

Presence of Growth Factors in Palmar and Plantar Fibromatoses

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Palmar and plantar fibromatoses are disease processes in which the presence of certain growth factors has not been defined. Monoclonal antibodies against transforming growth factor-beta, epidermal growth factor, procollagen type 1, fibronectin, phosphotyrosine residues, and CD41 platelet antigen were used in standard immunoperoxidase staining to study 36 nodules and 24 cords obtained from patients with fibromatoses. The specimens were studied via light microscopy, and staining intensity was quantitated using a computer-enhanced video system. Transforming growth factor-beta staining paralleled procollagen I, fibronectin, and phosphotyrosine staining within the nodule (early stages) but not the cord (late stages) tissue. These factors showed significant increased staining in the early stage of fibromatosis when compared to the late stage. This study is a preliminary demonstration of the presence of transforming growth factor-beta in palmar and plantar fibromatoses. (J Hand Surg 1994;19A:435-441.)

The exact cause of palmar and plantar fibromatoses is unknown, but they are known for their recurrence rates after resection and for a genetic predisposition. Initially, the disease is manifested by nodular thickening of the aponeurotic fascia and may proceed to contracted cords, which can lead to digital flexion and contracture as a late complication.¹ The factors that lead to the development and progression of the disease have yet to be delineated.

Growth factors are naturally occurring substances that have been shown to have dramatic effects on cellular proliferation and differentiation *in vitro* and in some *in vivo* models. Recent reports have linked

growth factors, such as transforming growth factor-beta (TGF- β), to disease processes that are characterized by excessive fibrosis, such as hepatic fibrosis and pulmonary fibrosis.^{2,3} This association raises the question of the possible presence of growth factors in other fibromatosis processes.

The purpose of this study was twofold: (1) to identify and localize growth factors and extracellular matrix components in palmar and plantar fibromatosis nodule and cord specimens and (2) to correlate the presence of these factors with previously reported histologic staging of fibromatosis tissue.

Materials and Methods

Histologic Staging and Evaluation

Histologically, the early stage is characterized by closely packed, rounded fibroblasts against a basophilic background.¹ Later stages show fusiform fibroblasts scattered in an eosinophilic background.¹ Specimen staging was based on the light microscopic appearance of cell density and morphology on hematoxylin and eosin-stained sections. A spectrum ranging from high cell density, round cells with a basophilic background to low cell density, fusi-

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form cells with an eosinophilic background was noted. Within a given specimen, varying degrees of staining were observed. Sequential histologic sections were carefully studied to include specific tissue location involving these same cells, which allowed evaluation of their staining characteristics with the monoclonal antibodies.

Immunohistochemical Studies

Immunohistochemical staining using monoclonal antibodies was used to study 10 fresh palmar (6 patients) and plantar (4 patients) fibromatosis specimens. Tissue from adjacent fascia in each patient, human tonsillar tissue (as a positive control factor), and nonimmune mouse sera (as a negative control factor) were also studied. Representative specimens were placed in histologic blanks (Baxter Health Care Corporation, McGaw Park, IL), covered with embedding compound (Miles Laboratories, Elkhart, IN) and quick-frozen in liquid nitrogen and stored in a -80° freezer prior to sectioning. Frozen sections 6- μ m thick obtained using a microtome (Tissue-Tek Cryostat, Miles Laboratories) and then mounted on a glass slide, fixed with cold acetone for 8 minutes, and allowed to air dry. The monoclonal antibodies were mouse-derived and directed against human antigens. The TGF- β monoclonal (gift from James R. Dasch, Section of Microbiology and Immunology, Collagen Corporation, Palo Alto, CA—reactive to both TGF- β 1 and TGF- β 2)⁴ and epidermal growth factor (EGF) monoclonal (Chemicon International, Temecula, CA) procollagen type I antibody (gift from John D. McDonald, Department of Pulmonary Medicine, Washington University School of Medicine, St. Louis, MO),⁵ commercially available murine monoclonal antibodies directed against fibronectin, CD41 (Chemicon International) and phosphotyrosine residues (Upstate Biotechnology, Lake Placid, NY) were used. An avidin-biotin immunoperoxidase kit and protocol (Vector, Burlingame, CA) were used with incubation of the specimens with horse serum to block nonspecific immune binding, including a quenching step for endogenous tissue immunoperoxidase. The color reagent used was 2-amino-ethyl carbazole, which creates a red-reaction product. A water-soluble hematoxylin counterstain was used to provide counterstain with the unstained regions of the specimen.

