

The Effect of Interferon- α_{2b} on an *In Vitro* Model of Dupuytren's Contracture

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The effects of interferon- α_{2b} (IFN- α_{2b}) on Dupuytren's and control palmar fibroblasts were evaluated using the fibroblast-populated collagen lattice model. Three paired strains of Dupuytren's and control fibroblasts were exposed to IFN- α_{2b} for 96 hours before incorporation into triplicate collagen lattices. Contraction of the lattices was recorded and Northern blot analysis of cytoskeletal mRNA was performed. Comparisons of Dupuytren's and control fibroblasts revealed significantly increased contractility of the Dupuytren's fibroblasts in 2 of the 3 strains. Treatment with IFN- α_{2b} significantly inhibited contraction in both Dupuytren's and control fibroblasts. In Dupuytren's fibroblasts, treatment with IFN- α_{2b} significantly down-regulated mRNA expression for cytoplasmic β -actin and γ -actin. (J Hand Surg 1999;24A:578-585. Copyright © 1999 by the American Society for Surgery of the Hand.)

Key words: Dupuytren's surgery, fibroblasts, contracture, interferon.

Dupuytren's contracture is characterized by progressive pathologic contraction of the palmar fascia that leads to digital flexion deformities. Although the cellular and molecular mechanisms of Dupuytren's contracture have yet to be fully explained, studies have shown that Dupuytren's fibroblasts develop an extensive microfilamentous apparatus that allows for generation of contractile forces.¹⁻⁴ This apparatus is characterized by the expression of intracellular bundles of actin filaments,¹ extracellular fibronectin

fibrils,² and a transmembranous fibronexus³ that links the actin and fibronectin fibrils.

The fibroblast-populated collagen lattice (FPCL) provides an *in vitro* system in which the contractility of Dupuytren's fibroblasts with a collagen matrix can be examined. When placed in a floating collagen lattice, fibroblasts reorganize and compact collagen fibers, resulting in contraction of the lattice.⁵ Previous studies have demonstrated the ability of Dupuytren's fibroblasts to generate contractile force and rapidly contract a 3-dimensional collagen lattice.^{6,7} The use of the FPCL allows for study of fibroblast contractile mechanisms and investigation of pharmacologic agents that may modulate contractility.

Interferon- α_{2b} (IFN- α_{2b}), an antifibrogenic cytokine produced by leukocytes, has been shown to suppress fibroblast contraction in fibroproliferative disorders such as hypertrophic scar and keloids.⁸ *In vitro* studies using hypertrophic scar fibroblasts have shown that IFN- α_{2b} significantly inhibits contraction and downregulates mRNA expression for both actin⁹ and fibronectin.¹⁰ Additionally, clinical trials of intralesional IFN- α_{2b} in hypertrophic scars¹¹ and IFN- γ in Dupuytren's disease¹² have shown benefi-

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cial effects. Although the effects of IFN- α_{2b} have been examined for normal dermal fibroblasts⁵ in an FPCL, its effects on Dupuytren's fibroblasts are unknown. The purpose of this study was to evaluate the effects of IFN- α_{2b} on contractility and cytoskeletal protein mRNA expression in Dupuytren's and control fibroblasts.

Materials and Methods

Cell Cultures

After approval by the institutional ethics review board, informed consent was obtained from the patients involved in the study. Dupuytren's fibroblast explant cultures were obtained from 3 adult male patients undergoing surgery for Dupuytren's contracture. In each patient, Dupuytren's fibroblast explant cultures were obtained from contracted cords of palmar fascia and control fibroblast explant cultures were obtained from nearby normal-appearing palmar fascia. The tissue was cultured in 75 cm² culture flasks (Corning, Inc, Corning, NY) containing Dulbecco's modified Eagle's medium with 10% fetal bovine serum (Gibco, Grand Island, NY) and antibiotics (penicillin G sodium 100 U/mL, streptomycin sulfate 100 μ g/mL, and amphotericin B 0.25 μ g/mL) as previously described.⁹ Culture flasks were incubated at 37°C in a water-jacketed humidified incubator in an atmosphere of 5% CO₂. The medium was replaced twice weekly and cells were subcultured 1:6 using 0.1% trypsin and 0.02% ethylenediaminetetraacetic acid (EDTA) (Sigma, St Louis, MO) when visual confluence was reached. Cell cultures from similar passage numbers (3 to 7) were used for the FPCL and RNA extraction.

