# Elevated Expression of the Genes for Transforming Growth Factor-$\beta_1$ and Type VI Collagen in Diffuse Fasciitis Associated with the Eosinophilia-Myalgia Syndrome

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Full-thickness skin biopsies obtained from four patients with rapidly progressive diffuse fasciitis associated with the Eosinophilia-Myalgia syndrome (EMS) were examined for the expression of transforming growth factor-$\beta_1$(TGF-$\beta_1$), type VI collagen, and fibronectin genes employing immunohistochemistry and in situ hybridizations. The immunohistochemical studies demonstrated increased deposition of TGF-$\beta$, type VI collagen, and fibronectin epitopes in the extracellular matrix of the fascia in comparison to the adjacent dermis in the same specimens. Increased levels of type VI collagen mRNA, as evidenced by positive in situ hybridization signals with an $\alpha$2(VI) collagen cDNA, were also found in the fascia in comparison with the dermis. In situ hybridizations of affected fascia with a human sequence-specific TGF-$\beta_1$ cDNA demonstrated numerous fibroblasts displaying positive hybridization signals indicative of high levels of transcripts for this cytokine. In contrast, no hybridization signal for TGF-$\beta_1$ was detected in fibroblasts in the adjacent dermis. These findings suggest that TGF-$\beta_1$ may play an important role in the development of the connective tissue alterations present in EMS-associated diffuse fasciitis. *J Invest Dermatol 96:20–25, 1991*

The Eosinophilia-Myalgia syndrome (EMS) is a recently recognized clinical entity characterized by the development of severe myalgias and peripheral blood eosinophilia in association with the consumption of L-tryptophan-containing products [1–8]. The disease reached epidemic proportions in the United States in late 1989, and to date more than 1500 cases have been reported to the Centers for Disease Control. The emerging clinical picture of patients affected with EMS indicates that in a substantial proportion of cases the disease evolves into a chronic, multi-systemic illness despite the discontinuation of L-tryptophan [8]. Among the clinical features that develop during the chronic phase of the illness, diffuse scleroderma-like cutaneous changes are prominent. Clinically, the affected skin appears hyperpigmented, markedly indurated, and tightly bound to the underlying tissues. Involvement is most common in the lower extremities and may affect the upper extremities and the trunk, whereas the hands, feet, and face are usually spared. The pathologic hallmarks of the chronic cutaneous involvement in this syndrome are inflammation and fibrosis of the fascia occasionally extending to the lower dermis and subjacent muscle. Tissue infiltration with eosinophils is frequently present, particularly in the early stages of the disease [4–8].

Although the precise etiologic agent and the mechanisms responsible for the clinical and pathologic manifestations of EMS are not known at the present time, it is apparent that excessive accumulation of connective tissue in the affected fascia is responsible for the remarkable cutaneous alterations observed in these patients. In a previous study, we demonstrated that fibroblasts in the affected fascia of patients with EMS display elevated expression of type I procollagen genes as detected by in situ hybridization with a human $\alpha$1(I) procollagen cDNA [4]. In the present study, we have investigated whether the alterations in the expression of extracellular matrix genes are confined to type I procollagen or whether genes for other connective tissue components, such as type VI collagen and fibronectin, also show increased expression. In addition, we have explored the hypothesis that transforming growth factor-$\beta_1$ (TGF-$\beta_1$), a pleiotropic cytokine released from activated lymphocytes, which has been shown to stimulate various fibroblast activities [9–13], plays a role in the development of the fascial fibrosis in EMS. For this purpose, we performed tissue immunolocalization employing human-specific anti-TGF-$\beta$ antibodies and in situ hybridizations with human sequence-specific TGF-$\beta_1$ cDNA.

## METHODS

**Patients** Four patients with L-tryptophan–associated EMS who developed severe and progressive scleroderma-like cutaneous involvement were studied at the Thomas Jefferson University Hospital (Table I). The details of their clinical features and laboratory abnormalities have been described previously [4] and are briefly summarized in Table I. Each case fulfilled the preliminary criteria

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Abbreviations:

cDNA: complementary deoxyribonucleic acid
EMS: eosinophilia-myalgia syndrome
mRNA: messenger ribonucleic acid
PAP: peroxidase anti-peroxidase
SSC: standard saline citrate
TBS: Tris-buffered saline
TGF-$\beta_1$: transforming growth factor-$\beta_1$

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**Table I.** Selected Clinical Characteristics of Four Patients with Diffuse Fasciitis Associated with EMS

| | Case 1 | Case 2 | Case 3 | Case 4 |
|---|---|---|---|---|
| Age (years)/sex | 59/F | 57/F | 74/F | 69/F |
| Onset of symptoms | 8/1989 | 10/1986 | 9/1989 | 5/1989 |
| Areas of cutaneous involvement[a] | A,T,L | A,T,L | A,T,L | A,L |
| Highest eosinophil count | $2.4 \times 10^9$/l | $0.8 \times 10^9$/l | $3.4 \times 10^9$/l | $6.4 \times 10^9$/l |

[a] A, arms/forearms; T, trunk/abdomen; L, legs/thighs.

for the definition of EMS suggested recently [3]. The clinical appearance of the affected skin was consistent with the diagnosis of diffuse fasciitis in each case.

