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Regulation of expression of α-smooth muscle actin in cells of Dupuytren's contracture

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Our aims were to describe the distribution of α-smooth muscle actin (SMA)-containing cells in Dupuytren's tissue in vivo and to determine the effects of selected agents in regulating the expression of SMA in Dupuytren's cells in vitro.

In selected hypercellular zones of Dupuytren's nodules up to 40% of the cells contained SMA, as shown by immunohistochemistry. A lower percentage (20%) of SMA-containing cells was found in regions of lower cellularity. A notable finding was that treatment in vitro of Dupuytren's cells with platelet-derived growth factor significantly reduced the content of SMA. Cells from the same patients showed a significant increase in expression of SMA in response to treatment with transforming growth factor, which confirmed recent findings. In addition, interferon-γ, which has been previously used as a treatment for Dupuytren's disease in a clinical study, had no reproducible effect on the expression of this actin isoform. Our findings are of significance for the conservative management of contractures.

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Dupuytren's contracture, or palmar fibromatosis, is one of several fibromatoses characterised by nodular or distributed aggregates of immature fibroblasts dispersed in a dense collagen matrix.[1] As a result of the proliferation and action of myofibroblasts in the palmar fascia, patients with Dupuytren's disease may have progressive and irreversible flexion contractures of the phalangeal joints. Myofibroblasts were first implicated as being responsible for contracture in Dupuytren's disease on the basis of their ultrastructural identification in transmission electron-microscopic studies.[2] Later investigations showed the expression of α-smooth muscle actin (SMA) in cells from tissue explants.[3] It was also shown that myofibroblasts were capable of generating a contractile force.[3,4]

Historically, surgery has been the main method of treatment of Dupuytren's disease, but the growing understanding of the role of cytokines in the aetiology of the disease suggests that non-surgical alternatives could be developed. Modification of the disease may result from regulation of the contractile cell phenotype by cytokines. A recent study[5] has shown the effectiveness of using pharmacological agents to reduce in vitro the contraction of Dupuytren's cells stimulated by lysophosphatidic acid.

Luck[6] described three phases through which Dupuytren's diseased tissue is believed to progress: proliferative, involutional, and residual. The relative abundance and distribution of myofibroblasts in Dupuytren's tissue vary according to the phase of the disease. During the proliferative stage, when fascial fibroplasia causes a nodular lesion, myofibroblasts appear and contribute extensively to the nodule. The involutional phase is marked by an alignment of the cells with the lines of stress within the tissue, and the myofibroblasts show a similar pattern. In the residual phase, the nodule is replaced by scar tissue and myofibroblasts can no longer be identified. They appear to have been replaced by mature fibroblasts.[7] During the progression of the disease, SMA is also expressed transiently. It appears and increases during the proliferative phase, remains during the involutional phase, and disappears during the residual phase.[8]

One objective of our study was to evaluate immunohistochemically the distribution of SMA-expressing cells in Dupuytren's tissue in order to determine the number of these cells in the various cellular regions which comprise Dupuytren's nodule and to establish the prevalence of this

Table I. Details of the 13 patients undergoing subtotal fasciectomy for Dupuytren's contracture

Case (specimen)	Age (yrs)	Gender
Immunohistochemistry		
1	26	F
2	44	M
3	58	M
4*	63	M
5	65	M
6	68	M
7*	73	M
8	74	M
Cell culture		
9	55	M
10	61	M
11	65	M
12	67	M
13	79	M

*recurrence of the disease

actin isoform in situ by direct examination of tissue samples. A second objective was to determine the effects of the following cytokines on the expression of SMA in Dupuytren's cells in vitro by Western blot analysis: transforming growth factor (TGF)-β1, platelet-derived growth factor (PDGF)-BB, and interferon (IFN)-γ. Recent immunofluorescence studies have shown that TGF-β1 can increase the expression of SMA in cells isolated from Dupuytren's tissue[9] and that IFN-γ can block the TGF-β1 stimulation of SMA.[10] Previous studies[11,12] have shown a reduction of SMA in fibroblasts from other sources treated with PDGF-BB by Western blot analysis.

A recent study[13] has used injections of purified clostridial collagenase to disrupt the Dupuytren's nodule in the non-surgical management of the disease. While the results are promising there is a concern about the incidence of recurrence of the contracture. A direct comparison of the effects of selected growth factors on the expression of SMA in Dupuytren's cells could be instructive in providing adjunctive therapy for the enzymic treatment of the disease and to reduce the incidence of recurrence. The value of our study extends beyond Dupuytren's disease. The development of a non-surgical treatment for Dupuytren's disease may also be implicated in the management of other contractures.