Interferon- α_{2b} Treatment

Once visual confluence was reached, Dupuytren's and control fibroblasts from each patient were divided into IFN-treated and -untreated groups. The IFN- α_{2b} -treated Dupuytren's and control fibroblasts were exposed to IFN- α_{2b} (Schering Corp, Bloomfield, NJ; specific activity 2×10^8 IU/mg protein) at 2,000 IU/mL. Fibroblasts were continually exposed to IFN- α_{2b} for 96 hours before harvesting for the FPCL assay. The dose and duration of treatment were based on earlier experiments in hypertrophic scar and normal dermal fibroblasts.¹⁰ Once polymerized within the collagen lattice, the treated group of fibroblasts received continuous exposure to IFN- α_{2b} at 2,000 IU/mL.

Fibroblast-Populated Collagen Lattice Assay

Fibroblast-populated collagen lattices were made using bovine skin type I collagen isolated and purified as described by Volpin and Veis¹³ by using a modification of the procedure of Bell et al.¹⁴ The lattices were produced in 12-mm wells within a 12-well tissue culture plate (Costar, Cambridge, MA). Each lattice contained 1.23 mL $3 \times$ Dulbecco's modified Eagle's medium + antibiotics, 417 μ L fetal bovine serum (for a final concentration of 10% fetal bovine serum), 1.5 mL sterile H₂O, 843 μ L acid-extracted fetal bovine type I collagen (3 mg/mL), 17 μ L 0.4 mol/L NaOH, and 1 mL cell suspension (300,000 cells/mL). The conditions produced rates of contraction in the control groups similar to those reported previously.^{9,14} For IFN-treated fibroblasts, IFN- α_{2b} (2,000 IU/mL) was added to the gels at the time of polymerization. Each treatment group was prepared in triplicate and immediately transferred to a humidified incubator at 37°C in an atmosphere of 5% CO₂. After 18 hours, the stabilized lattices were circumferentially released from the tissue culture wells with a sterile spatula.⁹ The surface area of the collagen gels was measured hourly for 12 hours using a transparency overlay (Fig. 1). The images were transferred using a scanner (ColorOne Scanner; Apple Inc, Cupertino, CA) connected to a computer equipped with a program (NIH Image Program, National Institutes of Health, Bethesda, MD) to quantify the area of free-form diagrams.

Cell viability was determined after 24 hours in the fibroblast-populated collagen lattice. Cells were released by digesting the lattice with bacterial collagenase (type IV; Sigma) in phosphate-buffered saline (1 mg/mL) at 37°C for 30 minutes. Isolated cells were pelleted by centrifugation at 1,600 rpm for 8 minutes and resuspended in 0.04% Trypan blue dye (Sigma) in phosphate-buffered saline. The cells were mounted on a hemocytometer and viability was determined by counting the percentage of cells that excluded the dye.

Northern Blot Analysis

Three paired strains of Dupuytren's and control fibroblasts were grown to confluence and treated with IFN- α_{2b} for 96 hours as described above. Total RNA was extracted from IFN- α_{2b} -treated and -untreated Dupuytren's and control fibroblasts using the GITC/CsCl procedure of Chirgwin et al.¹⁵ The RNA was then separated by electrophoresis on a 1% aga-

