance with previous results (30, 31). The relative increase after stimulation with TGF- β seemed somewhat less pronounced in SSc fibroblasts, probably reflecting the higher baseline staining in unstimulated control cultures. However, the intensity of the immunofluorescence appeared to be equally strong in both control and SSc fibroblasts after stimulation with TGF- β .

IIF of skin sections from healthy controls showed an intense staining (+++ or ++++) for type VII collagen at the dermal-epidermal junction, and similar staining was observed at the basement membrane zone of the skin specimens from SSc patients (Fig. 5). In addition, six out of eight SSc patients

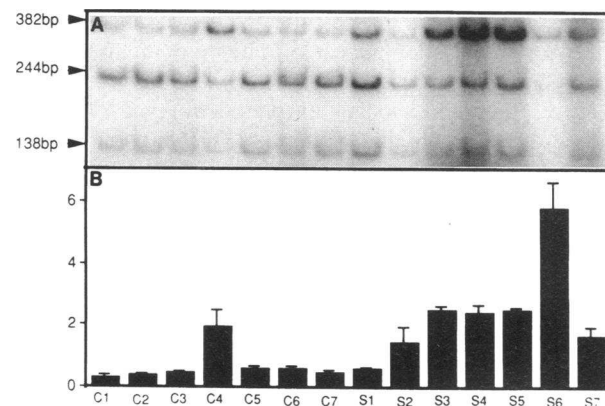


Figure 2. RT-PCR of RNA isolated from fibroblasts of normal healthy controls (C1-C7) or from patients with SSc (S1-S7). (A) Autoradiogram representing the 382-bp bands derived from amplification of control or SSc fibroblast mRNA. The 244- and 138-bp bands reflect the presence of fragments derived from the mutated internal standard cDNA after digestion with *EarI* (see Fig. 1). (B) Ratios of the 382- and 244-bp bands in each lane, as determined by densitometric scanning. The values represent the mean \pm SE from four separate determinations of the intensity of the bands at different levels of exposure to x-ray films. The mean value of SSc fibroblast type VII collagen transcripts, in comparison with controls, is statistically higher ($P < 0.02$) by Student's *t* test.

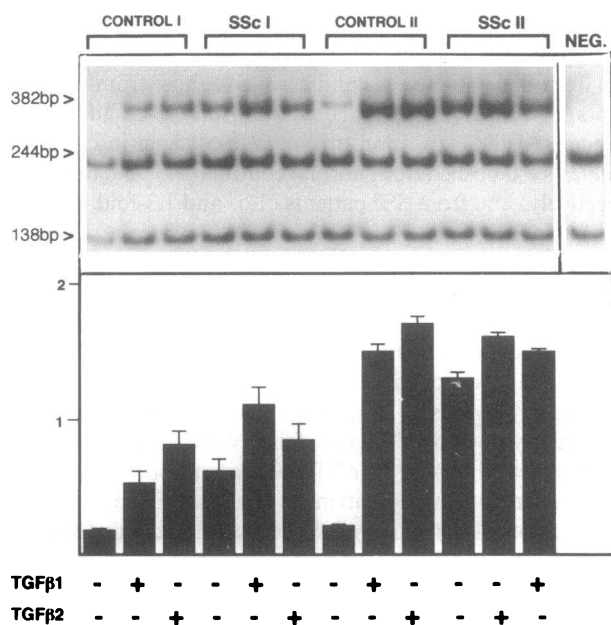


Figure 3. Demonstration that TGF- β 1 and TGF- β 2 increase type VII collagen gene expression in cultured fibroblasts. Cells from two SSc patients (SScI = S5 and SScII = S6 in Fig. 2) and matched control cultures were incubated without (-) or with (+) 10 ng/ml of TGF- β for 24 h. Type VII collagen mRNA levels were determined by RT-PCR, as described in Methods and in Fig. 2, using an internal cDNA standard. The 382-bp band represents endogenous mRNA, while the 244- and 138-bp bands result from digestion of the internal standard by Earl. The digestion of the internal standard was complete, as shown in the negative (NEG) control lane. The upper panel depicts the autoradiogram of the gel containing 32 P-labeled PCR products. The lower panel indicates the relative ratios of 382- and 244-bp bands, as determined by scanning densitometry (mean \pm SD of four separate determinations on the linear range of exposure of the autoradiograms).