Materials and Methods

Tissue specimens were obtained from 13 patients undergoing subtotal fasciectomy for Dupuytren's contracture (Table I). The indication for surgery was failure of conservative management. The excised tissue was obtained for study under a protocol which was approved by the Institutional Review Board. Two patients had recurrence of the disease. Samples from eight patients were allocated for SMA immunohistochemistry and specimens from the remaining five were designated for cell culture and Western blot analysis of the effects of selected cytokines on the expression of SMA.

Immunohistochemistry. The specimens were rinsed with phosphate-buffered saline solution (PBS) and fixed in formalin for seven days before being processed in the Tissue-Tek Vacuum Filtration Processor. The samples were embedded in paraffin. Microtomed sections, 7 μm thick, were mounted on to glass microscope slides. Several sections were fixed on each slide. The slides were stained with a monoclonal antibody for SMA.

In preparation for immunohistochemistry, the slides were first deparaffinised in xylene and then rehydrated in alcohol and water baths. The sections were digested for one hour with 0.1% trypsin (Sigma Chemical Co, St Louis, Missouri). After digestion, the slides were rinsed with PBS before 3% H_2O_2 was added for ten minutes to quench endogenous peroxidase. They were rinsed with PBS and incubated with 30% goat serum (#G9023; Sigma Chemical Co) for ten minutes to block non-specific sites. The primary antibody, mouse monoclonal anti-smooth muscle actin antibody (Cat A2547, clone 1A4, Sigma Chemical Co), was applied to all but one section on each slide. The last section was treated with mouse serum at the same protein concentration as that of the monoclonal antibody solution and served as a negative control. The slides remained incubated with these solutions for two hours and then were rinsed with PBS. They were then incubated with the secondary antibody, biotinylated goat anti-mouse immunoglobulin (#B7151, Sigma Chemical Co) for two minutes before being rinsed again with PBS. Extra Avidin-conjugated peroxidase was added for 20 minutes and the slides were rinsed further with PBS followed by distilled water. The labelling was developed by incubation with the substrate reagent (00-2007, Zymed) for ten minutes. The slides were then washed in distilled water before being placed in a bath of the counterstain, Mayer's haematoxylin, for 20 minutes. Coverslips were applied to the slides with warmed glycerol gelatin.

Immunohistochemical evaluation. The stained slides were examined using a Vanox AH-2 microscope (Olympus, Tokyo, Japan) by normal and polarised light. Four histological zones were identified on the basis of their cellularity.

Vascular. These zones comprised a unique category of cellularity since vascular smooth muscle always stained positive for SMA. This zone was not included in the evaluation of expression of SMA in myofibroblasts because of the confounding presence of a large number of vascular cells. Vascular zones were considered to be areas which contained one or more blood vessels.

Hypercellular. These contained more than 100 cells per mm^2.

Moderately cellular. These had between 50 and 99 cells per mm^2.

Hypocellular. These contained fewer than 50 cells per mm^2.

The percentage of the section represented by each of these four zones was estimated.

Cell morphology was determined according to nuclear aspect ratio. The cells were categorised as either round,

Table II. Summary of immunohistochemical results. For specimens 2,4 and 7, two regions of the biopsy were evaluated because of the marked morphological differences