Quantitative Analyses of Stained Sections

A Bio-Quant IV System (R & M Biometrics, Nashville, TN) was used to quantify tissue staining characteristics and intensity, as well as cell counts.⁷

Subtraction of background staining intensity from within each histologic section was done to control for differences in section preparation, amount of antigen, and the variations of illumination. Each section was stained using a single monoclonal antibody, with five measurements made by two independent individuals in a double-blinded fashion, and an average staining intensity was calculated for each section. Calculation of intensity was repeated in a like manner five times for the same section stained with the same antibody, and bar graphs were constructed with standard error bars. Statistical comparisons of staining intensities for each antibody between the tissue specimens were performed with a one-way analysis of variance and considered significant when p was $<.001$. The computer system allowed counting of the cells, which contributed to the staining intensity, and this was used to control for variations in cell number present on a given slide (i.e., early stages, highly cellular; later stages, less cellular).

Patients

Ten patients with palmar (6 patients) and plantar (4 patients) fibromatoses were included in the study. The age of the patients ranged from 35 to 60 years. There were 5 men and 5 women. The patients were divided into two groups, depending on their clinical presentation. The first group consisted of those who presented with painful nodules on the palmar surface of their hands or feet over period of 3–5 months. All of the nodules were noted to be tender on examination, but the four patients did not have associated joint contractures. The majority of the nodules in the hand were found palmar to the metacarpophalangeal and proximal interphalangeal joints. The nodules in the feet were found near the metatarsophalangeal joints. Four (2 palmar and 2 plantar) of the 10 patients presented with these findings on physical examination. The second group consisted of six patients who presented with progressive flexion contractures of digits of the hand (4 patients) or foot (4 patients) over a period of 1–3 years. These patients regained good digit extension of the hand and foot after surgery.

All patients had no other manifestations of fibromatosis and all underwent surgical resection of the palmar and plantar nodules and fibromatosis cords when present. Ten independent nodules were excised from each of the two patients with palmar fibromatosis, and eight independent nodules were excised from each of the two patients with plantar fibromatosis. Thirty-six nodules were thus examined and tested for the presence of growth factors from four patients. An average of four cords were excised

from each of the six patients who presented with flexion contractures. Therefore, a total of 24 cords were tested for the presence of growth factors.

Results

Histologic Staging

Histological staging of the specimens found 4 of the 10 cases (2 palmar and 2 plantar) to be early diseases and the remaining 6 to be late diseases and without nodules. Figure 1 shows a representative negative control for the early plump fibroblasts found within each of the fibromatosis nodules.

Transforming Growth Factor-beta Staining

Positive staining for TGF- β was seen in all nodular specimens from the four patients with early disease and was specific for regions of high cellular density. In Figures 2 and 3, early plump fibroblasts stained intracellularly with TGF- β antibodies in uniform fashion. No staining was noted in involutinal areas, cord tissue, or normal fascia. The calculated staining intensity for the four nodules relative to their respective controls are shown in Table 1. Figure 4A-D shows the staining intensity of early palmar and plantar fibromatoses with respect to each antibody and tissue sections. Figure 4E shows no staining differences between tissue sections with respect to each antibody in late fibromatosis tissue.

Epidermal Growth Factor Staining

No staining was noted for EGF in any of the specimens.



Figure 1. Representative negative control of the early fibroblasts from the palmar and plantar fibromatoses' nodules (oil immersion, original magnification $\times 1250$).

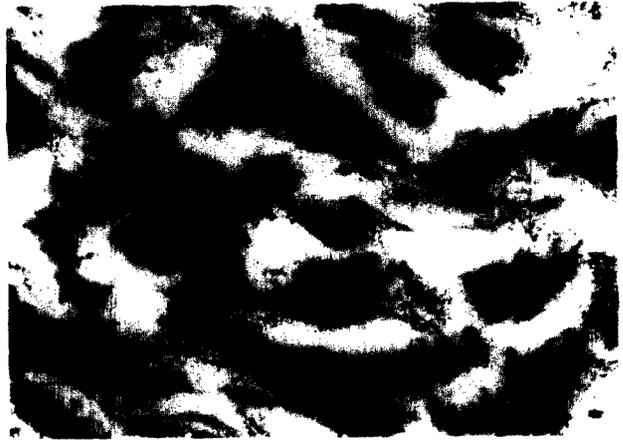


Figure 2. Immunohistochemical detection of transforming growth factor-beta 1. Intracellular staining of the early fibroblasts of the palmar fibromatosis' nodules (oil immersion, original magnification $\times 1250$).

