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Basic Fibroblast Growth Factor in Dupuytren's Contracture

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Lesions excised from nine patients undergoing surgery for Dupuytren's contracture (DC) and three normal fascia were examined for the presence of the angiogenic protein basic fibroblast growth factor (basic FGF). Endothelial cell proliferation assays established basic FGF-like activity in extracts of DC. Western blotting confirmed the presence of an 18,000-dalton protein which was localized in the lesions by immunohistochemical staining. All of the cells implicated in the progression of the disease (endothelial cells, fibroblasts, and myofibroblasts) contain the growth factor. Endothelial cells within the narrowed or occluded vessels, as well as fibroblasts surrounding these vessels, stained intensely positive. In situ ***hybridization using an antisense probe for human basic FGF and its receptor's (FGFR-1) mRNA established the major difference between normal and DC tissues: their levels are significantly higher than in the normal tissues. Thus the cells in DC also express both basic FGF and FGFR-1, suggesting a potential autocrine/paracrine role for basic FGF in the pathogenesis of DC. This finding is thus the first description of a nontumoral proliferative disease that can be directly associated with increased basic FGF mRNA. The possibility that therapies can be developed on the basis that basic FGF and its receptor are expressed in DC is discussed. (Am J Pathol 1992, 141: 661–671)***

Dupuytren's contracture (DC) is a proliferative disease of the palmar aponeurosis that induces increased fibrosis along the tendons of fingers and hands that often interferes with normal hand movement and may even lead to deformity. The disease is characterized by lesions containing nodules of proliferating endothelial cells, fibroblasts, and myofibroblasts.[1–3] Several studies have proposed that the proliferating myofibroblasts emanate from the perivascular cellular cuff of small vessels.[4,5] One of the most unusual features of the disease is the observation that most of the vessels contained in the lesion are narrowed or even occluded by proliferating endothelial cells[6] and the predominance of type III collagen.[7,8] The only current treatment is surgical excision of the fibroblastic mass, but recurrence after surgery is common.[3,9]

The characteristic proliferation of fibroblastoid and endothelial cells suggested to us that basic fibroblast growth factor (basic FGF) may be involved in DC. Basic FGF is an 18,000-dalton protein that has mitogenic activity for a large number of different cell types *in vitro*, including endothelial cells and fibroblasts.[10,11] Although it has been implicated in the microvascular complications of diabetes,[12] there are few instances in which this angiogenic factor has been firmly associated with a disease of cell proliferation. In the studies reported here, we have used several different approaches to examine the possibility that basic FGF is associated with the pathogenesis that characterizes DC. The results all support the hypothesis that basic FGF plays a role in the development of DC and suggest the possibility of developing a novel rationale for therapy of the disease.

Materials and Methods

Tissues

Lesions from patients undergoing surgery for DC and palmar fascia from normal patients were fixed in 4% paraformaldehyde or flash frozen on dry ice as described below. Patients were considered normal if they

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had no history or diagnosis of DC at the time of excision (eg, carpal tunnel syndrome).

Antibodies

Rabbit polyclonal antibodies were raised against a 1–24 synthetic fragment of bovine basic FGF(1–146). These antibodies detect basic FGF and do not recognize (<1%) acidic FGF. The peptide antigen has no homology with int-2,[13] hst/ks[14], FGF-5,[15] FGF-6,[16] or FGF-7.[17] IgG fractions of the antisera were prepared by ammonium sulfate precipitation (30%). They also were purified further by passage over a protein-A Sepharose column. The protein-A purified anti-(1–24) basic FGF was passed through an immunoaffinity column of basic FGF-Affigel 10 that had been constructed according to the manufacturer's specifications (Bio-Rad, Richmond, CA). Antibodies contained in the fractions that did not bind to the basic FGF column were collected and used for control studies.

Cell Proliferation Assays

The growth factor activity that is present in lesions of DC was tested using adrenal capillary endothelial cells. Tissues from five patients were obtained at the time of surgery for DC and frozen at −80°C. The tissues (460–840 mg) were homogenized in 3 ml extraction buffer (10 mmol/l [millimolar] TRIS-Cl, pH 7.0, with 2 mol/l NaCl, 1 mmol/l ethylenediaminetetra-acetic acid [EDTA], 1 mmol/l EDTA, 1 mmol/l phenyl-methyl-sulfonyl-fluoride (PMSF), 1 μg/mL aprotinin, 1 μg/mL leupeptin, 1 μg/mL pepstatin A). The homogenate was centrifuged (48,000*g*, 30 minutes) and diluted threefold with 10 mmol/l TRIS-Cl, pH 7.4, and incubated at 40°C overnight in the presence of 500 μL heparin-Sepharose gel (Pharmacia, Uppsala, Sweden). The following day the gel was centrifuged, washed twice with 1 mol/l NaCl in 10 mmol/l TRIS-Cl, pH 7.4, and the protein bound was eluted with two washes of 100 μL 2 mol/l NaCl in 10 mmol/l TRIS-Cl, pH 7.4. The elution volumes were pooled and the indicated aliquots added to adrenal capillary endothelial (ACE) cells.[18] Four days later, the cells were counted using a Coulter Particle counter. For comparison purposes, 2 ng/ml recombinant human basic FGF increased cell proliferation from 27,500 ± 2,100 cells/well to 133,300 ± 1,800 cells/well.