**Skin Histopathology** Full-thickness excisional biopsies that included the deep fascia were obtained from affected skin of each patient. Sections of paraffin-embedded tissue were stained with hematoxylin-eosin, Alcian blue at pH 2.5, Masson's trichrome, and Giemsa stains.

**In situ Hybridization** To investigate the expression of TGF-$\beta_1$ and type VI collagen genes, in situ hybridizations were performed with human sequence-specific cDNA, as previously described [13,14]. Briefly, 5-$\mu$m-thick cryosections were cut from snap-frozen biopsy samples, fixed immediately with paraformaldehyde in phosphate-buffered saline for 20 min, and prehybridized under conditions described previously [13,14]. The tissue specimens were hybridized for 16 h at 42°C in a solution containing the $^{32}$P-labeled cDNA probe. After each hybridization, the specimens were washed at a final stringency of 0.2 × SSC. The [$^{32}$P]cDNA-mRNA hybrids were detected by immersing the samples in autoradiographic emulsion (NTB-3, Eastman Kodak, Rochester, NY) and exposing them at 4°C in a desiccant-containing box for 3–5 d. The samples were developed with Kodak D-19 developer, stained with hematoxylin, dehydrated with ethanol, cleared with xylene, and mounted. For detection of type VI collagen mRNA transcripts, a 1.4-kb human $\alpha$2(VI) collagen cDNA was used [15]. A TGF-$\beta_1$ cDNA was developed by polymerase chain reaction amplification. For this purpose, mRNA from cultured human fetal skin fibroblasts was used as template for synthesis of cDNA, and a 450-bp region defined by synthetic oligonucleotide primers corresponding to the published TGF-$\beta_1$ sequences [16] was amplified by polymerase chain reaction [17]. The 0.45-kb cDNA was isolated by agarose gel electrophoresis and subcloned into Bluescript KS-vector, and its identity was confirmed by nucleotide sequencing [18].

**Immunolocalization of Type VI Collagen, Fibronectin, and TGF-$\beta$ Epitopes** For indirect immunofluorescence, 5-$\mu$m-thick frozen sections were rinsed with Tris-buffered saline (TBS, pH 7.6) and preincubated for 15 min in TBS containing 1% bovine serum albumin. The samples were then exposed overnight at 4°C to affinity-purified rabbit antibodies to human type VI collagen [19] and human plasma fibronectin (Accurate Chemical & Scientific Corp., Westbury, NY). The sections were washed in TBS for 60 min with five changes, and then incubated with tetramethyl rhodamine-isothiocyanate–conjugated goat anti-rabbit IgG secondary antibodies (Miles Laboratories, Inc., Elkhart, IN). After a 60-min incubation at room temperature, the sections were washed in TBS with five changes for 60 min, rinsed with distilled water, air dried, mounted with Fluoromount (Fisher Scientific, Fairlawn, NJ), and examined with a fluorescence microscope (Optiphot, Nikon Inc., Garden City, NY), equipped with filters for detection of fluorescein isothiocyanate and tetramethyl rhodamine-isothiocyanate. Representative sections were photographed using Tri-X film (Eastman Kodak Co.). In control reactions, the primary antibody was omitted or replaced with sera from non-immunized animals. Only a faint uniform background was observed in all controls.

Peroxidase–anti-peroxidase (PAP) immunostaining was a slight modification of the method previously described in detail [20]. Briefly, 5-$\mu$m-thick sections were incubated in 0.01 M HCl containing 10 U/ml pepsin (Sigma Chemical Co., St. Louis, MO). Endogenous peroxidase activity was blocked by incubating the sections in 0.3% hydrogen peroxide in methyl alcohol. To prevent non-specific antibody binding, the sections were pre-incubated for 30 min in TBS containing 1% bovine serum albumin. For detection of TGF-$\beta$ protein by immunostaining, sections were first incubated with an affinity-purified rabbit anti–TGF-$\beta$ antibody (R and D Systems Inc., Minneapolis, MN). Swine anti-rabbit antiserum (Accurate Chemical & Scientific Corp.) was then used as the linking antibody, and the sections were incubated with rabbit PAP (Accurate Chemical & Scientific Corp.). Peroxidase activity was detected by incubation with 0.05% 3,3′-diaminobenzidine tetrahydrochloride in 0.01% $H_2O_2$.

## RESULTS

The biopsy specimens obtained from the four patients studied here displayed the characteristic features of diffuse fasciitis with eosinophilic tissue infiltrates as described previously in patients with EMS [4,5,7]. In all cases, the epidermis appeared normal on histopathologic examination. A striking inflammatory cell infiltrate consisting mainly of plasma cells, lymphocytes, and abundant eosinophils was present throughout the dermis and fascia. Many eosinophils appeared to be degranulating, and eosinophilic granules were seen scattered throughout the tissue. Moderate perivascular inflammation without vessel wall necrosis was present. The fascia was markedly thickened, and heavily infiltrated with thick bundles of collagen that merged with the thickened interlobular septa of the panniculus (Fig 1).