showed an intense (++) or (+++) staining reaction throughout the dermis (Fig. 5, B and C, and Table II). In two cases (S4 and S5), the staining reaction appeared to be more intense in the reticular dermis, as compared with the papillary dermis (Fig. 5 B). In two patients (S2 and S3), the presence of type VII collagen was also noted within the epidermis (Fig. 5 C), and in one patient (S3) distinct staining reaction for type VII collagen was also detected in the subcutaneous tissue (Fig. 6 A). Parallel staining of control skin specimens revealed essentially negative staining reaction (- or \pm) in the epidermis and dermis (Fig. 5 A) and the subcutaneous adipose tissue (Fig. 6 B).

Detection of TGF- β epitopes in the affected skin of SSc patients. Since TGF- β has previously been shown to upregulate type VII collagen gene expression both at the protein and mRNA levels (30, 31), the presence of TGF- β 1 and TGF- β 2 epitopes was also evaluated in the skin specimens of the eight patients with SSc and compared with control skin samples from three healthy individuals. In healthy individuals, the presence of TGF- β 1 and TGF- β 2 epitopes could be observed in the epidermis (\pm , +, or ++), while the staining reaction in the dermis was essentially negative (Fig. 7, A and B). In SSc patients, TGF- β 1 and TGF- β 2 epitopes were clearly detectable in the epidermis and dermis of all patients studied (Fig. 7, C and D, and Table II). Staining for TGF- β 1 and TGF- β 2 epitopes in subcutaneous tissue was also performed in one healthy control and three patients with SSc. In the healthy individual, staining

for both forms of TGF- β was entirely negative (Fig. 6 D and Table II). In contrast, in two out of three patients with SSc, both TGF- β 1 and TGF- β 2 epitopes were detected (Fig. 6 C and Table II). In general, a correlation could be observed between the relative staining intensity of TGF- β and type VII collagen epitopes in the dermis of SSc patients (Table II).

Electron microscopy. Ultrastructural analysis of the skin from two affected patients revealed a normal appearing epidermis with a continuous basement membrane and anchoring fibrils, and the subepidermal region, as expected, contained an increased amount of collagen arranged in thick interweaving bundles (not shown). Of particular interest was the observation that the deeper dermis contained numerous aggregates of filamentous structures which were interspersed between the collagen bundles and the elastic fibers. These filaments were 15–20 nm in thickness and up to 200 nm in length. In some instances, these filaments interacted end-to-end, formed elongated structures, and occasionally showed a curvilinear conformation similar to that observed in the anchoring fibrils associated with the basement membrane at the dermal-epidermal junction (28). These findings suggest that these filaments may represent abnormal type VII collagen aggregates, but their exact biochemical composition could not be determined.

Discussion

Type VII collagen is the major, if not the exclusive, constituent of anchoring fibrils (38). These fibrils play an essential role in