Immunologic Detection of Basic FGF

Western blotting for basic FGF were performed according to previously described methods[19,20] with slight modifications. The heparin-Sepharose–associated material, prepared as described above, was eluted with 60 μL fivefold concentrated Laemmli's sample buffer,[21] and the eluate was electrophoresed on 15% sodium dodecyl sulfate polyacrylamide gel electrophoresis, electroblotted onto nitrocellulose, and incubated with protein A-purified anti-basic FGF(1–24).[20] Binding was visualized with goat anti-rabbit IgG conjugated to horseradish peroxidase (Bio-Rad Laboratories, Richmond, CA). In the radioimmunoassay (RIA), the salt eluate from the heparin-Sepharose column was diluted in RIA buffer (10 mmol/l sodium phosphate, pH 7.2, 25 mmol/l EDTA, 0.15 mol/l NaCl, 0.3% bovine serum albumin, 0.1% sodium azide) and the amounts of immunoreactive basic FGF were determined in serial dilutions.

Tissue Preparation for Histology

Lesions excised from the hands of nine patients undergoing surgery for DC were examined. Biopsies were fixed in 4% paraformaldehyde for 4 to 6 hours, or frozen in ornithine-carbamoyltransferase (OCT) after collection and stored at −80°C. Both techniques gave similar staining patterns. Cryostat sections (12 μm) were mounted on slides, and when necessary, fixed for 15 minutes in 4% paraformaldehyde. They were stored at −80° until needed. For *in situ* hybridization, the sections were processed as described.[22] Briefly, they were acetylated for 10 minutes, rinsed in 2 × (0.3 mol/l NaCl, 0.03 mol/l Na Citrate), dehydrated through a graded series of ethanol washes, and then air dried for 2 hours before hybridization (see below).

Immunohistochemistry

The immunohistochemical techniques used for the detection of basic FGF have been previously described.[23] In the human tissues described here, endogenous peroxidase activity was quenched by incubating the sections at room temperature for 30 minutes in 0.3% hydrogen peroxide in absolute methanol. The tissue sections then were incubated with 1.5% goat serum (Vector, Burlingame, CA) for 30 minutes to reduce nonspecific staining. After an incubation overnight (20 hours) at 4°C with protein-A purified anti-FGF (2.5 μg/ml diluted in phosphate-buffered saline [PBS], 0.3% Triton, and 5% bovine serum albumin), the sections were washed and incubated with a 1/200 dilution of biotinylated goat anti-rabbit IgG (Vector, CA) for 1 hour, followed by a 30-minute incubation with a biotin-avidin-peroxidase complex (Vector, CA). Finally, basic FGF was visualized by treating the sections for 5 minutes with 0.5 mg/ml diaminobenzidine (Sigma Chemical Co., St. Louis, MO) prepared in 10 mmol/l PBS, pH 7.6, and containing 0.01% hydrogen

peroxide. Unless described otherwise, each step of the staining procedure was performed at room temperature. All steps were separated by buffer washes consisting of PBS-0.3% Triton. The sections were counterstained in Harris hematoxylin, dehydrated, cleared, and mounted.

In Situ *Hybridization*

The methods for the detection of basic FGF mRNA were essentially as described by Emoto et al.,[22] except that a 0.46 Kb cDNA encoding human basic FGF was used. A full-length cDNA for the basic FGF receptor[24] was cut at Bgl-II sites to yield the 1.6-Kb fragment coding the extracellular domain, and this fragment was inserted into bluescript (Stratagene, San Diego, CA). The probes were labeled with $\alpha-{}^{35}$S-UTP in a transcriptional run-off reaction[25] using the T_3 or T_7 polymerases to yield labeled sense and antisense RNA. The hybridizing solution contained 50% formamide, 0.3 mol/l NaCl, 10 mmol/l TRIS (pH 8.0), 1 mmol/l EDTA, 10 mmol/l dithiothreitol (DTT), 1× Denhardt's solution, and 10% dextran sulfate (wt/vol). Total yeast RNA and tRNA (0.5 μg/ml) were used as blocking RNA.

After hybridization, sections were washed with 4× SSC (0.6 mol/l NaCl/0.06 mol/l NaCitrate, pH 7.0) at room temperature and treated with ribonuclease A (20 μg/ml) for 30 minutes at 37°C. The sections then were thoroughly washed in 0.1 × SSC, 1 mmol/l DTT at 65°C, dehydrated with absolute alcohol, dried, coated with Kodak NTB2 liquid autoradiographic emulsion, and exposed at 4°C for 2 to 3 weeks. The slides were developed in Kodak D-19 (3.5 minutes), rinsed briefly in water, and fixed for 2 minutes. After washing in distilled water, the slides were counterstained with hematoxylin and eosin as indicated and the silver grains examined by bright- and dark-field microscopy.

Results

DC Tissue Contains an Endothelial Cell Growth Factor

As shown in Figure 1, extracts of DC lesions contain a factor capable of stimulating endothelial cell proliferation. Because the ACE cells were tested with material that elutes from heparin-Sepharose columns with 2 mol/l NaCl, the results are consistent with the activity being due to basic FGF.[18] All of the five tissues examined had the capacity to stimulate cell proliferation, although there were clear quantitative differences in the amounts of biologic activity detected in each partially purified extract. Extracts of tissues from samples of normal hand (free of DC) also showed the presence of levels of an endothelial cell growth factor similar to the levels of the DC tissues (data not shown).