When the affected tissues were examined for immunolocalization of type VI collagen, a marked accumulation of epitopes for this

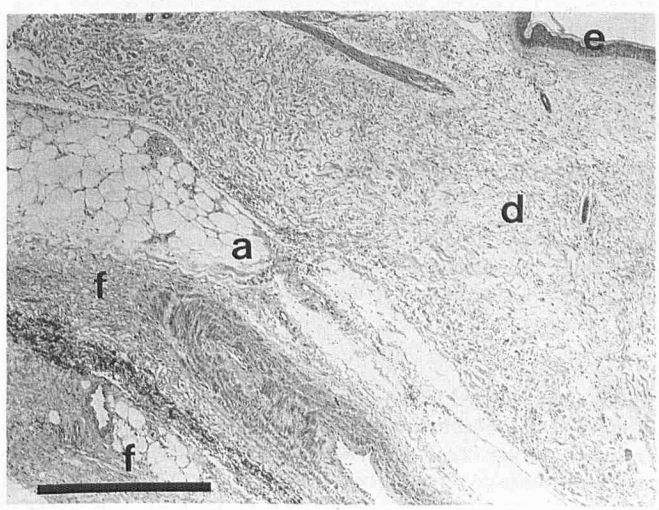


**Figure 1.** Histopathology of the full-thickness skin biopsy specimen from case 1. The dermis is moderately thickened without apparent dermal sclerosis. The fascia is markedly thickened and demonstrates excessive collagen deposition. An intense inflammatory cell infiltrate containing lymphocytes, plasma cells, and abundant eosinophils is observed throughout the tissue although it is more prominent in the fascia (hematoxylin and eosin). e, epidermis; d, dermis; a, adipose tissue; f, fascia. *Bar,* 1 mm.

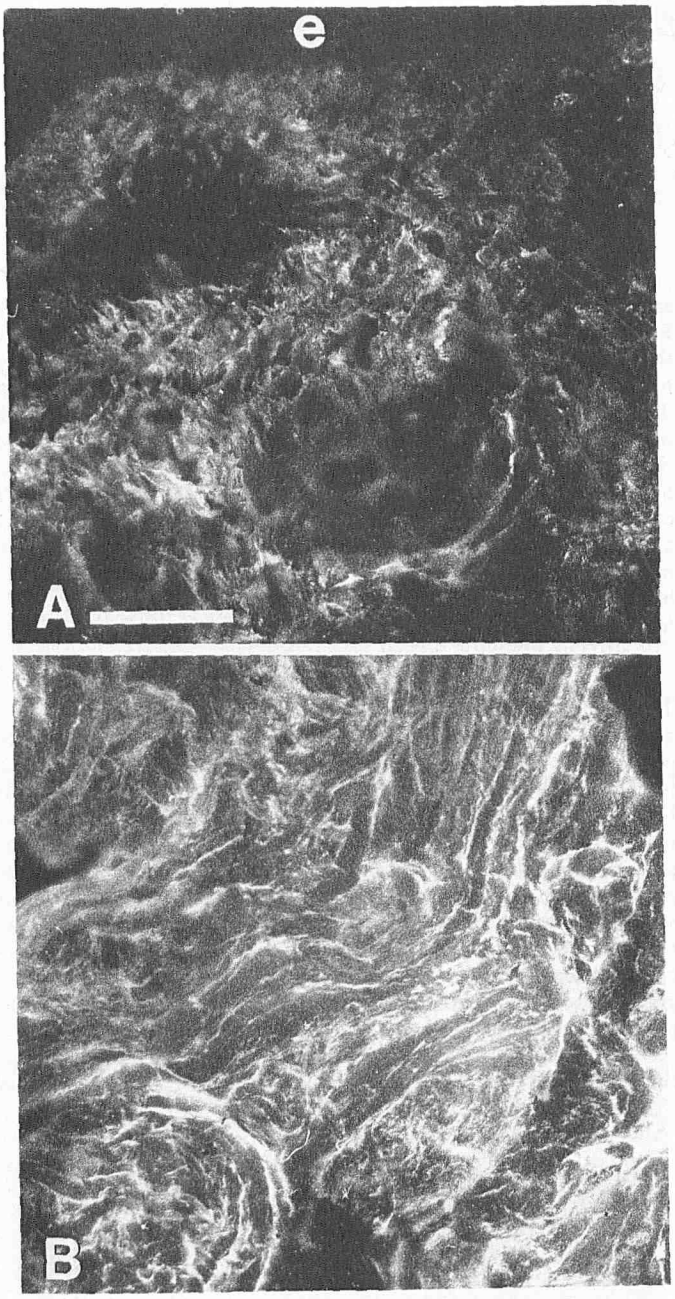


**Figure 2.** Indirect immunofluorescence staining for type VI collagen. A full-thickness skin-biopsy specimen was excised from a patient with diffuse fasciitis. *A*, upper dermis and epidermis (e); *B*, fascial region of the same tissue section as in *A*. The exposure time was the same in *A* and *B*, and the prints were reproduced under identical conditions. The immunoreaction in fascia is more intense than in dermis. *Bar*, 50 μm.