Fibronectin, Procollagen I, and Phosphotyrosine Staining

Staining with the monoclonal antibodies paralleled TGF- β . Figure 5 shows intracellular staining of the early fibroblasts by antiphosphotyrosine antibodies within the palmar nodules. Similarly, Figure 6 shows intracellular staining of the early fibroblasts with antiphosphotyrosine antibodies within the plantar nodules. Figure 7 shows the reticular pattern of extracellular staining for fibronectin in close proximity to the early fibroblasts within the nodule. Figure 8 shows the intracellular staining of the early fibroblasts by antiprocollagen I antibodies within the palmar nodules. The calculated staining intensity for representative nodules relative to their re-



Figure 3. Immunohistochemical detection of transforming growth factor-beta 1. Intracellular staining of the early fibroblasts of the plantar fibromatosis' nodules (oil immersion, original magnification $\times 1250$).

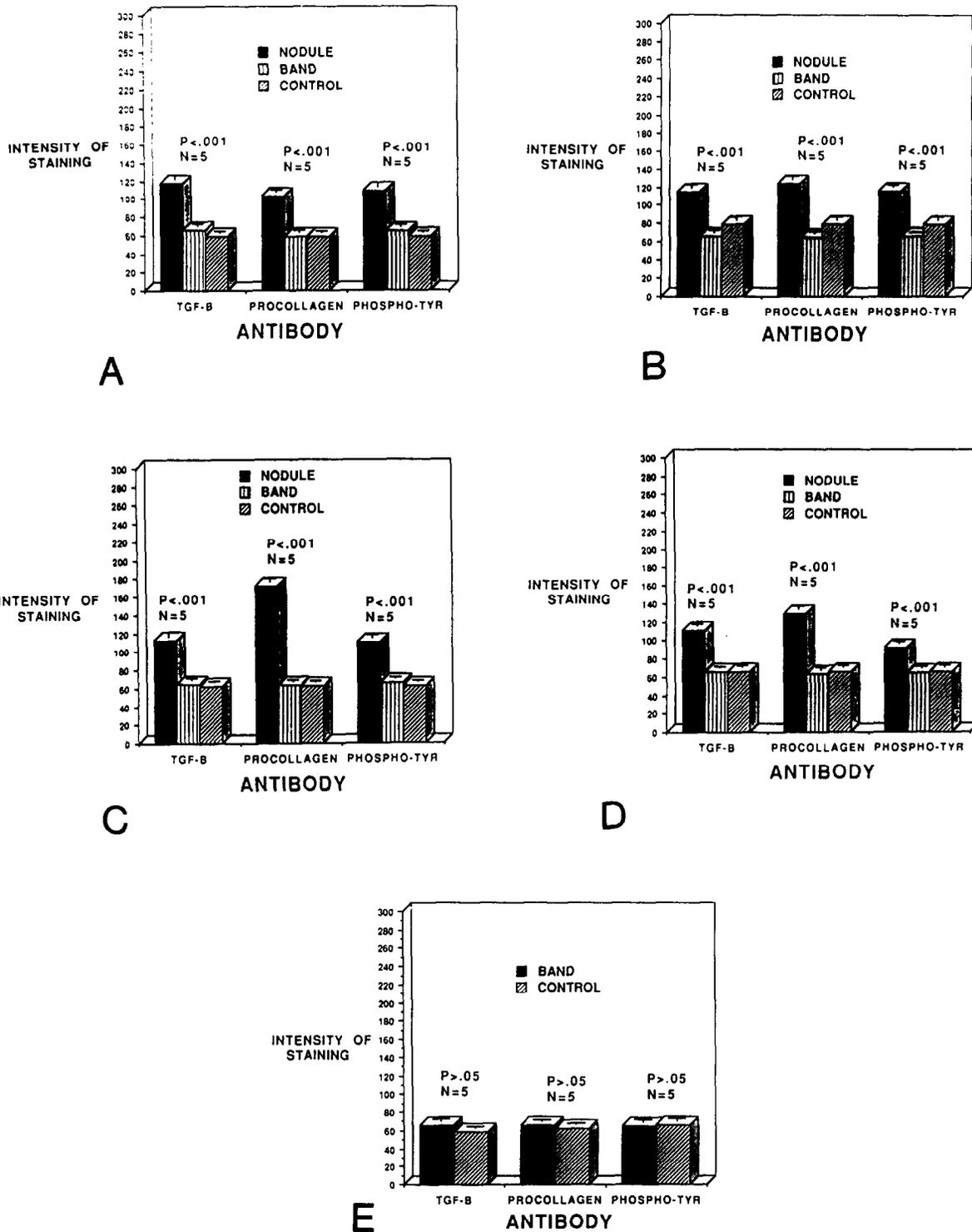


Figure 4. Calculated staining intensities, comparing nodule, band, and control tissue from early palmar and plantar fibromatoses (n = number of patients). (A) Patient 1: early palmar fibromatosis; (B) patient 2: early plantar fibromatosis; (C) patient 3: early plantar fibromatosis; (D) patient 4: early palmar fibromatosis; (E) patient 5: late palmar fibromatosis.