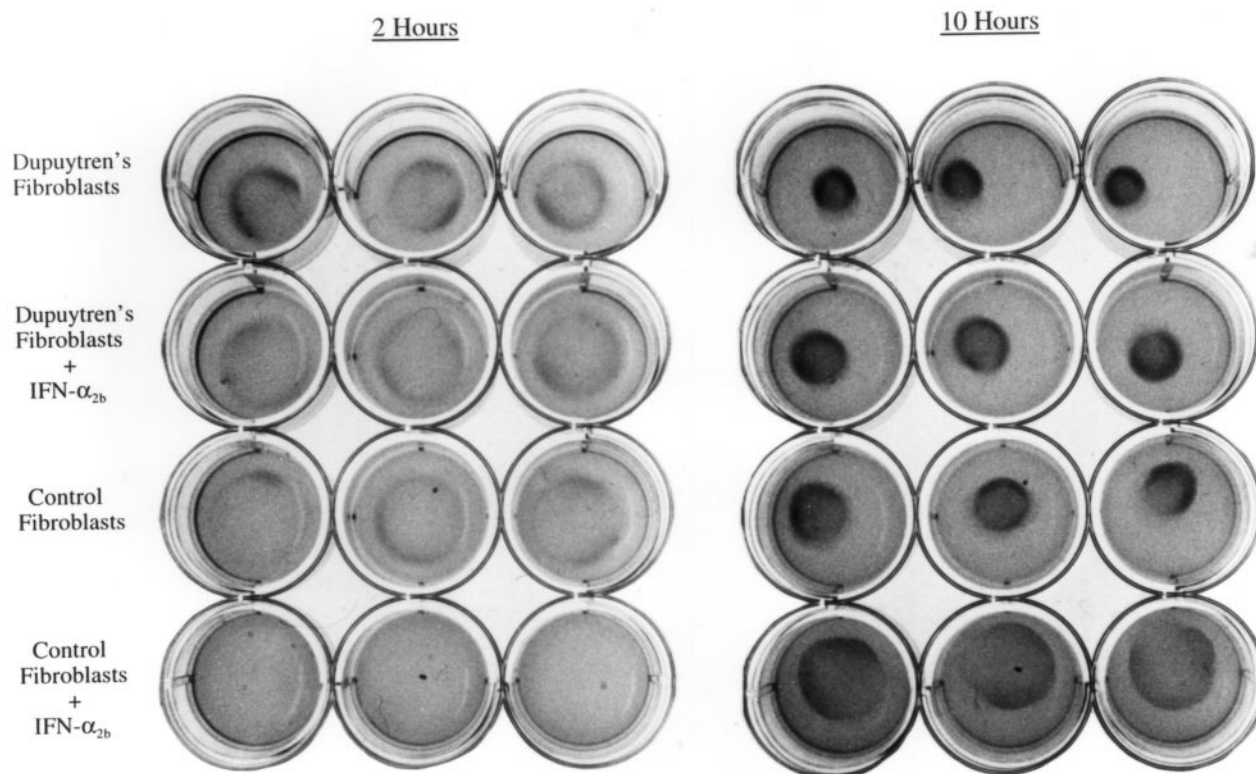


Figure 1. Fibroblast-populated collagen lattices (shown for patient 2). Each row represents one treatment group in triplicate. In all strains, treatment of Dupuytren's and control fibroblasts with IFN- α_{2b} resulted in significantly less contraction of the collagen lattices ($p < .0001$).

rose gel containing ethidium bromide and 2.2 mol/L formaldehyde. Gels were then blotted onto nitrocellulose membranes.¹⁶ Membranes were baked in a vacuum oven for 2 hours at 80°C, then incubated in prehybridization solution containing 50% formamide, 0.3 mol/L sodium chloride, 20 mmol/L Tris HCL (pH 8), 1 mmol/L EDTA, 1× Denhardt's solution (1× = 0.02% bovine serum albumin, Ficoll, and polyvinylpyrrolidone), 0.005% salmon sperm DNA, and 0.005% poly (A) for 2 to 4 hours at 45°C. Hybridization was performed in the same solution at 45°C for 16 to 20 hours using cDNA probes for α -actin, β -actin, γ -actin, and 18S ribosomal RNA (American Type Culture Collection, Rockville, MD). The probes were labeled with ^{32}P - α -dCTP (DuPont Canada, Streetsville, Mississauga, Ontario) by nick translation. Filters were initially washed at room temperature with 2× sodium chloride sodium citrate (1× = 0.15 mol/L sodium chloride, 0.015 mol/L sodium citrate) and 0.1% sodium dodecyl sulphate for 30 minutes, then for 20 minutes at 65°C in 0.1× sodium chloride sodium citrate and 0.1% sodium dodecyl sulphate solution. Autoradiography was per-