protein was observed in the fascia (Fig 2). To compare the relative amounts of the protein epitopes detectable in dermis and fascia, all specimens were processed in parallel and photographed under identical conditions of exposure time. This approach permitted a semiquantitative assessment of the intensity of immunofluorescence. As shown in Table II, there was a marked increase in the staining of the fascia with antibodies for type VI collagen. When in situ hybridization studies were performed employing an α2(VI) collagen-specific cDNA, a clearly positive hybridization signal was observed throughout the fascia (Fig 3). The hybridization signal was considerably more intense in the fascia, as compared to the adjacent dermis. Immunolocalization of fibronectin epitopes also showed much higher intensity of immunofluorescence in the fascia compared to dermis (Fig 4), indicating increased deposition of fibronectin in the fascia.

**Table II.** Comparison of the Relative Expression of Type VI Collagen and TGF-$\beta_1$ Genes and the Respective Proteins in the Dermis and Fascia from Four Patients with Diffuse Fasciitis Associated with EMS[a]

| | Dermis | Fascia |
|---|---|---|
| Type VI collagen | + | +++ |
| α2(VI) collagen mRNA | + | ++ |
| TGF-β protein | 0 | ++ |
| TGF-$\beta_1$ mRNA | 0 | +++ |

[a] Semiquantitative assessment of the levels of protein epitopes, as detected by indirect immunofluorescence, and the presence of the corresponding mRNA, as detected by in situ hybridizations, was performed as described in the text. The comparisons are based on evaluation of the same tissue specimens.

Recent studies have implicated TGF-$\beta_1$ in the pathogenesis of a variety of fibrotic diseases [21–24]. These observations coupled to our recent demonstration of elevated TGF-$\beta_1$ gene expression in involved tissue in cases of localized scleroderma (generalized morphea) and diffuse fasciitis not associated with L-tryptophan ingestion [13] prompted us to investigate the presence and expression of TGF-$\beta_1$ in dermal and fascial tissues from the patients studied here. As shown in Fig 5*A, B*, the presence of TGF-β protein was demonstrated immunohistochemically in association with a subpopulation of fibroblastic cells in the affected fascia, whereas the upper dermis was largely devoid of TGF-β epitopes. As reported previously, the basal keratinocytes of the epidermis were found to contain protein epitopes reacting with antibodies to TGF-β (Fig 5*A*). To examine if the selective deposition of TGF-β protein in the fascia demonstrated by immunohistochemistry was due to local synthesis of the cytokine, we performed in situ hybridizations with a human sequence-specific TGF-$\beta_1$ cDNA. The results, illustrated in Fig 5*C*, showed that the expression of the TGF-$\beta_1$ gene was clearly detectable in the affected fascia. In contrast, no cells with positive hybridization signals could be demonstrated in the dermis or in the sub-epidermal region (not shown).

There was no preferential localization of fibroblasts with positive hybridization signals for type VI collagen or TGF-$\beta_1$ mRNA in proximity of eosinophils. Thus, the presence of eosinophils and the activation of type VI collagen and TGF-$\beta_1$ gene expression in fibroblasts displayed a discordant spatial distribution within the tissues. Furthermore, the presence of abundant eosinophils at locations distant from activated fibroblasts and in areas displaying no hybridization signals suggests that direct eosinophil/fibroblast interactions may not be involved in the stimulation of fibroblast collagen synthesis or in the enhanced expression of TGF-$\beta_1$ gene in these patients. However, these observations do not exclude the possible role of eosinophil-derived soluble products in such activation.

## DISCUSSION

Cutaneous involvement is one of the most frequent and prominent clinical manifestations of EMS [4–8]. The most characteristic cutaneous change in the chronic phase of the disease is diffuse fasciitis with extensive thickening, inflammation, and fibrosis of the fascia. The mechanisms responsible for the excessive deposition of extracellular matrix components in the affected fascia of patients with EMS are not known at the present time. In a previous study, we demonstrated that the affected integument of patients with EMS displayed elevated expression of the genes for type I procollagen [4]. The expansion of knowledge regarding the phenotypic heterogeneity of collagens has renewed the interest in the potential role of newly discovered genetically distinct collagens in human pathologic processes. Recent studies have suggested that type VI collagen represents a major fraction of connective tissue collagens in a variety