	Zone		Morphology (% of totals)					
Case specimen	Cellularity	% of section	Round	Oval	Elongated	Elongated and crimp	SMA+ of total cells (%)	SMA + of crimped cells (%)
1								
	Vascular	2	60	40	0	0	0	
	Hyper	2						
	Moderate	4	40	60	0	0	0	
	Hypo	92	0	20	70	10	0	0
2a								
	Vascular	15	40	50	8	2	94	90
	Hyper	40						
	Moderate	15	20	30	30	20	40	50
	Hypo	30	15	20	15	50	10	20
2b								
	Vascular	2	60	40	0	0	100	
	Hyper	28						
	Moderate	10	35	50	8	7	35	0
	Hypo	60	10	20	30	40	5	2
3								
	Vascular	1	28	72	0	0	10	
	Hyper	52						
	Moderate	35	0	75	25	40	0	0
	Hypo	12	0	32	17	51	0	0
4a								
	Vascular	1						
	Hyper	15	85	15	0	0	0	
	Moderate	20	72	28	0	0	0	
	Hypo	64	0	5	5	90	20	20
4b								
	Vascular	5	100	0	0	0	40	
	Hyper	3						
	Moderate	2	0	100	0	0	10	
	Hypo	90	0	0	5	95	2	0
5								
	Vascular	2						
	Hyper	28	95	5	0	0	30	
	Moderate	30	10	80	10	0	20	
	Hypo	40	5	5	85	5	0	0
6								
	Vascular	1	85	15	0	0	90	
	Hyper	75						
	Moderate	14	0	85	10	5	20	0
	Hypo	10	0	10	40	60	0	0
7a								
	Vascular	15	30	70	0	0	95	
	Hyper	5						
	Moderate	45	0	15	15	60	5	2
	Hypo	35	40	45	10	5	0	0
7b								
	Vascular	2	30	70	0	0	10	
	Hyper	20						
	Moderate	30	10	10	10	70	80	90
	Hypo	48	10	20	65	15	10	0
8								
	Vascular	5	92	8	0	0	25	
	Hyper	40						
	Moderate	20	10	75	15	10	30	2
	Hypo	35	5	30	23	42	1	1

oval, or elongated according to the following criteria: 1) round, length:width = 1; 2) oval, <1 length:width < 5; and 3) elongated, length:width > 5.

Elongated cells were also analysed for crimp. Cells were considered to be crimped if they had at least one fold along their length, the period of which was generally comparable to that of the crimp of the adjacent collagen matrix. The percentage of cells with each of the three morphologies was determined in each of the four zones.

We evaluated only immunohistochemically prepared slides on which the negative control section displayed no noticeable presence of the chromogen. The smooth muscle cells in the vascular zones were used as a positive control. Cells were considered to be positive for smooth muscle actin only if they stained with the same chromogen intensity which was found in the vascular smooth muscle. The percentage of SMA-positive cells was determined by dividing the number of positively-stained cells by the total number of cells in the zone and rounding the results to the nearest 5% (for values greater than 5%). The percentage of the SMA-containing cells among those which were crimped was also determined.

Examination of the biopsied tissues from three patients (cases 2, 4 and 7) showed notable differences in selected regions, and these regions were evaluated separately.

Cell culture. The tissue samples were placed in PBS until they were prepared for cell culture within three hours of removal. After rinsing the tissue samples thoroughly with PBS the fat and soft tissues surrounding the fibrotic lesion were removed. Each sample was then divided into 1 x 1 x 2 mm sections. These were used as explants and placed into two-dimensional six-well culture plates containing 2 ml of Dulbecco's modified medium (D-MEM F12) supplemented with 10% heat-inactivated fetal bovine serum (Australian Certified; Hyclone, Logan, Utah) and 1% antibiotic-antimycotic solution prepared with 10000 U/ml of penicillin G sodium, 10000 µg/ml of streptomycin sulphate, and 25 µg/ml of amphotericin B in 0.85% saline (100X; Gibco BRL, Grand Island, New York). The culture plates were placed in an incubator at 37˚C in an atmosphere of 5% CO_2 and 95% air. The media were changed three times a week. The explants were observed daily for signs of outgrowth. When the fibroblasts from the explants achieved confluence, the cell number was estimated using a haemocytometer, and the cells were subcultured into four 25 ml culture flasks. The amount of prepared medium added increased to 5 ml per flask and the media continued to be changed three times a week. When the cells in the flasks from the first or second passage were 75% confluent, they were treated with the selected cytokines (see below).

Cytokine treatment. The growth factors and doses which we used included: TGF-β1, 1 ng/ml (Sigma Chemical Co); PDGF-BB, 10 ng/ml (BioMimetic Pharmaceuticals Inc, Franklin, Tennessee), and interferon-γ, 1000 U/ml (Sigma Chemical Co). These doses were based on previous studies performed on human gingival fibroblasts,[11] cells isolated from torn human rotator cuffs[12] and on smooth muscle cells.[14] One or two 25 ml flasks were prepared for each agent and the untreated control group. The flasks were incubated with the agent and 5 ml of prepared medium for four days. The control cultures received only the culture medium. The cells were then removed from the flasks by digestion with trypsin. They were centrifuged, resuspended in PBS and counted. The solution was then centrifuged again, the PBS removed, and lysis buffer added at 0.5 ml per 2 million cells and placed in the freezer until a Western blot analysis was performed.