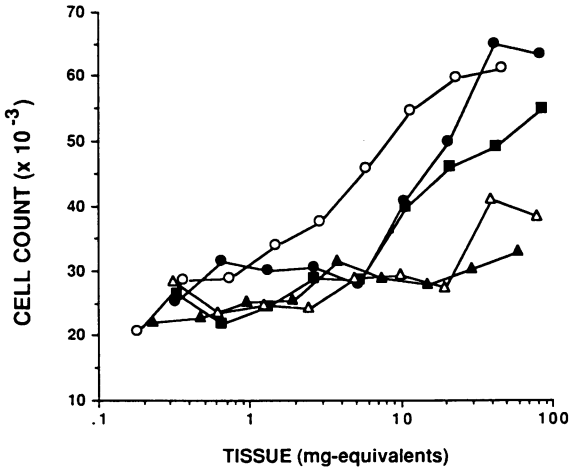

Figure 1. *Proliferative activity in extracts from Dupuytren's lesions. Extracts of DC lesions from five biopsies were prepared and assayed as in Materials and Methods and tested for their ability to stimulate endothelial cell proliferation as described in the text. Each symbol corresponds to the biopsy of one patient, standard deviations from triplicate determinations were all within 15% of the mean. Patient codes are JA –○–; TH –●–; HE –△–; HN –■–; and SE –▲–.*

DC Tissue Contains Basic FGF

To further characterize the proliferative activity contained in the DC lesions, we extracted the tissues from four patients, absorbed the extract to heparin-Sepharose as described in Materials and Methods and submitted them to Western blotting analyses. The extracts were eluted from the heparin-Sepharose gel with denaturing electrophoresis sample buffer and examined for the presence of basic FGF. Figure 2 shows that in each tissue, a single 18,000-dalton protein can be detected. In each instance, the protein appears indistinguishable from the basic FGF standard. A similar pattern is obtained when normal fascia is extracted and processed (Figure 2).

Histopathology of the DC Lesions

Our results are in good agreement with the previous histopathologic descriptions of the fascia obtained from lesions of DC.[26–28] First, there is a significant increase of connective tissue (Figure 3) that is characterized by the presence of bands and nodules of collagen with interwoven fibroblasts. The density of fibroblasts is considerably higher in nodules than in bands and the connective tissue of the DC nodules is also infiltrated by characteristic

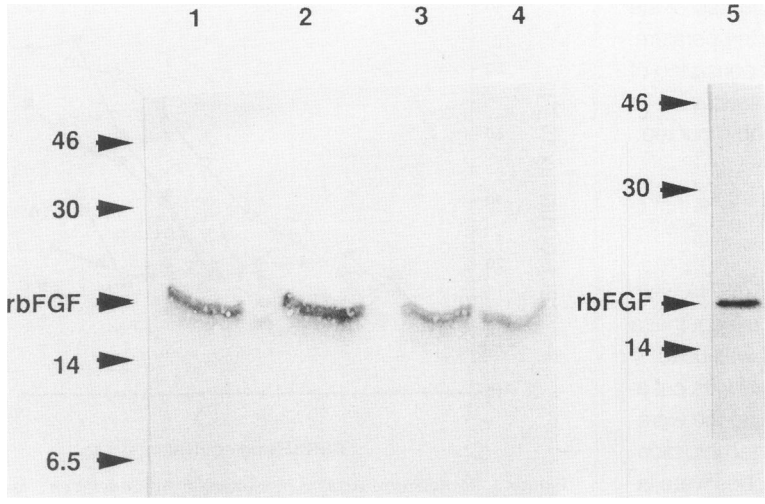

Figure 2. *Western blotting of heparin-Sepharose purified extracts of lesions. Extracts of DC lesions were processed and analyzed by SDS-PAGE and immunoblotting as described in the text. Numbers refer to molecular weight of protein standards as expressed in kilodaltons. The arrow corresponding to rbFGF refers to the migration of recombinant human basic FGF. Lane 1: Patient HE (800 mg of original tissue); Lane 2: Patient JA (991 mg of original tissue); Lane 3: Patient HN (513 mg of original tissue); Lane 4: Patient SE (724 mg of original tissue); Lane 5: Normal palmar fascia (560 mg of original tissue).*

microvessels. The capillaries and small vessels appear narrowed and even in some instances occluded by what appears to be proliferating endothelial cells in the lumen.

Immunohistochemical Localization of Basic FGF in DC

Immunostaining of DC tissue with the anti-basic FGF antibody shows significant positive staining in several structures that characterize the lesions of DC (Figures 3 and 4). The fibroblastoid cells and myofibroblasts that are organized in bundles surrounding the vessels all contain basic FGF. The difference is particularly dramatic when compared with adjacent connective tissue. Of particular interest, the microvessels that are narrowed and occluded with proliferating endothelial cells (Figures 3A, C, E, 4A) are perhaps the most intensely stained structures, second only to sweat glands (Figure 4C). The results presented in Figures 3A, E, and 4B, C show high magnification fields of characteristic DC structures. As an example, Figures 3A, E, and 4A show a localization of basic FGF associated with occluded vessels. They often surround the larger vessels in a constellation-like structure and show strongly positive staining for basic FGF (Figure 4A). In the small vessels, the cells forming the wall (pericytes, muscle cells, and endothelial cells) are strongly positive (Figures 3A, C, E and 4A). As expected, fibroblasts and the extracellular matrix associated with the microvasculature show the presence of basic FGF, a feature also seen in controls. Interestingly, this staining does not appear elevated, particularly when the cellular stainings are compared. Even some of the vessels that have no indication of narrowing or even abnormal proliferation are strongly stained. It is interesting that although many of the fibroblasts appear to be completely devoid of staining, others within the same area and almost side by side are strongly stained (Figure 3E). It is thus possible that this heterogeneous population in DC reflects a mixture of "transformed"[29] and normal fibroblasts in the lesion. This finding supports the notion that the source of basic FGF is local and derived from within the lesion. Immunostaining of normal fascia (Figure 4D), however, also established the presence of basic FGF in the extracellular matrix and in specific cellular structures like fibroblasts, blood vessels, and sweat glands. Thus, although there is no major difference between the intensity of staining of each of these individual structures in normal fascia and in DC lesions, there is an overall increased staining in DC lesions because of the increase in the density of these structures. In many areas of the lesions a considerable nuclear staining was observed, but it did not appear to correlate with any specific area.