formed by exposing Kodak X-Omat film (Rochester, NY) to the nitrocellulose fibers at -70°C in the presence of an enhancing screen.

Statistical Analysis

Data from the FPCL contraction measurements and Northern blot analysis were analyzed using a paired *t*-test (2-tailed *p* values) performed with the GraphPad Prism statistical package (San Diego, CA). $P < .05$ was considered significant. Ninety-five percent confidence intervals are shown in parenthesis.

Results

Fibroblast-Populated Collagen Lattice Contraction

The data from the paired strains of fibroblasts (Dupuytren's vs control) revealed significant differences in contractility for all 3 strains (Fig. 2). Comparing the overall mean of the differences at each time point, patient 1 demonstrated a 4.3% ($p < .05$, 0.1% to 8.4%) increase in contractility of the control

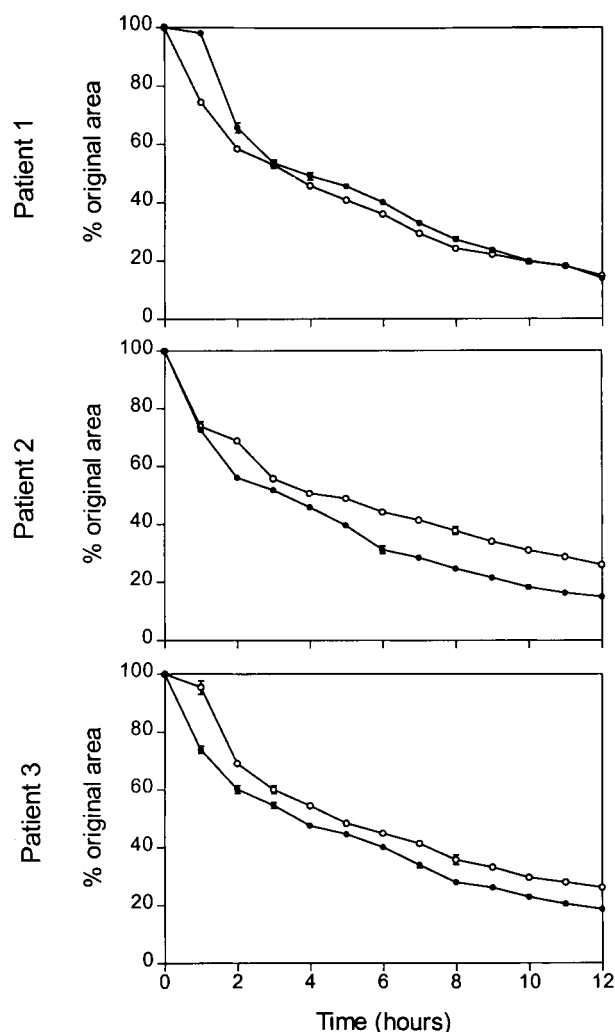


Figure 2. Contraction of FPCL by Dupuytren's (solid circles) and control (open circles) fibroblasts. Dupuytren's fibroblasts exhibited significantly decreased contractility in patient 1 ($p < .05$) and increased contractility in patients 2 and 3 ($p < .0001$). Each point represents the mean value \pm SEM for triplicate lattices.

fibroblasts compared with Dupuytren's fibroblasts. In contrast, Dupuytren's fibroblasts from patients 2 and 3 demonstrated a 10% ($p < .0001$, 7.3% to 12.6%) and 8% ($p < .0001$, 5.1% to 10.8%) increase in contractility compared with control fibroblasts.