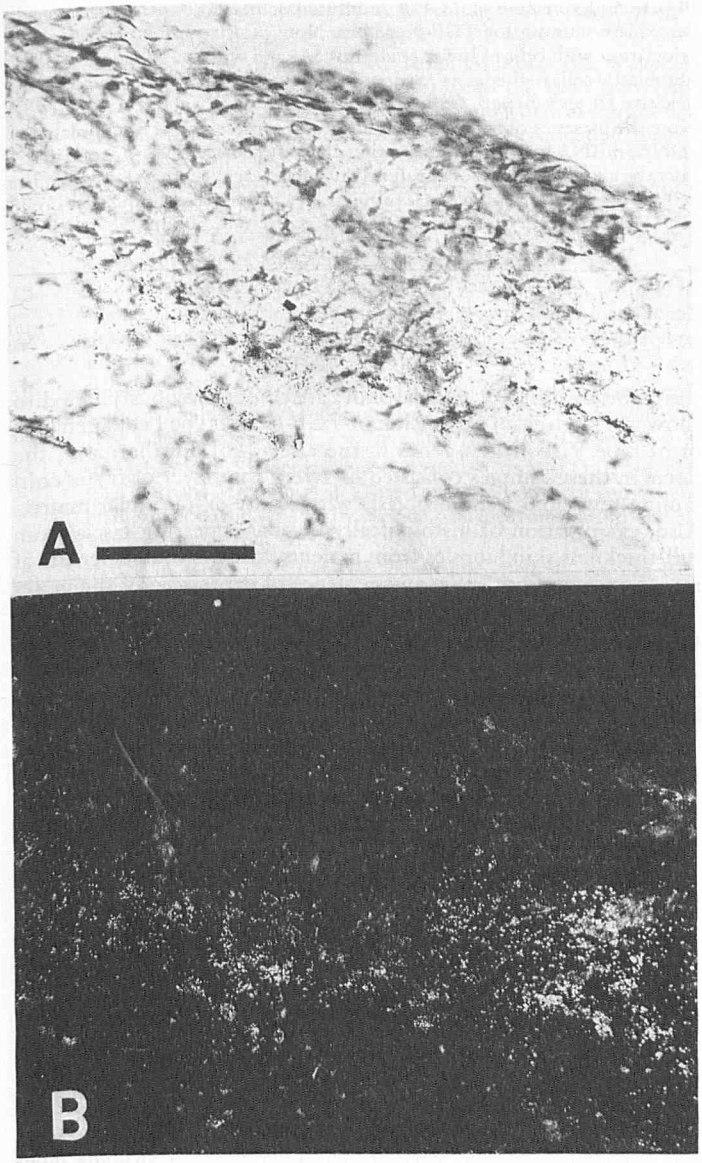


**Figure 3.** In situ hybridization of fascia with an $\alpha 2$(VI) collagen cDNA. Note the presence of autoradiographic grains representative of radiolabeled cDNA/mRNA hybrids indicating expression of type VI collagen genes within the lesional area. *A*, bright field; *B*, dark field image of *A* (hematoxylin counterstain). *Bar,* 100 $\mu$m.