Localization of Basic FGF Synthesis in DC

In contrast to the immunohistochemical results, there was a dramatic difference between basic FGF mRNA expression in normal and DC-derived fascia. *In situ* hybridization using an antisense RNA probe to human basic FGF was performed to determine if there is synthesis of basic FGF in DC that might account for its immunologic and biologic presence in the lesions. As seen in Figure 5A, mRNA for basic FGF can be seen in specific restricted loci in the Dupuytren's tissue. Fibroblastoid cells dispersed in the collagen matrix are overlaid by a signal indicating the presence of basic FGF mRNA. The signal is specific and was not seen in adjacent sections that were hybridized with the sense strand of cRNA for human basic FGF (Figure 5B). For comparative purposes, the bright field corresponding to Figures 5A and B is

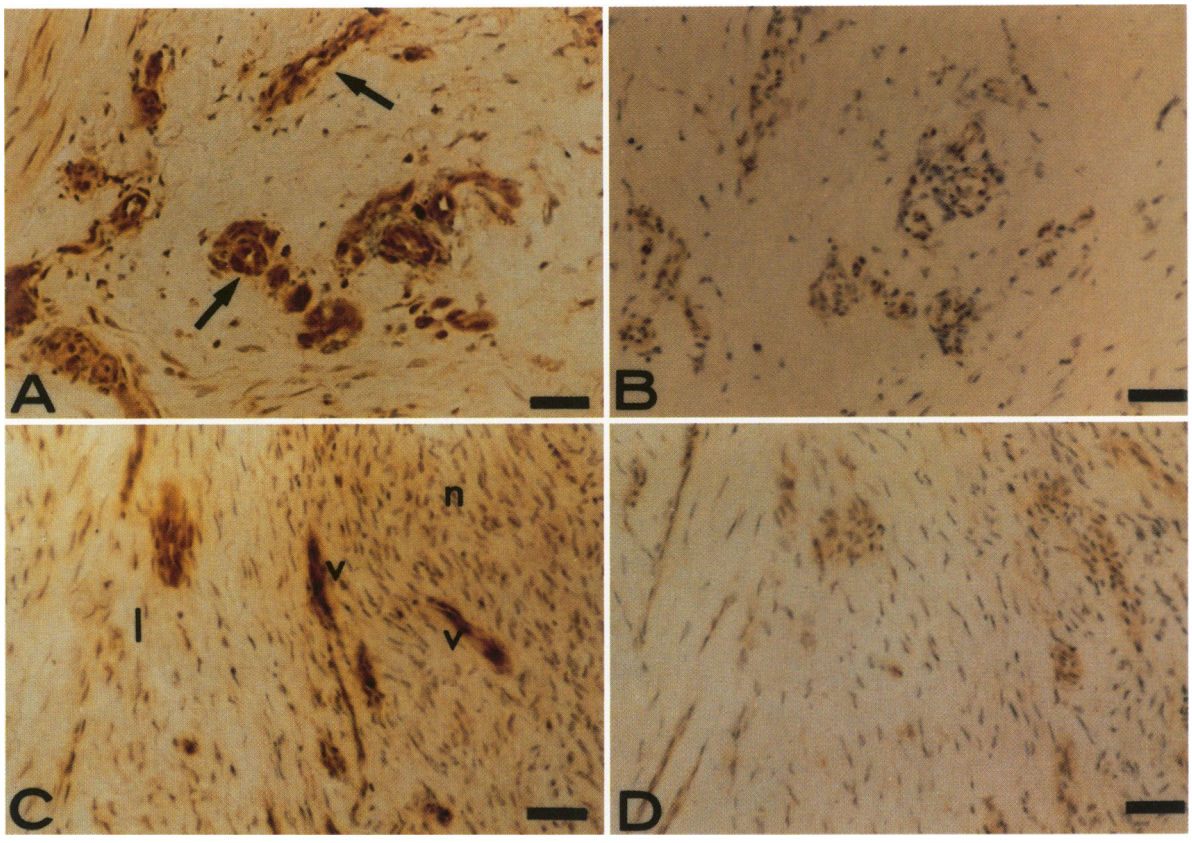

Figure 3. *Immunoreactive basic FGF in DC.* **a**: *Section of a Dupuytren's nodule shows, at low magnification, the specific immunostaining for basic FGF. Numerous small spastic and occluded vessels are organized in neovascular areas dispersed throughout the connective stroma. Endotheloid vessel is indicated by an arrow (Bar = 50 μm).* **B**: *An adjacent section is shown that was treated with a preparation of antiserum depleted of anti-basic FGF IgG by FGF affinity chromatography. (Bar = 50 μm).* **C**: *Staining of basic FGF in a section of Dupuytren's tissue shows fibrous lamellar and fibrous nodular areas. In the lamellar structure, the fibroblasts are longitudinal and separated by collagen* stroma. *The immunostaining is weak in this area. The nodular area has stronger immunostaining, especially over the very abundant fibroblasts. The reaction is moderate, but significant over the stroma. There are also numerous highly immunostained blood vessels that have exceptionally thick walls and some even occluded. Lamellar connective tissue (l), nodular and cellular connective tissue (n), vessels (v) (Bar = 50 μm).* **D**: *An adjacent section stained with antiserum that was depleted of anti-FGF IgG by basic FGF affinity chromatography (Bar = 1 mm).*

shown in Figure 5C. At higher magnification, it is clear that the specific labeling is of the fibroblastoid cells and that it is not randomly distributed (Figure 5D). The adjacent section, hybridized with the sense strand, has no specific signal (Figure 5E). In normal fascia, (Figure 5F), and in contrast to fascia obtained from DC, the amounts of basic FGF mRNA detected by *in situ* hybridization are barely detectable. Thus, DC lesions are actively expressing the basic FGF gene.