Smooth muscle actin Western blot analysis. Tubes containing the lysis buffer and cells from the tissue digest and cell cultures were microcentrifuged and the supernatant removed. Protein extracted from smooth muscle cells (human aorta) served as the positive control. To perform the protein assay, 0.15 µg/µl of bovine serum albumin was used in amounts of 10, 20, 40 and 60 µl, with 200 µl of dye. dd H_2O was added to bring the total amount to 1000 µl and a standard curve plotted. Then 20 µl of supernatant from each sample was added to 780 µl of ddH_2O and 200 µl of dye. The mixture was incubated for ten minutes at room temperature and readings were taken at a wavelength of 595 nm.

The following amounts of protein were analysed: 5 µg of the smooth muscle cell control, 10 µg of specimens 9 to 12 and 15 µg of specimen 13. Stacking and resolving gels were prepared for gel electrophoresis. The protein standard and sample were prepared with sample buffer, heated in boiling water for five minutes, and loaded into wells of the gel. The gel was run in 1X running buffer (Tris/Glycine/SDS) for 1.5 hours at 110 volts.

The blot was transferred by soaking the gel, membrane, filter paper, and sponge in the transfer buffer for ten minutes. After loading the system into the holder, it was run for one hour at 100 volts. After removal, the membranes were washed in the transfer buffer for five minutes and placed in 5% dry milk overnight. They were then incubated with the primary anti-SMA antibody for two hours and rinsed three times in transfer buffer for ten minutes each. This was followed by application of the secondary antibody and a luminol-based chemiluminescent detection system. Films were digitised for densitometric analysis using NIH Image processing and analysis software (National Institutes of Health, Bethesda, Maryland). The results were reported as a percentage of the densitometric readings of the positive smooth muscle cell control.

Results

Histological examination revealed wide variation in the composition of the tissue within individual histological sections and among samples from different patients (Table II). The regions stratified on the basis of cell number density as hypercellular, moderately cellular, and hypocellular, generally had histological features which coincided with the three stages of progression of the disease, namely proliferative, involutional, and residual.