Interferon- α_{2b} significantly inhibited contraction of both Dupuytren's and control FPCL in all strains ($p < .0001$) (Fig. 3). For Dupuytren's fibroblasts, IFN- α_{2b} treatment resulted in a mean reduction of contractility of 11.1% (8.1% to 14.1%), 15.5% (13.6% to 17.5%), and 17.8% (14.9% to 20.8%) for patients 1, 2, and 3, respectively. For control fibro-

blasts, IFN- α_{2b} treatment resulted in a mean reduction of contractility of 11.8% (9.3% to 14.3%), 21.9% (18.8% to 24.9%), and 14.7% (11.2% to 18.1%), respectively.

Cell counts performed after lattice contraction showed no significant difference in number or viability of cells between IFN-treated and untreated lattices (data not shown). Therefore, IFN- α_{2b} did not appear to have direct cytotoxic effects that could account for differences in contractility.

Expression of mRNA for Cytoskeletal Proteins

Northern blot analysis of total mRNA extracted from 3 paired cells strains of untreated and IFN- α_{2b} -treated fibroblasts was performed. The autoradiograms were quantified by densitometry and relative expression was adjusted for loading using the cDNA probe for 18S ribosomal RNA.

The β - and γ -actin isoform mRNAs had apparent sizes of 2.1 kb and were less abundant after treatment with IFN- α_{2b} (Figs. 4, 5). In Dupuytren's fibroblasts, IFN- α_{2b} treatment significantly reduced γ -actin mRNA (from 0.62 to 1.79; $p < .05$) and α -actin mRNA (from 1.20 to 2.17; $p < .005$). In control fibroblasts, IFN- α_{2b} treatment appeared to reduce β - and γ -actin mRNA, but these differences were not statistically significant. When comparing Dupuytren's and control fibroblasts without treatment, there again appeared to be less β - and γ -actin mRNA in control fibroblasts; for β -actin this difference was marginally significant (from -0.02 to 2.14; $p = .05$) and for γ -actin the difference was not statistically significant.

Discussion

The purpose of this study was to evaluate the effects of IFN- α_{2b} on contractility and cytoskeletal protein mRNA expression in Dupuytren's and control fibroblasts. This study demonstrates that (1) in 2 of 3 strains tested, Dupuytren's fibroblasts showed a significant increase in contractility compared with control fibroblasts, (2) IFN- α_{2b} significantly inhibits contraction of Dupuytren's and control fibroblasts in FPCL, and (3) treatment of Dupuytren's fibroblasts with IFN- α_{2b} significantly downregulates mRNA levels of cytoplasmic β - and γ -actin isoforms.

Previous studies have reported varying results when comparing the ability of Dupuytren's and control fibroblasts to contract a collagen lattice. Tarpila et al⁶ found that control dermal fibroblasts from the forearm caused significantly more contraction of col-