Localization of FGFR-1 Synthesis

A cRNA probe complementary to human basic FGFR-1 was used to determine if there is an increased expression of the basic FGF receptor in DC. As seen in Figure 6A, a mRNA for basic FGFR-1 can be seen in specific loci in the Dupuytren's tissue. Fibroblastoid cells dispersed in the collagen matrix contain a strong signal for FGFR-1 mRNA (Figure 6C) and were not seen in adjacent sections that were hybridized with the corresponding sense strand of cRNA (Figure 6B). The signal is particularly high over the occluded vessels. In the normal fascia, the level of FGFR-1 mRNA is present over selected fibroblasts and is clearly significantly lower than in the DC tissues (Figure 6D for comparison).

Discussion

Dupuytren's contracture is associated with multiple functional changes in the cell populations and in the extracellular matrix of the palmar fascia. It is characterized by several unusual phenomena, including the presence of fibroblasts expressing myofibroblastic phenotype, the predominance of type III collagen,[7,8] and an angiogenic

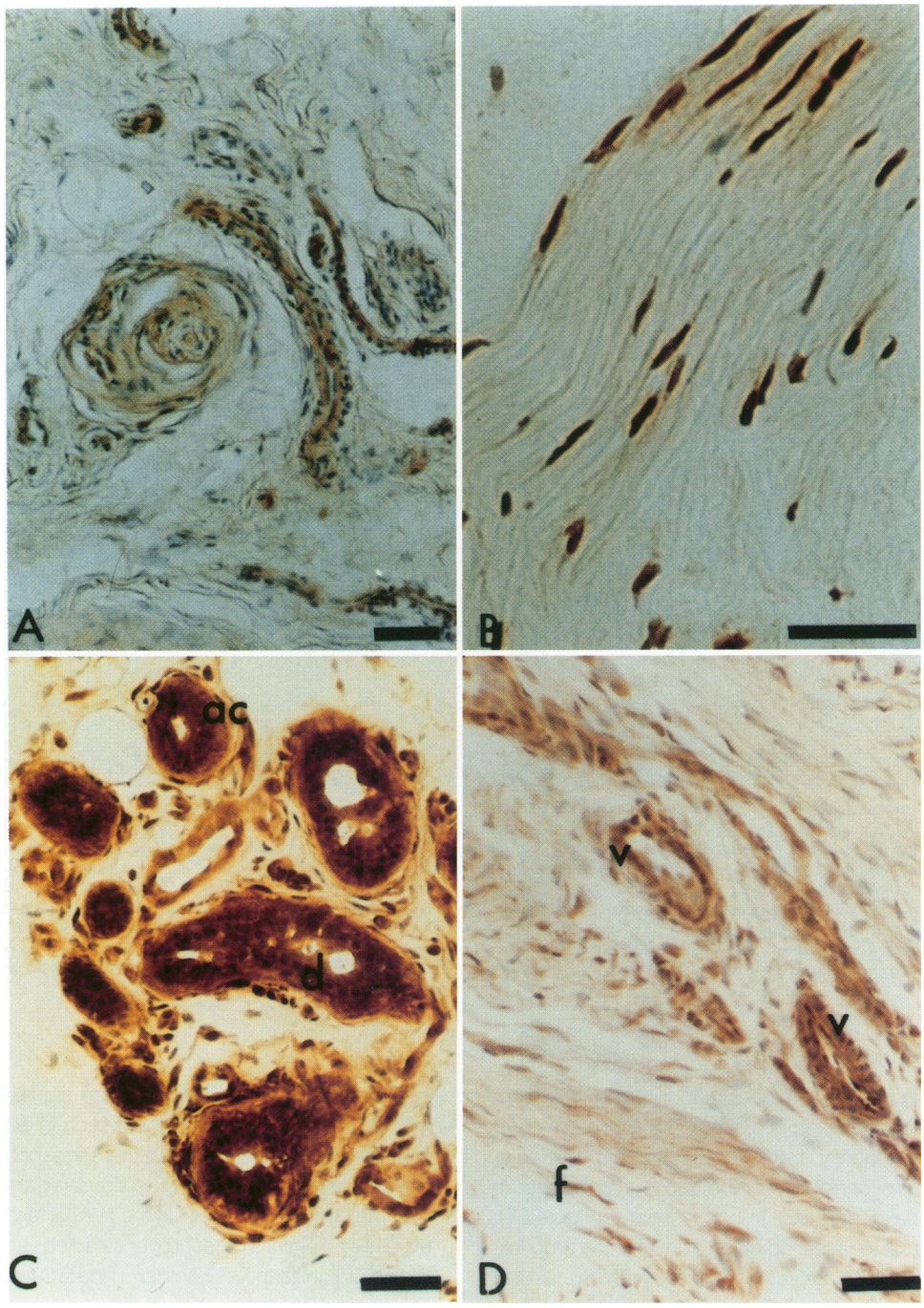

Figure 4. *Immunolocalization of basic FGF in DC.* **A**: *The immunolocalization of basic FGF in an area that shows a major vessel partially occluded, but with extended vascular wall. Numerous small vessels show wall thickening and there is a strong immunoreaction in the neovascular ring at the periphery of the major vessel (Bar = 50 μm).* **B**: *Localization of basic FGF in a DC biopsy shows moderate immunoreactivity over the collagen stroma especially surrounding the myofibroblasts and an intense staining in the cytoplasm and nucleus of the fibroblasts (Bar = 50 μm).* **C**: *Localization of basic FGF over the acini and the ducts of a sweat gland shows positive staining for basic FGF acini (ac), ducts (d) (Bar = 50 μm).* **D**: *Localization of basic FGF in normal hand fascia, fibroblasts (f), vessels (v) (Bar = 50 μm).*