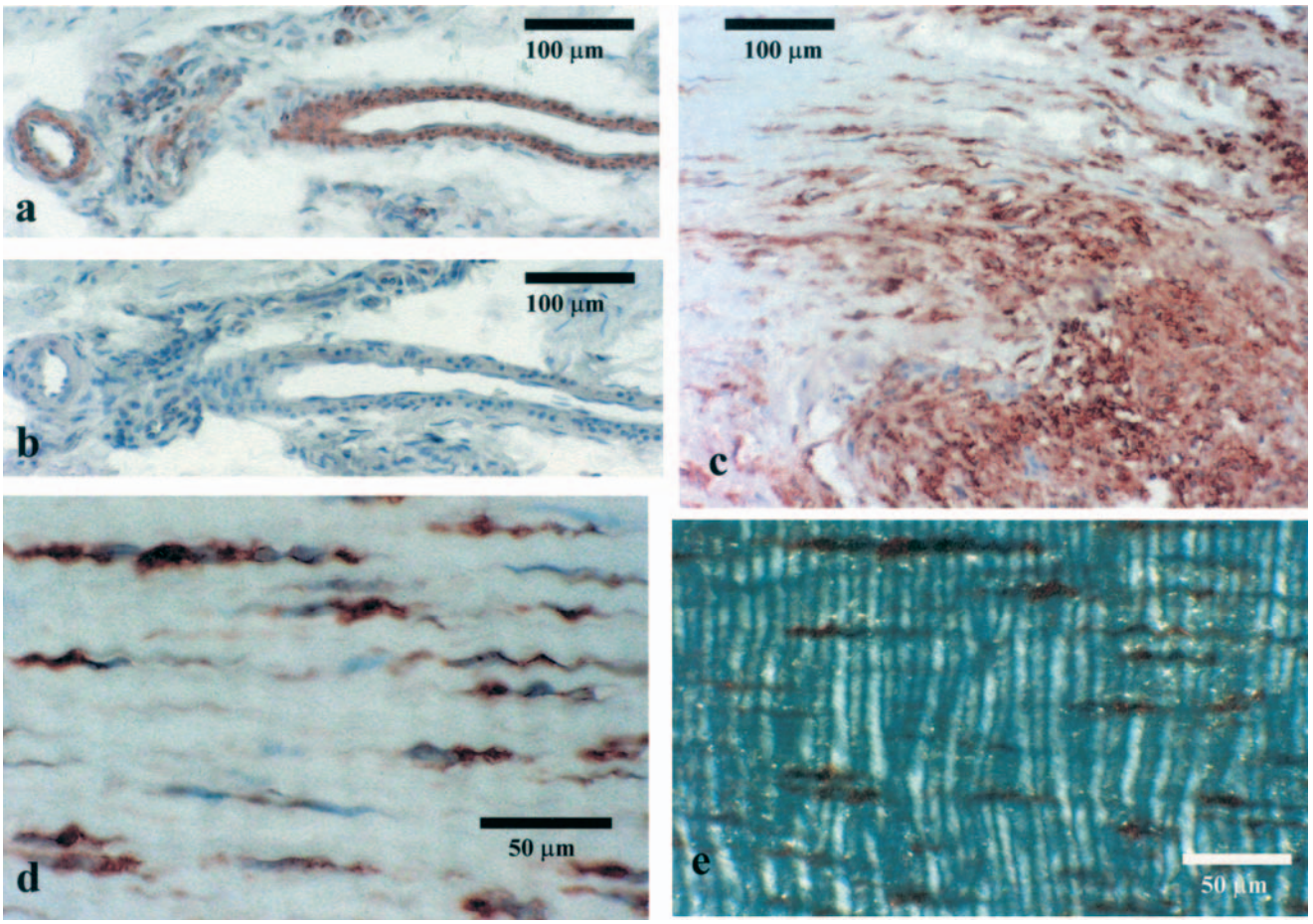

Fig. 1

SMA immunohistochemical micrographs from selected regions in specimen 2. Figure 1a – Section showing SMA-containing vascular smooth muscle cells around vessels cut in transverse (left) and longitudinal section (running horizontally). Figure 1b – Negative control section (a serial section) for Figure 1a stained with a mouse serum instead of the SMA antibody. Figure 1c – A hypercellular region consisting of a large percentage of SMA-expressing cells which can be seen towards the bottom right. More elongated and crimped cells, many of which contain SMA, can be seen running horizontally in the middle of the section and to the left. A hypocellular zone can be seen in the upper left. Figure 1d – SMA containing elongated cells show the same crimp pattern as that of the fibrocollagenous matrix. The period of the crimp is about 10 μm. Figure 1e – Polarised light micrograph of an area similar to that in Figure 1d showing the crimp of the tissue.

The specimens were generally of low vascularity with the vascular area representing 5% or less of the section, except in two cases where it reached 15% (Figs 1a and b). Two specimens (1 and 4b) had tissue with few cells. Most of the histological sections comprised hypercellular and moderately cellular material. The relative amounts of these two tissues varied among samples. The greatest amount of hypercellular material in a section was 75% (specimen 6).

Cell morphology. The cells found throughout the regions of high and low cellularity had an appearance consistent with fibroblasts, some plump (rounded) and others elongated (Fig. 1c). The hypervascular regions were generally populated by round or oval cells while the areas of low cell number density contained elongated cells (Table II). The rounded cells did not appear to be transverse views of elongated cells because the diameter of the nucleus was greater than the thickness of the nucleus of the elongated cells. These plump cells were more likely to be fibroblasts with abundant cytoplasm, immature fibroblasts or undifferentiated mesenchymal cells. In some sections rounded cells in a hypercellular material blended into a hypocellular fibrocollagenous matrix with elongated and crimped cells (Fig. 1c). Elongated cells with crimp (Fig. 1d) were only found in the moderately cellular and hypocellular regions (Table II).

The fibrocollagenous regions in areas of low cellularity showed a crimp with a peak-to-peak spacing (period) of 10 to 20 μm (Fig. 1d) which was prominent in polarised light microscopy (Fig. 1e). The crimped region often occupied a large percentage of the tissue area. Many of the elongated cells in zones of crimped collagen showed the same period of the crimp along their length (Table II; Fig. 1d).