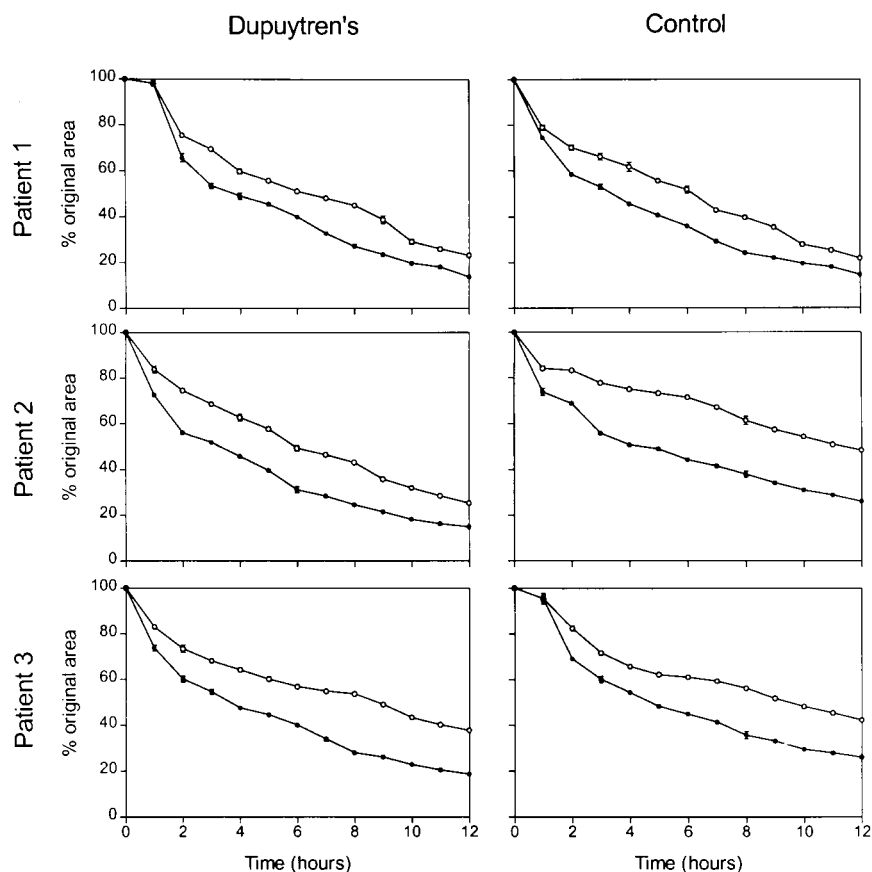


Figure 3. Contraction of FPCL by untreated (solid circles) and IFN- α_{2b} -treated (open circles) Dupuytren's and control fibroblasts. Treatment with IFN- α_{2b} significantly decreased contractility of Dupuytren's and control fibroblasts ($p < .0001$) for all 3 patients. Each point represents the mean value \pm SEM for triplicate lattices.

lagen lattices compared with fibroblasts from nodules of Dupuytren's contracture. Rayan and Tomasek⁴ previously demonstrated no difference in the ability of Dupuytren's fibroblasts and normal palmar fibroblasts to contract a collagen lattice. More recently, however, Tomasek and Rayan¹⁷ observed differences in these 2 populations when Dupuytren's cell strains were divided into 2 populations based on expression of α -smooth muscle actin. In our study, we observed that 2 of the 3 strains of Dupuytren's fibroblasts were significantly more contractile than control fibroblasts. In one strain, control fibroblasts showed increased contractility compared with Dupuytren's fibroblasts. It is possible that these variations in contractility reflect explant cultures of fibroblasts at varying stages of the disease process, subclinical involvement of the tissue obtained as control palmar fascia, phenotypic variability of some cell strains during the cell culturing process, or other unknown mechanisms that modulate contractility. Further studies are needed to clarify these issues.

In this study, control tissue was obtained from the nearby normal-appearing palmar fascia in Dupuytren's disease patients. It is important to note that this was chosen to represent control fascia rather than normal fascia. Previous studies have documented lesser pathologic changes in apparently uninvolved palmar fascia in Dupuytren's disease.¹⁸ Although many previous studies use palmar fascia obtained from normal healthy patients as controls, these studies may be confounded by interindividual variables such as age, sex, systemic influences, and genetic factors, which also likely contribute to the diathesis of Dupuytren's disease. While the possibility of subclinical involvement of control fibroblasts from normal-appearing palmar fascia remains, our data suggest that rates of contraction differ in at least 2 of 3 fibroblast pairs, suggesting that differences exist between contracted and noncontracted palmar fascia in patients with Dupuytren's disease.

Fibroblast-populated collagen lattices provide a useful model for studying Dupuytren's contracture.