response that leads to the development of spastic structures narrowing and occluding microvessels.[6,26] The results presented here all support the hypothesis that the endothelial cell growth factor, basic FGF, may be involved in the pathogenesis of DC. Furthermore, they suggest that this disease may be autocrine in nature and that cell proliferation is due to the local production and utilization of this growth factor.

Despite intense experimental investigation, the cause and pathogenesis of DC remains unknown. We have recently studied the presence and synthesis of basic FGF and FGFR-1 in cells isolated from lesions of DC. These

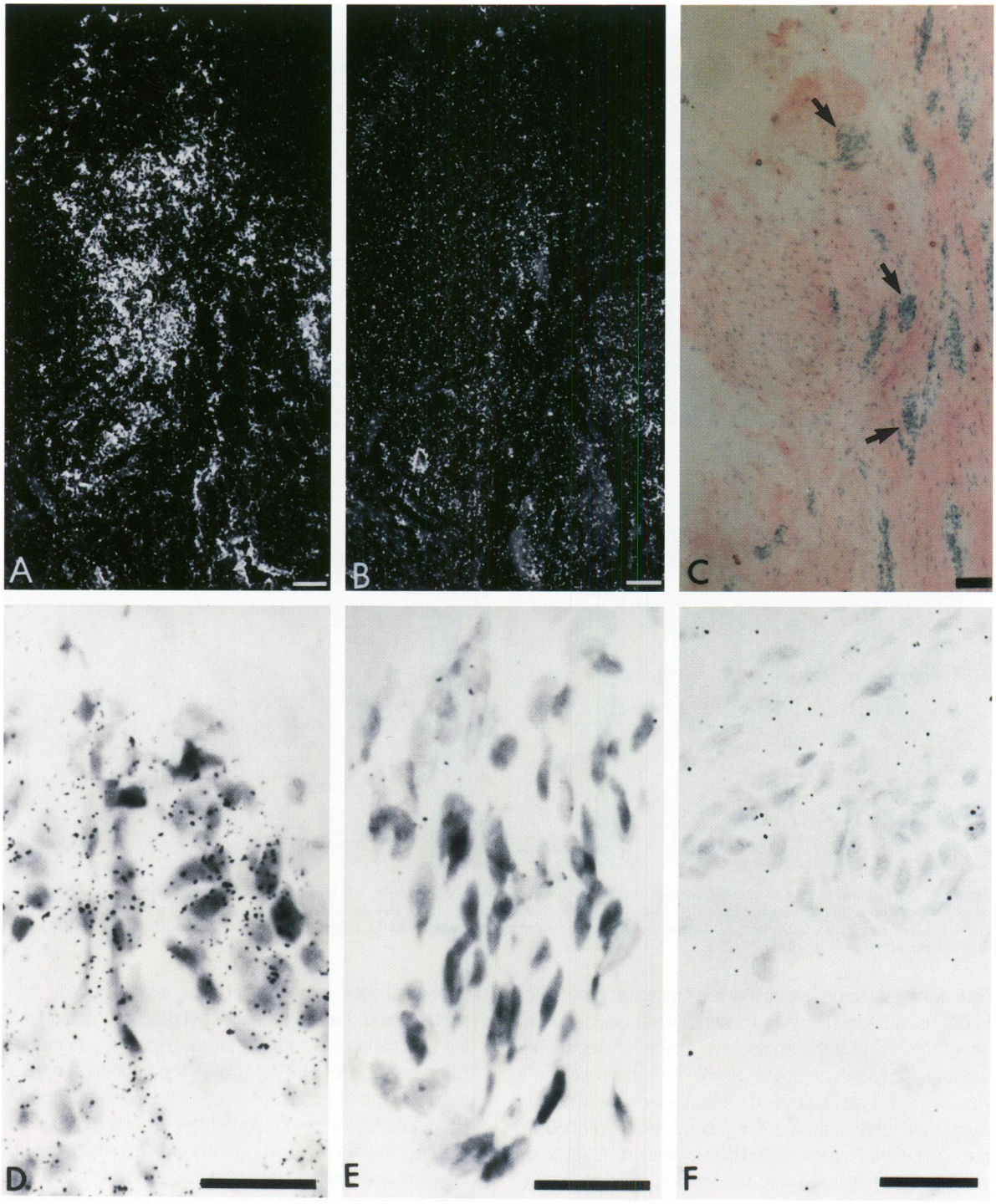

Figure 5. *Localization of FGF synthesis in DC.* **A:** *A low magnification of a dark-field micrograph shows the distribution of the basic FGF mRNA in a Dupuytren's lesion using an antisense mRNA probe for basic FBGF.* **B:** *shows the control section using the sense strand probe.* **C:** *A bright field of the adjacent section corresponding to Figure 5A and B stained with hematoxylin-eosin. The arrows highlight the cellular structures shown in Figure 5A that express the basic FGF mRNA and FGFR-1 (Bar = 100 μm);* **(D,E)** *are high magnification images of* **(A)** *and* **(B),** *respectively, of the* in situ *hybridization to the Dupuytren's tissue that shows the labeled fibroblasts with the human basic FGF antisense cRNA probe* **(D)** *and control sense probe* **(E)** *(Bar = 25 μm);* **(F)** *shows the presence of a low intensity signal in the normal hand. Bright field photographs (Bar = 25 μm).*