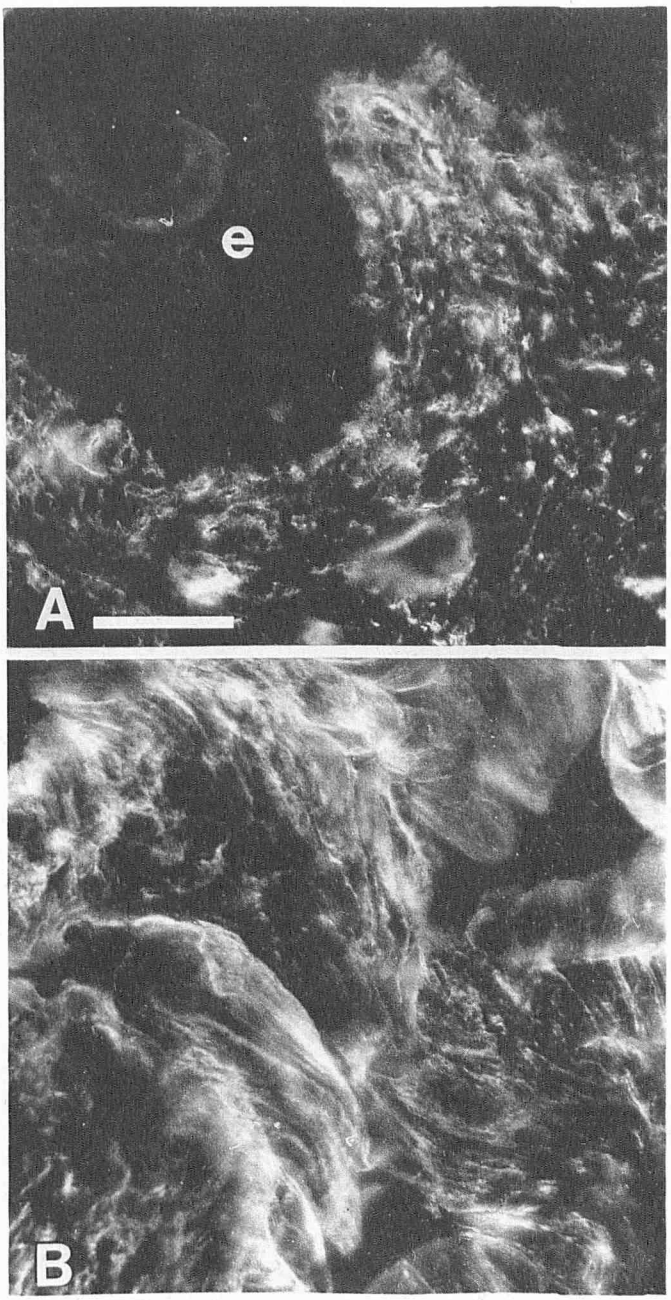


**Figure 4.** Indirect immunofluorescence staining for fibronectin of a full-thickness skin-biopsy specimen excised from a patient with diffuse fasciitis. *A*, upper dermis and epidermis (e); *B*, fascial region of the same tissue section as in *A*. The exposure time was the same in *A* and *B*, and the prints were reproduced under identical conditions. The immunoreaction is more intense in fascia than in dermis. *Bar,* 50 $\mu$m.

of tissues, including the skin [15,19,25]. In addition, we recently found that type VI collagen gene expression is increased in affected skin from patients with diffuse scleroderma [26], a disease that, as with diffuse fasciitis, is also characterized by severe cutaneous fibrosis. It was of interest, therefore, to determine if fascia from patients with EMS displayed similar abnormalities in the expression of type VI collagen. Our results clearly indicate that the increased expression of extracellular matrix genes in EMS is not confined to type I procollagen, because increased expression of type VI collagen genes as well as accumulation of the corresponding protein epitopes could be demonstrated in the affected fascia.

The prominent role that TGF-$\beta_1$ plays in the regulation of fibroblast connective tissue production [9–12,21] has suggested that this pleiotropic cytokine may be involved in the initiation, development, or progression of fibrosis in a variety of diseases of humans and experimental animals. Elevated expression of TGF-$\beta_1$ genes has been shown in generalized morphea and in diffuse fasciitis not associated with L-tryptophan ingestion [13], diffuse scleroderma [22], vitreo-retinal fibrosis [23], bleomycin-induced pulmonary fibrosis in hamsters [24], and liver injury following $CCl_4$ administration to rats [27]. Increased expression of the TGF-$\beta_1$ gene and accumulation of this cytokine in the extracellular matrix of the affected fascia of patients with EMS were demonstrated in the present study. These results suggest, therefore, that TGF-$\beta_1$ may be intimately involved in the pathogenesis of the diffuse fasciitis in patients with EMS. Furthermore, our studies demonstrated that the elevated expression of the TGF-$\beta_1$ gene was confined to fibroblastic cells in the affected