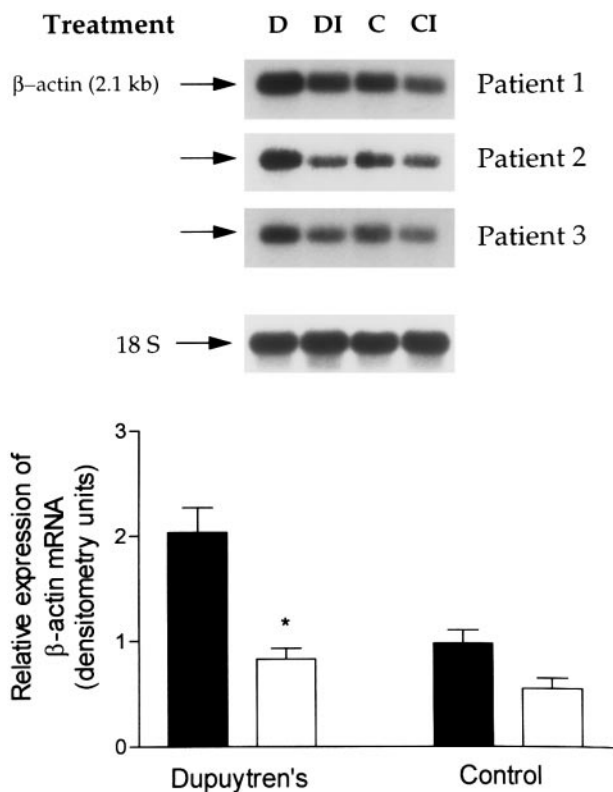


Figure 4. Northern blot analysis of β -actin mRNA expression. Loading was corrected with 18S ribosomal RNA (shown for patient 1). Interferon- α_{2b} treatment (open bars) significantly reduced β -actin mRNA in Dupuytren's fibroblasts. D, Dupuytren's fibroblasts; DI, Dupuytren's fibroblasts + IFN- α_{2b} ; C, control fibroblasts; CI, control fibroblasts + IFN- α_{2b} . * $p < .05$.

Fibroblasts in collagen lattices function in a more *in vivo*-like environment, proliferate more slowly, and acquire a more *in vivo*-like morphology than fibroblasts grown in monolayer culture.⁵ In the stress-relaxed collagen lattice model similar to our experimental model, fibroblasts organize their actin into microfilament bundles (stress fibers), generate isometric tension, and rapidly contract on release of the lattice from its points of attachment.¹⁹ This rapid phase of contraction is followed by a slower phase of contraction that is similar to the contraction of a free-floating collagen lattice model. During this slow phase of contraction, traction forces are produced by elongation and spreading of fibroblasts, resulting in compaction of collagen fibrils.²⁰

Previous studies have used the FPCL to investigate the effects of pharmacologic agents on contractility of Dupuytren's fibroblasts.^{12,21} The suggestion that the IFN family of cytokines may function as anti-

brogenic factors has led to investigation of its effects in several fibroproliferative disorders, including hypertrophic scar, keloid, scleroderma, and Dupuytren's contracture. Using the FPCL model, IFN- α_{2b} , IFN- β , and IFN- γ have all been shown to reduce the rate and extent of lattice contraction.⁵ In an open pilot study, Pittet et al¹² demonstrated the beneficial effects of intralesional injections of γ -IFN in Dupuytren's disease. However, we are unaware of previous *in vitro* studies exploring the effect of IFN- α_{2b} on Dupuytren's fibroblasts. Our findings demonstrate that IFN- α_{2b} significantly ($p = .0001$) inhibits the contraction of Dupuytren's and control fibroblasts in an FPCL. This inhibition of contractility does not appear to be due to a direct cytotoxic effect of IFN- α_{2b} as cell counts performed after gel contraction showed no significant difference ($p = .15$) in cell counts or cell viability between untreated and IFN- α_{2b} -treated fibroblasts.