Table 1. Mean Staining Intensity of Fibromatosis Specimens

Case No.	Antibody						p	Negative Control (nonimmune mouse sera)
	TGF- β		Procollagen I		Phosphotyrosine			
	N	B	N	B	N	B		
1	117 \pm 5	66 \pm 3	103 \pm 2	59 \pm 1	109 \pm 5	65 \pm 2	<.001	59 \pm 1
2	115 \pm 3	66 \pm 3	124 \pm 3	65 \pm 2	116 \pm 3	65 \pm 1	<.001	78 \pm 4
3	112 \pm 4	65 \pm 2	172 \pm 4	63 \pm 1	110 \pm 3	65 \pm 1	<.001	63 \pm 1
4	112 \pm 1	65 \pm 1	129 \pm 3	64 \pm 2	93 \pm 2	65 \pm 2	<.001	65 \pm 3
5	—	66 \pm 3	—	65 \pm 1	—	65 \pm 3	NS	59 \pm 1
6	—	65 \pm 2	—	64 \pm 1	—	63 \pm 2	NS	63 \pm 1
7	—	64 \pm 1	—	63 \pm 2	—	66 \pm 1	NS	65 \pm 3
8	—	63 \pm 1	—	65 \pm 3	—	64 \pm 2	NS	62 \pm 1
9	—	62 \pm 1	—	65 \pm 1	—	65 \pm 1	NS	60 \pm 2
10	—	61 \pm 2	—	63 \pm 2	—	64 \pm 1	NS	61 \pm 1

All values mean \pm SEM. TGF- β , transforming growth factor-beta; N, Dupuytren's nodule; B, Dupuytren's band.

spective controls is shown in Table 1. Figure 4A-D demonstrates significantly different staining intensities between the nodule and band tissue for each of the antibodies. In contrast, no difference was noted for late fibromatosis tissue in Figure 4E.

CD41 (Anti-platelet) Staining

CD41 staining was present and confined to the blood vessels and was otherwise absent.

Discussion

Palmar and plantar fibromatoses have been described as an orderly progression of disease stages, from early stages where the cells are dense and enlarged to later stages where the cells are fusiform and diffuse. The etiology of fibromatosis is elusive.

Chiu and MacFarlane reported three stages of Dupuytren's disease: early, active, and advanced.¹ The cell of the early disease was hypothesized to be the perivascular fibroblast, whereas the cell of active and advanced disease was hypothesized to be the myofibroblast.¹ Andrew et al. provided another hypothesis when they found an increased number of macrophages in the early stages of Dupuytren's disease and suggested that the macrophages may release growth factors that could promote the progression of the fibrotic process.⁶ A better understanding of these growth factors and myofibroblasts and how they interact could help prevent late contracture problems, which are often difficult to treat.

The role of growth factors in wound healing has been demonstrated in both *in vitro* and *in vivo* models. TGF- β has been shown to promote extracellular matrix formation in wound repair.⁸ Ignatz



Figure 5. Immunohistochemical detection of phosphotyrosine residues. Intracellular staining of the early fibroblasts in the palmar fibromatosis' nodules (oil immersion, original magnification \times 1250).



Figure 6. Immunohistochemical detection of phosphotyrosine residues. Intracellular staining of the early fibroblasts in the plantar fibromatosis' nodules (oil immersion, original magnification \times 1250).

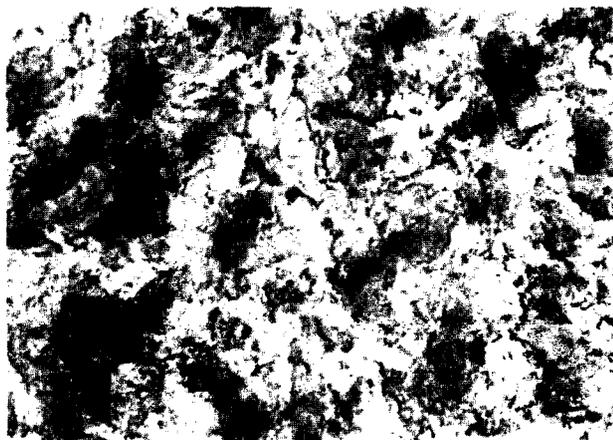


Figure 7. Immunohistochemical detection of fibronectin residues. Reticular pattern of extracellular staining in close proximity to the early fibroblasts of the palmar fibromatosis' nodules (oil immersion, original magnification $\times 1250$).