SMA immunohistochemistry. Virtually all the cells in some regions of the tissues stained positive for SMA (Table II; Fig. 1c). There was, however, a wide variability in the percentage of SMA-positive cells in the regions with various cellularities, with the greatest percentages in the hypercellular and moderately cellular zones (Table II). There was difficulty, however, in definitively identifying SMA-staining

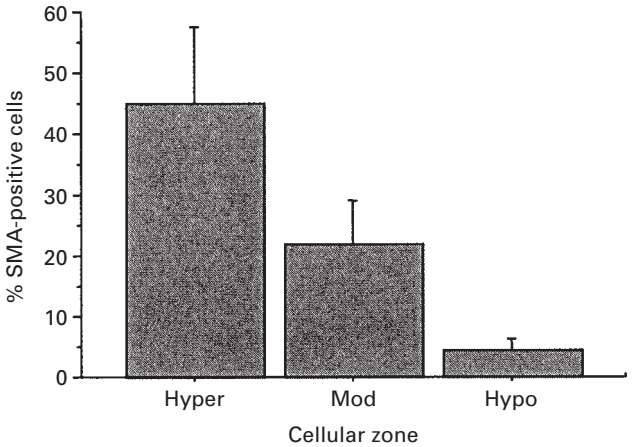

Fig. 2

Chart showing the mean percentage (± SEM) of SMA-containing cells in the three cellular zones in Table II (Hyper, hypercellular zone; Mod, moderately cellular zone; and Hypo, hypocellular zone (n = 11 including the two samples from specimens 2, 4, and 7)).

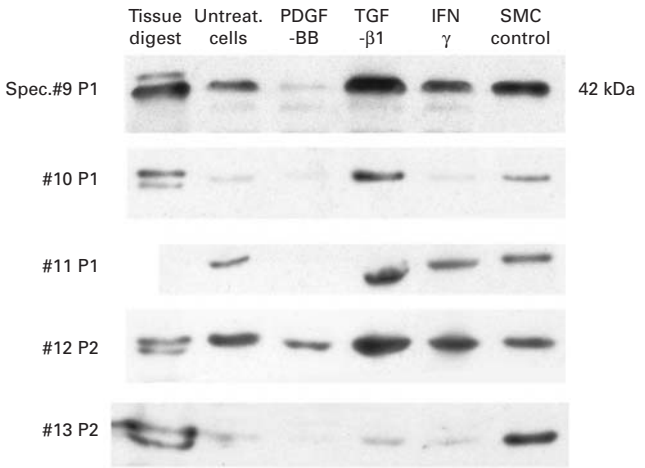

Fig. 3

Western blot films showing the SMA content of cells from the five specimens allocated for this analysis after the first or second passage.

cells in the hypocellular regions because of the very narrow profile of many of the elongated cells, as shown by some of the cells in Figure 1d. Therefore there may have been a greater percentage of SMA-expressing cells in this zone than had been recorded. There was, however, a marked difference in the percentage of SMA-staining cells in the hypercellular, moderately cellular, and hypocellular zones (Fig. 2). One-factor analysis of variance (ANOVA) showed a significant effect of zone on the percentage of SMA-expressing cells ($p = 0.007$).

In some specimens (2a and 7b) most of the elongated/crimped cells contained SMA (Table II; Fig. 1d). There was, however, no systematic finding in this regard since many crimped cells did not stain for SMA in other samples.

SMA Western blot analysis and the effects of selected cytokines. In four of the five specimens allocated for Western blot analysis cytoplasmic protein extracted from cells immediately after isolation from the tissue digests showed the presence of SMA (Fig. 3). The most prominent band in the film was at the 42kDa location coincident with the SMA extracted from the smooth muscle cell controls. In each of these cases, however, an unexplained second band was also present which did not appear in the cytoplasmic protein extracted from the cultured cells (Fig. 3). SMA was detectable in varying amounts in the cells from the three patients (specimens 9, 10 and 11) evaluated after the first passage and in both specimens (12 and 13) analysed after passage 2.

A consistent finding was the decrease in the SMA content of cells treated with PDGF-BB and an increase in those treated with TGF-β1 (Fig. 3). Treatment with IFN-γ had a variable effect in these cells. Comparison of the relative amounts of SMA in the untreated and treated Dupuytren's cells was performed using densitometry of the Western blot films. The intensity of a band of SMA from an untreated or treated Dupuytren cell sample was divided by the intensity of the SMA band from a sample of the smooth muscle cell control on the same gel. In this way the amount of SMA in the Dupuytren's cells was expressed as a percentage of that found in smooth muscle cell controls. This method of comparison was used for the samples from four patients (9 to 12) for which the same amount of cytoplasmic protein (10 μg) extracted from the Dupuytren's samples and control smooth muscle cells was analysed (Fig. 4). The 10 μg sample of cytoplasmic protein from the Dupuytren's cells contained more than 50% of the content of SMA found in 5 μg of protein from the smooth muscle cell control. The difference of a factor of two in the amount of protein analysed suggested that the cells from the Dupuytren's specimens had approximately 25% of the SMA content of smooth muscle cells. Treatment with PDGF-BB resulted in a reduction of 65% in the SMA content of the cells (Fig. 4), and treatment with TGF-1 an increase of 3.4-fold in the content of SMA.