cells contain biologically active growth factor, express large quantities of FGFR-1 on the cell surface, and proliferate in response to exogenous basic FGF.[30] In DC, however, there are various cell types involved that express a large number of distinct phenotypes at different stages of DC. We therefore have set out to investigate the possibility that the DC lesion *in vivo* contains a specific, yet pluripotent, endothelial cell growth factor like basic

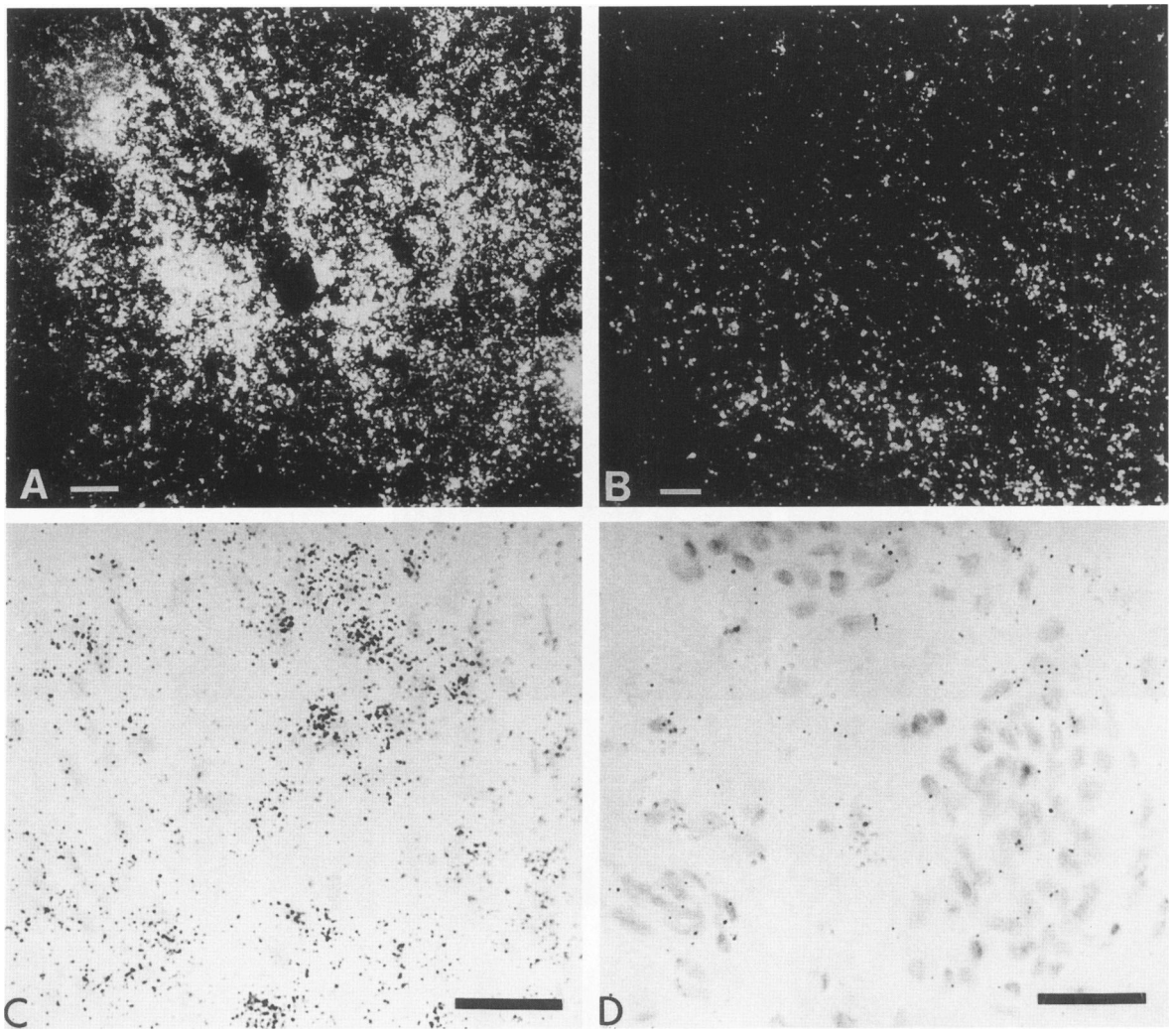

Figure 6. *FGFR-1 in DC. The* in situ *hybridization with antisense cRNA described in the text* **(A)**, *but not with sense strand* **(B)** *identifies the same lesions that synthesize basic FGF as the sites of receptor synthesis (dark field);* **(C)** *shows the presence of a higher transcription level of FGFR-1 mRNA in the fibroblasts of the DC tissues when compared to the normal fascia* **(D**; *bright field). The hybridization signal in* **(C)** *is high over the vessels (Bar = 25 μm).*

FGF. Many of the cell types that are functionally modified in DC have been shown to be sensitive to basic FGF *in vitro,*[11,18,31,32] and the angiogenic effects of basic FGF are well established in both *in vivo*[31,33–36] and *in vitro*[37,38] models. Because Dupuytren's lesions can maintain their pathologic characteristics for months when grafted in nude mice,[6] we hypothesized that local factors, acting in an autocrine fashion, could be contributing significantly to the progression (and recurrence) of the lesions.