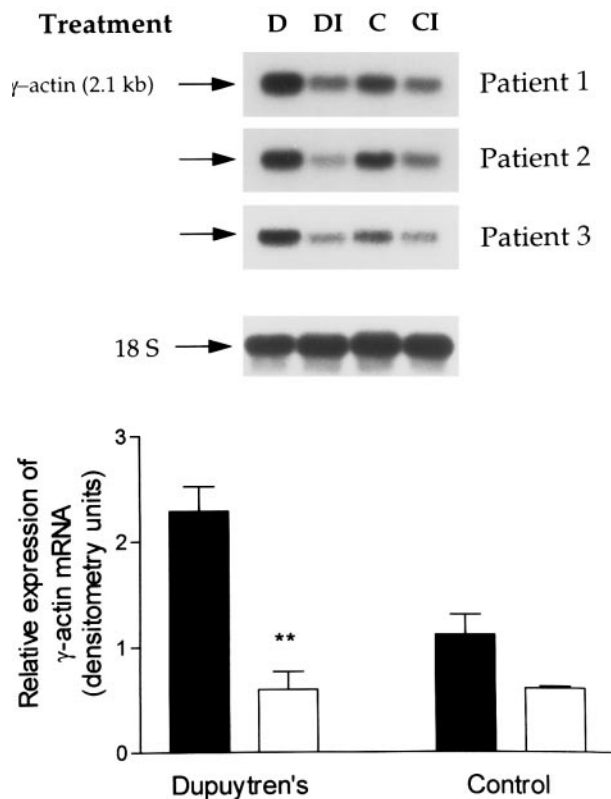


Figure 5. Northern blot analysis of γ -actin mRNA expression. Loading was corrected with 18S ribosomal RNA (shown for patient 2). Interferon- α_{2b} treatment (open bars) significantly reduced γ -actin mRNA in Dupuytren's fibroblasts. D, Dupuytren's fibroblasts; DI, Dupuytren's fibroblasts + IFN- α_{2b} ; C, control fibroblasts; CI, control fibroblasts + IFN- α_{2b} . ** $p < .005$.

Although the exact cellular mechanisms of Dupuytren's contracture are unknown, 2 main theories exist. Myofibroblasts have been proposed as a subtype of fibroblasts that acquire phenotypic features of smooth muscle cells and cause shortening of the palmar fascial. These myofibroblasts express increased amounts of α -smooth muscle actin (α -SMA) rather than β and γ cytoplasmic actin isoforms, which are characteristically expressed by normal fibroblasts. It has been previously demonstrated that Dupuytren's fibroblasts can acquire smooth muscle characteristics and that increased expression of α -SMA correlates with increased contractility.¹⁷ However, the same investigators noted that the expression of large amounts of α -SMA does not appear to be required for cellular contraction. Indeed, normal palmar fibroblasts, which express little α -SMA, can still generate considerable amounts of contractile force, as do strains of Dupuytren's contracture expressing little α -SMA.¹⁷ Additionally, Leavitt et al²² have demonstrated that transformed cell strains expressing α -SMA phenotypically exhibit greater contact inhibition and lower invasive and motility capabilities (features of fibroblasts required during wound healing and contractile situations) than do others lacking α -SMA expression, further calling into question the correlation of α -SMA expression and contraction.

An alternative theory of contraction proposes that fibroblasts exert a traction force generated by the continual extension and retraction of filipodia in a treadmill fashion rather than progressive shortening of their cytoskeleton.²³ Based on antibody and gene localization studies, filipodia and regions of moving cytoplasm predominantly consist of class I isoactins (β and γ) whereas microfilament bundles (stress fibers) are predominantly composed of class II actins (α -actins).²⁴ Previous studies also have shown that β - and γ -actin isoforms can interact with myosin and generate contractile forces.¹⁷ It is possible that a reduction in cytoplasmic β - and γ -actin impedes the formation of filipodia and subsequently reduced the force producing lattice contraction. Further studies are needed to explore the interaction of cytoplasmic proteins and their relationship to contractility in Dupuytren's contracture.

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