One-factor ANOVA showed that the effect of treatment with cytokine on the content of SMA was statistically significant ($p = 0.0006$, Fig. 4). Two-tailed, paired Student's *t*-tests showed that the PDGF-BB-induced decrease and TGF-β1-induced increase in expression of SMA were statistically significant ($p = 0.028$ and $p = 0.049$, respectively). There was no statistically significant effect of IFN- ($p = 0.2$) on the content of SMA of the cells.

The effect of treatment with PDGF-BB on cell proliferation was marked (Fig. 5). There was a ten-fold increase in cell number after the four-day treatment. This compared with an approximate twofold increase in the untreated control cells. One-factor ANOVA showed a significant effect of treatment with cytokine on the increase in cell number ($p < 0.0001$). *Post-hoc* testing using Fisher's protected least-squares differences showed that the difference between the PDGF-BB group and the other three groups was statistically significant ($p < 0.0001$).

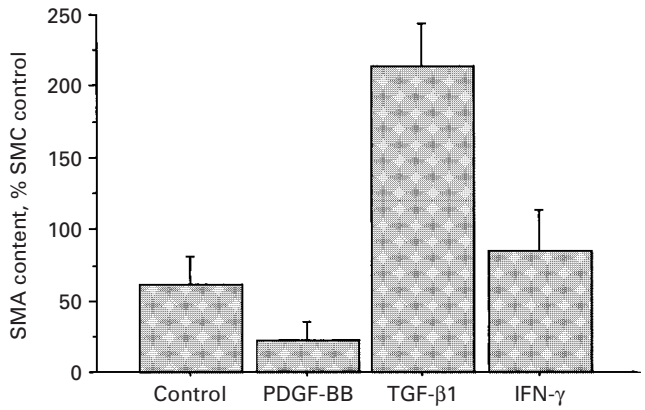

Fig. 4

Chart showing the densitometry results from the Western blot analyses (n = 4) of the mean (± SEM) SMA content of untreated Dupuytren's cells and cells treated with PDGF-BB, TGFβ-1 or IFN-γ, expressed as a percentage of the SMA content of smooth muscle cell (SMC) controls run in the same gels. The same amount of cytoplasmic protein (10 μg) extracted from the Dupuytren's cells and SMC controls was run on the Western blots. The statistically significant increase in the content of SMA resulting from treatment with TGF-β1 and the decrease due to treatment with PDGF-BB are evident. The slight increase in content resulting from treatment with IGF-γ was not statistically significant.

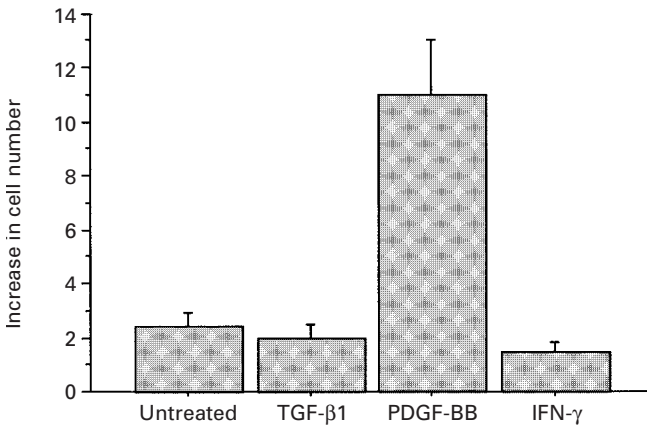

Fig. 5

Bar chart showing the mean (± SEM) increase in cell number after treatment for four days with the selected growth factors, relative to the initial value. The untreated control group is also shown.

Discussion

The variability of the cellularity of Dupuytren's nodules was clearly in evidence in our study, within sections from the same nodule. SMA-expressing cells were found in regions with different cellularity, with the percentage of SMA-containing cells decreasing with decreasing cellularity of the region. As has been previously found,[8] the percentage of SMA-containing cells was elevated in the proliferative phase. SMA-expressing cells all but disappeared during the residual phase[8] which was characterised by a low cell-number density. The zones with uniform crimp of the fibrocollagenous matrix were of interest. The fact that the constituent SMA-containing cells in these regions showed the same crimp suggests that they have a role in producing the buckling force which may have been responsible for creating this architectural feature.