and Massague found that both collagen and fibronectin production were stimulated by TGF- β .⁹ In our study, TGF- β was present in the environment of early fibromatosis but not in the later stages. Also, early plump fibroblasts within a fibromatosis nodule demonstrated the presence of TGF- β intracellularly. This result may be consistent with the findings of Kane et al. who showed, using *in situ* hybridization techniques, that fibroblasts are one of the cells responsible for synthesizing TGF- β in growth factor-augmented healing processes.¹⁰ This result may also be consistent with those of Andrew et al., who found that early nodules stained positive for macrophages.⁶ They suggested that macrophages, which have been shown to be a significant

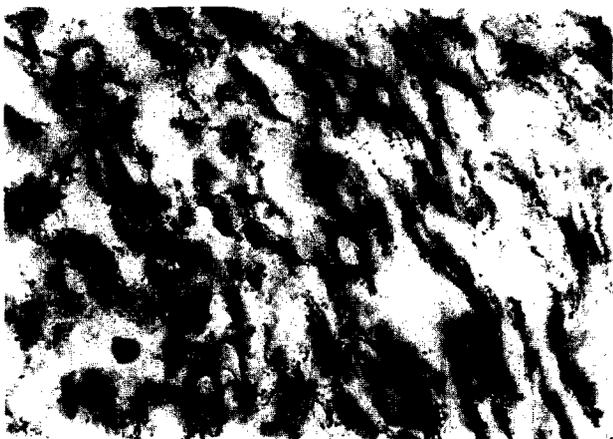


Figure 8. Immunohistochemical detection of Procollagen I. Intracellular staining of the early fibroblasts in the palmar fibromatosis' nodules (oil immersion, original magnification $\times 1250$).

source of TGF- β , may release growth factors that might contribute etiologically to the development of Dupuytren's disease.⁶ One plausible reason for the presence of TGF- β in early nodules is that it may be involved in the activation of these early cells. The initiation of this process would be through synthesis of new collagen and fibronectin, until formation of cord tissue ensues. Support for this hypothesis comes from the staining of newly synthesized intracellular collagen (procollagen type I) and extracellular fibronectin, which paralleled TGF- β staining in the early nodules. The source of TGF- β remains to be demonstrated. In the normal healing wound, the three important sources of TGF- β are platelets in the early stages and macrophages and fibroblasts in the later stages.¹¹ The CD41 platelet antigen stain was confined to the blood vessels, suggesting that the TGF- β may be either from the macrophages or the fibroblasts. This requires further investigation to better determine the source. If TGF- β does play a role in the development of fibromatosis, then local blocking of the producing cell or its TGF- β product could offer a form of treatment of the disease.

Tyrosine kinase activation is one of the known intracellular messengers of various growth factors, such as EGF, basic fibroblast-derived growth factor, and platelet-derived growth factor.¹² We demonstrated parallel staining of phosphotyrosine residues in the same cells where TGF- β was found. Two hypotheses might explain this finding. First, TGF- β may act through a tyrosine kinase process. This is less likely because recent studies *in vitro* have shown this not to be the case.¹³ The other is that yet another growth factor acting through a tyrosine kinase receptor is involved in the process. This other growth factor may act independently or in addition to TGF- β , as found by recent studies on platelet-derived growth factor-AA in cell culture.¹⁴ Intracellular transduction is another point of possible medical intervention, as shown by experiments that mutate the tyrosine kinase activity of the EGF and insulin receptor, which lead to loss of function.¹² Again, it is important to note that the phosphotyrosine residues paralleled the presence of TGF- β , but the data do not provide information about function or importance in the particular stages.

This preliminary study raises many questions. First, given that TGF- β was present along with procollagen I and fibronectin in early fibromatosis nodules, what is its role in these cells and how might this contribute to the final maturation of these cells eventually leading to scar tissue? Second, given that phosphotyrosine residues also paralleled the presence of TGF- β , could there be other growth factors

important in the early process of differentiation? Gonzalez et al. and Lappi et al. have shown *in vitro* that basic fibroblast-derived growth factor may be implicated in the progression of Dupuytren's contracture.^{15,16} Similarly, Badalamante et al. reported the possible role of platelet-derived growth factor in Dupuytren's disease.¹⁷ Third, if these growth factors do exist, do they act with or without TGF- β ? The answers to these questions require an *in vivo* model for investigating the formation of fibromatosis tissue.

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