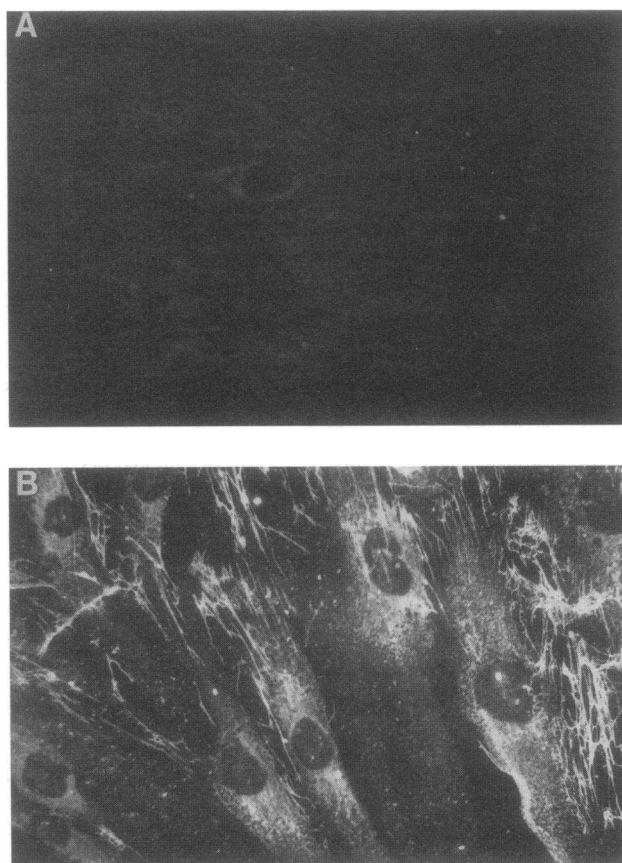


Figure 4. IIF staining with anti-type VII collagen mAb L₃D of fibroblast cultures established from a healthy control, C5 (A), and a sex- and age-matched SSc patient, S5 (B).

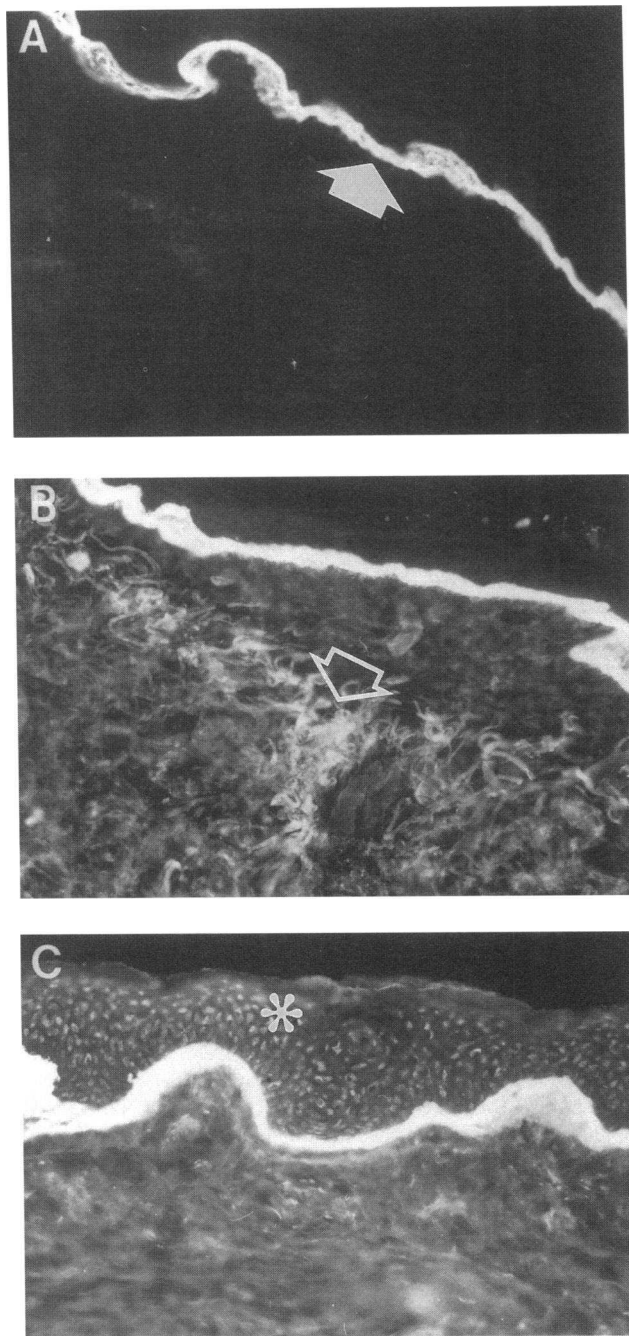


Figure 5. IIF staining of type VII collagen epitopes within normal skin (A) and in two patients with SSc, S4 and S2 (B and C, respectively). Note the strong linear staining for type VII collagen epitopes in the cutaneous basement membrane zone in all three specimens, as indicated by an arrow in A. The presence of type VII collagen epitopes is demonstrated in the reticular dermis in a patient with SSc (B, open arrow) and also within the epidermis (C, asterisk).