Immunohistochemistry may thus be of value in demonstrating the percentage of SMA-containing cells and give an indication of their state of contraction based on their buckled appearance. It is not clear, however, that such a study of biopsied material could be of value in providing a guide to the prognosis of the disease in a particular patient. This is due in part to the variability of the histological composition of the tissue comprising the lesion and to the known transience of SMA expression.[15] Thus while it is known that SMA-expressing connective-tissue cells can stay in a contracted state indefinitely in much the same way that smooth muscle cells maintain tone,[16] in some cases there may be a down-regulation of SMA expression or apoptosis of the SMA-containing cells.[17]

The demonstration of the prevalence of SMA-containing cells in Dupuytren's tissues in our study further focuses attention on the regulation of the expression of this isoform of actin as a means of controlling the disease. A notable finding was the significant decrease in SMA in PDGF-BB-treated Dupuytren's cells. A comparable result had been previously obtained in a study using human gingival fibroblasts.[11] PDGF-α and PDGF-β along with TGF-β have been found to be elevated in Dupuytren's nodules, compared with healthy tissues.[18] PDGF is produced in the diseased tissue[19] and it appears to have a role in the proliferative stage of this disease.[20] It binds to cell-membrane receptors on the myofibroblasts during both the proliferative and involutional stages of Dupuytren's.[20] Furthermore, PDGF has been shown to have dose-dependent mitogenic effects.[21] Other effects attributed to PDGF include increased synthesis of type-III collagen,[22] the reorganisation of actin filaments[23] and the stimulation of the production of arachidonic acid which can be converted to prostaglandin.[23] It is possible that the effect of PDGF in down-regulating expression of SMA may be a homeostatic mechanism to counter the up-regulating effect of TGF-β. Alternatively, it may be that the rapid cell proliferation stimulated by PDGF precludes the expression of this particular actin isoform. These findings warrant further study including a dose-response analysis.

Our finding of a significant increase in expression of SMA by treatment with TGF-β1 of Dupuytren's cells in vitro, coincided with recent immunofluorescence studies which have shown that TGF-β1 increased the percentage of SMA-containing cells in Dupuytren's tissue.[24] The expression of TGF-β in Dupuytren's tissue has been considered to be important because of its multiple effects which could promote the processes underlying the pathogenesis of the disease: mitogenesis,[25] chemotaxis[26] and the synthesis of collagen and fibronectin.[27] TGF-β can also induce expression of SMA,[28] suggesting that stimulation of fibroblasts by

TGF-β may induce their differentiation into myofibroblasts. Furthermore, in a released collagen lattice contraction assay, TGF-β1 has been shown to increase the force of contraction generated by myofibroblasts obtained from Dupuytren's tissue.[9] It would be interesting in future studies to determine if PDGF-BB could reduce or prevent the stimulation by TGF-β of the expression of SMA in Dupuytren's cells.

A cytokine produced by helper T lymphocytes, IFN-γ, is believed to suppress the differentiation of myofibroblasts. Treatment with IFN-γ has been shown to decrease expression of SMA in cultured fibroblasts[29] and in addition, to block effectively changes in the expression of SMA and the formation of fibronectin fibrils and fibronexus which are normally incurred by exposure to TGF-β1.[9] Furthermore, IFN-γ can reduce the contractile force caused by Dupuytren's myofibroblasts treated with TGF-β1. A pilot study to determine the effects in vivo of the treatment of Dupuytren's disease with IFN-γ has shown that this can decrease the amount of SMA expressed in the myofibroblasts, decrease the size of the nodule, and decrease symptoms.[30] In contrast to these previous findings our study did not reveal a meaningful effect of IFN-γ in reducing the expression of SMA in Dupuytren's cells. Additional work will be necessary to resolve the differences in response to IFN-γ found in this and previous studies.

Our study may provide a useful basis for the comparison of the relative effects of selected cytokines on the expression of SMA in Dupuytren's disease. This could be helpful in identifying novel strategies for the non-surgical treatment of this often debilitating problem. Moreover, our findings could provide insights in new approaches to the management of other fibrotic contractures such as joint contractures.

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