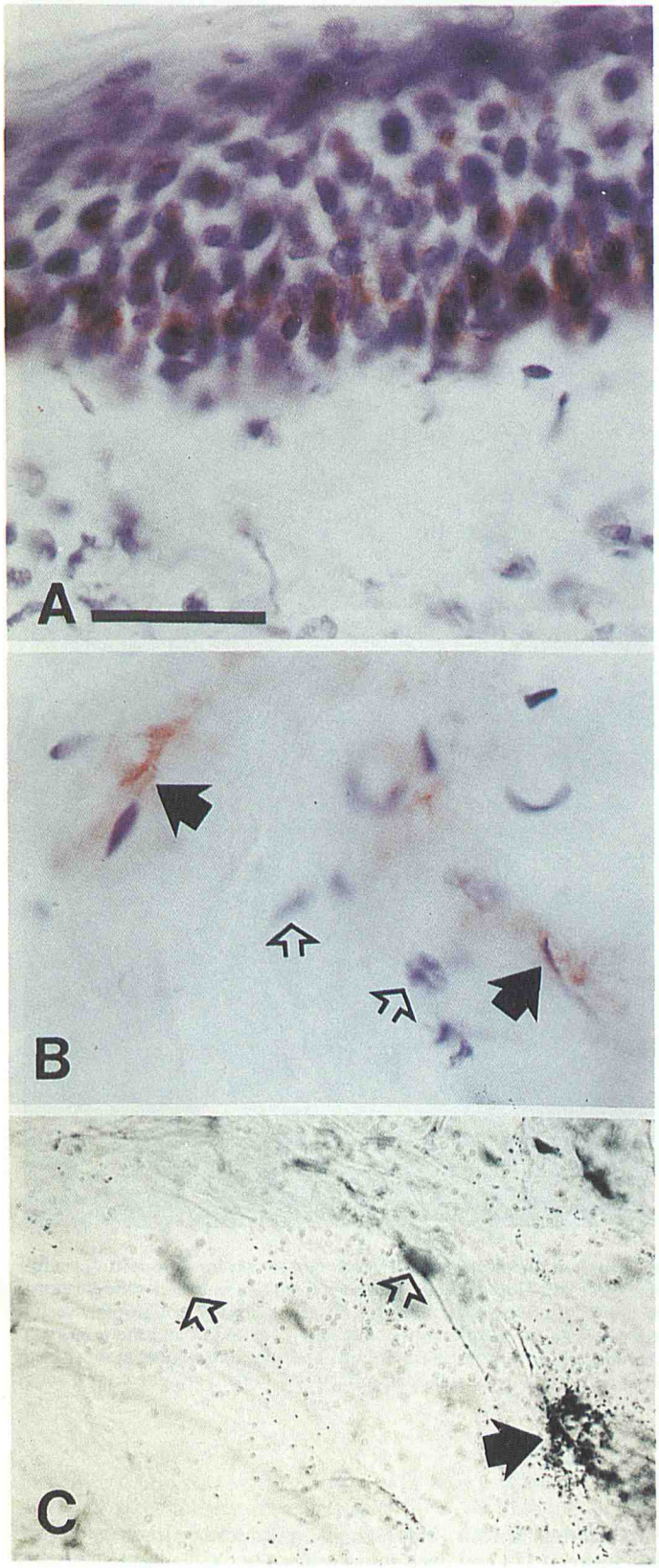


**Figure 5.** Expression of TGF-$\beta$ in diffuse fasciitis. *A, B*: peroxidase–anti-peroxidase staining for TGF-$\beta$ protein. Note positive immunoreaction in association with cells of lower epidermis *(A)* and within a subpopulation of fibroblastic cells in the fascia *(B, arrow)*, whereas the majority of the cells are negative *(B, open arrows)*. *C*: In situ hybridization with a cDNA for TGF-$\beta_1$. Note the presence of autoradiographic grains representative of radiolabeled cDNA/mRNA hybrids demonstrating expression of the TGF-$\beta_1$ gene in the fascia by a subpopulation of apparently fibroblastic cells *(arrows)*. Most of the cells, however, are negative *(open arrows)*, in a pattern resembling that noted for TGF-$\beta_1$ protein (hematoxylin counterstain). *Bar*, 50 $\mu$m.

fascia. These results are in agreement with previous in vitro observations indicating that fibroblasts are capable of the production of TGF-$\beta_1$ and that they can increase the expression of TGF-$\beta_1$ genes in an autocrine manner upon exposure to this growth factor [28]. Although normal fascia was not examined in the present study, in situ hybridizations of normal skin carried out previously failed to show any significant expression of TGF-$\beta_1$ and type I collagen [13] or of type VI collagen genes in the fascia [26]. Furthermore, the fascia in these samples consisted of a very thin layer of tissue containing few elongated fibroblasts with scanty extracellular matrix. Also, examination of histologically normal-appearing fascia from full-thickness skin biopsies from patients with systemic sclerosis of recent onset did not show any evidence of expression of the genes for type I collagen and TGF-$\beta_1$ [13] or for type VI collagen [26]. The results presented here suggest, therefore, that activated T cells present in the affected tissues in EMS-associated diffuse fasciitis produce TGF-$\beta_1$ locally. The cytokine may then interact with fibroblasts and induce its own expression in these cells. The locally produced TGF-$\beta_1$ may also be released and may bind to the extracellular matrix. The continued presence of this cytokine would cause persistent stimulation of adjacent fibroblastic cells resulting in increased production of type I and VI collagens and fibronectin leading to the development of tissue fibrosis.

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## REFERENCES

1. Eosinophilia-myalgia syndrome—New Mexico. MMWR 38:765–767, 1989
2. Eosinophilia-myalgia syndrome and L-tryptophan containing products in New Mexico, Minnesota, Oregon and New York. MMWR 38:785–788, 1989
3. Clinical spectrum of eosinophilia-myalgia syndrome—California. MMWR 39:89–91, 1990
4. Varga J, Peltonen J, Uitto J, Jimenez SA: Development of diffuse fasciitis with eosinophilia during L-tryptophan treatment: demonstration of elevated type I collagen gene expression in affected tissues. A clinicopathologic study of four patients. Ann Intern Med 112:344–352, 1990
5. Hertzman PA, Blevins WL, Mayer J, Greenfield B, Ting M, Gleich GJ: Association of the eosinophilia-myalgia syndrome with the ingestion of tryptophan. N Engl J Med 322:869–873, 1990
6. Kilbourne EM, Swygert LA, Philen RM, Sun RK, Auerbach SB, Miller L, Nelson DE, Falk H: Interim guidance on the eosinophilia-myalgia syndrome. Ann Intern Med 112:85–86, 1990
7. Silver RM, Heyes MP, Maize JC, Quearry B, Vionnet-Fuasset M, Sternberg EM: Scleroderma, fasciitis and eosinophilia associated with the ingestion of tryptophan. N Engl J Med 322:874–881, 1990
8. Varga J, Heiman-Patterson TD, Emery DL, Griffin R, Lally EV, Uitto JJ, Jimenez SA: Clinical spectrum of the systemic manifestations of the eosinophilia-myalgia syndrome. Semin Arthritis Rheum 19:313–328, 1990
9. Ignotz RA, Massague J: Transforming growth factor-$\beta$ stimulates the expression of fibronectin and collagen and their incorporation into the extracellular matrix. J Biol Chem 261:4337–4345, 1986
10. Varga J, Jimenez SA: Stimulation of normal human fibroblast collagen

production and processing by transforming growth factor-$\beta$. Biochem Biophys Res Commun 138:974–980, 1986