The presence of basic FGF has been established in a large number of normal tissues, and it is not surprising to find immunostaining for basic FGF in normal fascia. It appears to us that, if this growth factor is associated with DC, an elevated expression of basic FGF mRNA and FGFR-1 might be underlying the pathogenesis in DC. The results using *in situ* hybridization with a human probe for the basic FGF mRNA confirm this hypothesis and show that their corresponding genes are locally transcribed, and that the signal of hybridization is higher in the DC than in the normal tissues. Similar *in situ* studies using a probe for FGFR-1 mRNA show identical results, suggesting that an autocrine/paracrine loop may be responsible for the stimulation of the excessive growth of the endothelial cells and myofibroblasts in DC. Accordingly, we suggest that the detection of basic FGF mRNA and its receptor in DC is an unusual phenomenon that may be directly related to the disease. To date, these techniques have only detected basic FGF mRNA in the brain[22,39] and the developing embryo (unpublished results).

In the current work, the immunohistochemical staining with an antibody to basic FGF clearly establishes that the protein is present in both normal and DC tissues. There is no evidence, however, that this basic FGF is bioavailable

to FGFR-1. It is interesting that the staining for basic FGF in the occluded microvasculature that develops in the periphery of the major vessels appears particularly intense. The nodular area of the lesions, which is thought to be the organizing center, is densely populated with intensely positive immunostaining fibroblasts.[5,40] Although the results presented here do not show any differences in the intensity of the immunostaining of the specific individual structures that are present in DC and normal fascia (vessels, sweat glands, fibroblasts), there is an increase in their number and clustering in DC. Accordingly, the disease may be the result of an increase in basic FGF availability. If this is the case, the pathogenesis of DC would be related to the regulation of FGF activity. Although the methods do not differentiate between increased transcription and increased mRNA stability, *in situ* studies with basic FGF and FGFR-1 mRNA show the true differences in the disease state. The observation that there is active synthesis of FGF and FGFR-1 in DC-derived, but not normal, fascia supports a role for basic FGF in the progression and development of DC and suggests that the disease is fundamentally autocrine/paracrine in nature.

It can only be presumed that there exists some still-unidentified primary inducer of DC that increases basic FGF mRNA in fascia, perhaps its local release by endothelial and related perivascular cells, and thus results in a stimulation of the angiogenic response and the proliferation of myoblasts and fibroblasts. As such, the pathogenesis of DC ultimately may be mediated through an increased local production of basic FGF. As an example, consider the possibility that the generation of oxygen free radicals might induce the expression of angiogenic factors like basic FGF. Murrell and Hueston[41] have proposed that the generation of oxygen free radical damage in DC tissues causes the pericyte necrosis that precedes local proliferation of fibroblasts and fibroblastoid cells. A similar pericyte drop-out is observed in the retinas of diabetics, appears to predispose the development of proliferative diabetic retinopathy, and is associated with basic FGF.[10,12,42] Thus, like in inflammatory arthritic joints,[43] an FGF can be implicated in a proliferative disease. The microcirculation of the hand and retina thus may be particularly susceptible to oxygen free radical damage, leading to a sustained induction of angiogenic factors. This might even suggest a molecular basis to the increased incidence of DC in patients with diabetes mellitus.

Hypoxia is another event that is often associated with DC[44] and that can stimulate the proliferation of fibroblasts, their transformation into myofibroblasts, collagen synthesis, and release. We thus would predict that basic FGF-dependent proliferation of cells in the capillary lumen increases occlusion and thus increases the level of hypoxia and the progression of the disease. Experimental models of ischemia certainly increase basic FGF mRNA in the heart as does injury in the brain (unpublished observations). Because injury appears to be sufficient to induce basic FGF (and FGFR-1) expression,[45–48] the high recurrence of DC after surgery might represent the failure of this procedure to resolve local ischemia, which in turn is exasperated by the local subclinical injury that is induced by normal finger movement. Accordingly, it may be worth considering FGF receptor-mediated therapies as an adjunct to surgery. Such therapies include the use of competitive receptor specific antagonists[49,50] or basic FGF mitotoxins.[20,51] In this latter instance, because FGF mitotoxins selectively mediate the death of any cell that expresses the FGFR-1, these reagents may be valuable tools to medically treat DC. We have shown that a chemical conjugate composed of basic FGF and the ribosome-inactivating protein saporin is cytotoxic to cells derived from lesions of DC.[30]

Although the results presented here implicate basic FGF in the pathogenesis of DC, they certainly do not preclude the possibility that other growth factors are also involved. Indeed, we are currently determining the possible role of the platelet-derived growth factor, the transforming growth factors, the vascular endothelial growth factors, and other cytokines in this disease. Presumably, if it were possible to determine the mechanism responsible for increased basic FGF expression in DC, it might be possible to understand the cause of this disease and perhaps the reason that it is often associated with vision threatening diabetic retinopathy,[52,53] a proliferative disease recently linked to the same angiogenic factor, basic FGF.[12,42] Understanding the molecular events responsible for the onset, progression, and recurrence of DC is thus the first step toward the development of a novel rationale for medical intervention.

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