stabilizing the association of the basement membrane to the underlying dermis and, consequently, provide integrity to the cutaneous basement membrane zone (28). In normal skin, the distribution of type VII collagen is essentially restricted to the dermal-epidermal basement membrane zone, the site of anchoring fibrils. Decreased expression of type VII collagen and/or impaired assembly of this collagen into anchoring fibrils has been associated with blistering skin diseases, including the heri-

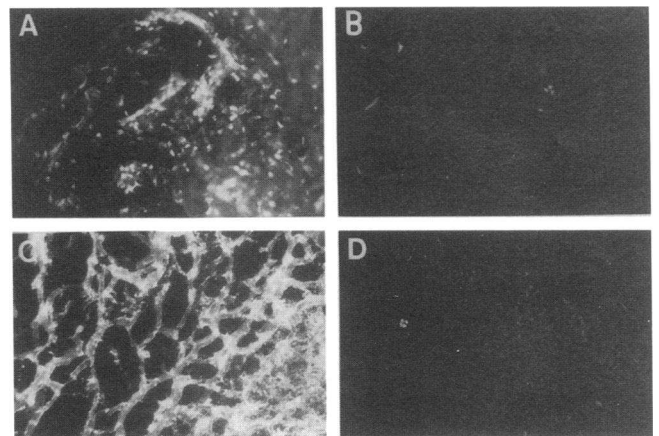


Figure 6. The presence of type VII collagen and TGF- β 1 epitopes in the subcutaneous tissue of a patient, S3 (A and C, respectively), while the immunostaining of the subcutaneous adipose tissue from a normal control skin with respective antibodies (B and D) is entirely negative.

table forms of dystrophic epidermolysis bullosa (27, 39, 40), and an acquired autoimmune disease, epidermolysis bullosa acquisita (41). To date, no example of pathologic upregulation of type VII collagen in an ectopic topographic location has been described. However, a previous ultrastructural study has indicated the presence of anchoring fibrils in the lung tissue of patients with idiopathic pulmonary fibrosis; these fibrils were not detected in normal lungs (42). Since the anchoring fibrils observed in the lung tissue resemble those found in normal skin, it is conceivable that type VII collagen levels may also be increased in the fibrotic lung tissue in SSc.

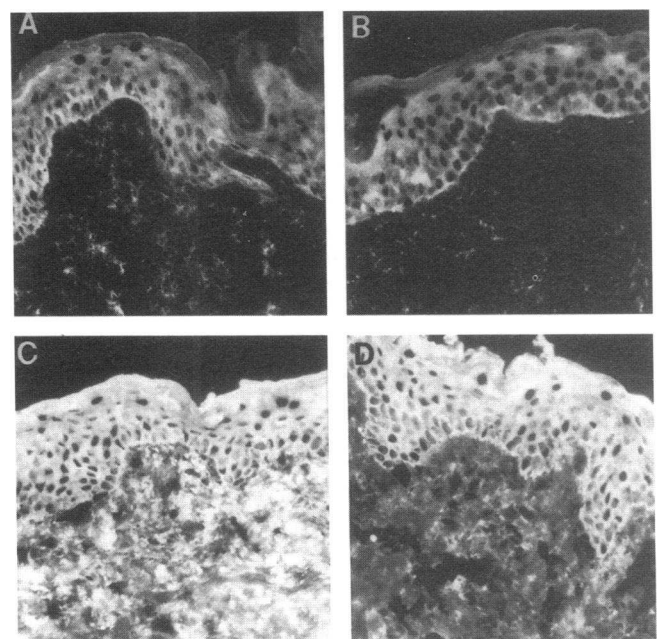


Figure 7. Immunofluorescence staining of normal human skin (A and B) or skin from a patient (S3) with SSc (C and D) with antibodies recognizing the epitopes for TGF- β 1 (A and C) or TGF- β 2 (B and D). Note the presence of a relatively faint, yet clearly detectable, staining in the epidermis of normal skin (A and B), while the staining is markedly enhanced in the SSc patient's skin (C and D).