11. Roberts AB, Sporn MB, Assoian RK, Smith JM, Roche NS, Wakefield LM, Heine UI, Liotta LA, Falanga V, Kehrl JH, Fauci AS: Transforming growth factor type-beta: rapid induction of fibrosis and angiogenesis in vivo and stimulation of collagen formation in vitro. Proc Natl Acad Sci USA 83:4167–4171, 1986
12. Varga J, Rosenbloom J, Jimenez SA: Transforming growth factor $\beta$ (TGF$\beta$) causes a persistent increase in steady-state amounts of type I and type III collagen and fibronectin mRNAs in normal human dermal fibroblasts. Biochem J 247:597–604, 1987
13. Peltonen J, Kähäri L, Jaakkola S, Kähäri V-M, Varga J, Uitto J, Jimenez, SA: Evaluation of transforming growth factor $\beta$ and type I procollagen gene expression in fibrotic skin diseases by in situ hybridization. J Invest Dermatol 94:365–371, 1990
14. Peltonen J, Jaakkola S, Lebwohl M, Renvall S, Risteli L, Virtanen I, Uitto J: Cellular differentiation and expression of matrix genes in type 1 neurofibromatosis. Lab Invest 59:760–771, 1988
15. Chu M-L, Mann K, Deutzmann R, Pribula-Conway D, Hsu-Chen CC, Bernard MP, Timpl R: Characterization of three constituent chains of collagen type VI by peptide sequences and cDNA clones. Eur J Biochem 168:309–317, 1987
16. Derynck R, Jarret JA, Chen BY, Baton DH, Bell JR, Assoian RK, Roberts AB, Sporn MB, Goeddel DV: Human transforming growth factor-$\beta$ cDNA sequence and expression in tumor cell lines. Nature 316:701–705, 1985
17. Oste C: Polymerase chain reaction. Biotechniques 6:162–167, 1988
18. Sanger F, Niclen S, Coulson AR: DNA sequencing with chain-terminating inhibitors. Proc Natl Acad Sci USA 74:5463–5467, 1977
19. von der Mark H, Aumailley M, Wick G, Fleischmajer R, Timpl R: Immunochemistry, genuine size and tissue localization of collagen VI. Eur J Biochem 142:493–502, 1984
20. Sternberger LA: The unlabeled antibody peroxidase-antiperoxidase (PAP) method. In: Immunocytochemistry, 3rd ed., John Wiley & Sons, NY, 1986, pp 90–209
21. Roberts AB, Heine UI, Flanders KC, Sporn MB: Transforming growth factor-$\beta$. In: Fleischmajer R, Olsen BR, Kuhn K (eds.). Structure, Molecular Biology and Pathology of Collagen. Ann NY Acad Sci 1990, pp 225–232
22. Gruschwitz M, Müller PU, Sepp N, Hofer E, Fontana A, Wick G: Transcription and expression of transforming growth factor type beta in the skin of progressive systemic sclerosis: a mediator of fibrosis? J Invest Dermatol 94:197–203, 1990
23. Connor TB, Roberts AB, Sporn MB, Danielpour D, Dart LL, Michels RG, de Bustros S, Enger C, Kato H, Lansing M, Hayashi H, Glaser BM: Correlation of fibrosis and transforming growth factor-$\beta$ type 2 levels in the eye. J Clin Invest 83:1661–1666, 1989
24. Raghow R, Irish P, Kang AH: Coordinate regulation of transforming growth factor $\beta$ gene expression and cell proliferation in hamster lungs undergoing bleomycin-induced pulmonary fibrosis. J Clin Invest 84:1836–1842, 1989
25. Olsen DR, Peltonen J, Jaakkola S, Chu M-L, Uitto J: Collagen gene expression by human skin fibroblasts: abundant steady-state levels of type VI procollagen mRNAs. J Clin Invest 83:791–795, 1989
26. Peltonen J, Kähäri L, Uitto J, Jimenez SA: Increased expression of type VI collagen genes in progressive systemic sclerosis lesions in situ. Arthritis Rheum 33:11829–1835, 1990
27. Borunda-Armendariz J, Seyer JM, Kang AH, Raghow R: Regulation of TGF$\beta$ gene expression in rat liver intoxicated with carbon tetrachloride. FASEB J 4:215–221, 1990
28. van Obberghen-Schilling E, Roche NS, Flanders KC, Sporn MB, Roberts AB: Transforming growth factor $\beta_1$ positively regulates its own expression in normal and transformed cells. J Biol Chem 263:7741–7746, 1988