Table II. Presence of Type VII Collagen, TGF- β 1, and TGF- β 2 Epitopes in the Skin and Subcutaneous Tissue of SSc Patients and Healthy Controls*

Patient	Type VII collagen				TGF- β 1			TGF- β 2		
	Epidermis	BMZ [‡]	Dermis	Subcutaneous	Epidermis	Dermis	Subcutaneous	Epidermis	Dermis	Subcutaneous
Controls										
C1	-	++++	-	NS	++	+/-	NS	+	-	NS
C2	-	++++	+/-	NS	++	+	NS	+	+/-	NS
C3	-	++++	+/-	-	+	+/-	-	+/-	+/-	-
SSc patients										
S2	++	++++	+++	+/-	++++	++++	+	+++	+++	+
S9	-	++++	+/-	NS	++	+	NS	+	+	NS
S10	++	++++	+++	++	++++	+++	+	+++	++	+
S11	-	++++	+++	+/-	++	++	NS	++	+	NS
S12	+/-	+++	++	+/-	++	++	+/-	++	+	+
S13	+/-	+++	++	NS	++	++	NS	++	+	NS
S14	-	+++	++	NS	++	++	NS	++	+	NS
S15	-	+++	+/-	NS	+	+	NS	+	+/-	NS

* The intensity of the staining reaction was semiquantitatively expressed as follows: -, negative; +/-, very faint; +, faint; ++, intense; +++ or +++++, very intense; NS, not studied. [‡] BMZ, basement membrane zone.

The results of our study demonstrate that in SSc skin type VII collagen is present in high amounts, not only in its expected location at the basement membrane zone but also in the dermis. Ultrastructural studies showed an abundance of fibrillar structures, which may represent type VII collagen, in the dermis of SSc patients. The accumulation of type VII collagen in SSc skin was accompanied by the presence of TGF- β 1 and TGF- β 2 epitopes. Further studies performed in vitro on dermal fibroblasts showed increased expression of type VII collagen in SSc cells at the mRNA and protein levels. Also, TGF- β 1 and TGF- β 2 further upregulated the expression of type VII collagen both at the mRNA and protein levels. Although upregulation was noted in both normal and SSc fibroblasts, the normal cells appeared to be more responsive to TGF- β stimulation, probably because of lower baseline level of type VII collagen expression in unstimulated control cultures. The higher baseline level of expression of type VII collagen in SSc cultures may result from the exposure of the cells to TGF- β in vivo, and the attenuated response of SSc fibroblasts to TGF- β is consistent with earlier data demonstrating unresponsiveness of SSc cells to growth factors (15, 43). Also, there was a general correlation between the relative intensity of staining reaction of TGF- β and type VII collagen epitopes within individual skin specimens. Collectively, these results suggest that the abundance of type VII collagen in SSc dermis may result from increased local expression of TGF- β .

Clinically, induration and thickening of the skin which is tightly bound to the underlying tissue are the hallmark of SSc. The presence of type VII collagen and fibrillar structures in the dermis may provide an explanation for this appearance of the affected skin in SSc patients, and this effect could be expected to be detectable only in the affected area of the skin. The accumulation of type VII collagen in the skin could result from upregulation of the corresponding gene by TGF- β . In this context, it is of interest to note that TGF- β 1 has been shown to increase its own expression in an autocrine manner, while TGF- β 2 increases the expression of TGF- β 1 and TGF- β 2 (44). Consequently, an early increase in the local expression of TGF-

β 1 or TGF- β 2 could perpetuate their expression, thus resulting in continued expression of type VII collagen and other TGF- β -responsive genes in chronic fibrotic conditions. Thus, attempts to counteract the effects of TGF- β with other cytokines (45, 46) or to inhibit type VII collagen gene expression by novel approaches, such as the use of antisense oligomers (47), might be helpful in preventing the progression or even in reversing some of the clinical manifestations of SSc.

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