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Histogenesis of Dupuytren's disease: An immunohistochemical study of 30 cases

Thirty-seven specimens from the hands of 30 patients with Dupuytren's disease were examined by light microscopy after immunohistochemical staining for the presence of desmin intermediate filaments. Results indicated that desmin-positive cells were present in the proliferative Dupuytren's nodules, and that the number of desmin-positive cells decreased significantly in the fibrous phase of the disease. Also, on the basis of the pattern of distribution of the desmin-positive cells around vessels, we postulate that the desmin-positive cells in Dupuytren's nodules were migrating perivascular smooth muscle cells from the vessel wall. The exact fate of these cells is uncertain, but we hypothesize that these displaced perivascular smooth muscle cells are capable of transforming into collagen-producing, desmin-negative myofibroblasts that form the cellular basis of Dupuytren's lesions. (J HAND SURG 1988;13A:61-7.)

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In 1968 Ishikawa, Bischoff, and Holtzer[1] first coined the term *intermediate-sized filaments* to denote a set of cytoplasmic fibers that were approximately 10 nanometers (nm) in diameter, which were distinct from the 5 to 7 nm myofilaments and the much thicker 21 to 24 nm microtubules. Since then, it has become evident that intermediate filaments are ubiquitous in mammalian tissues[2-4] and that most cells are found to have only one of five possible intermediate filament types, i.e., cytokeratins are found in epithelial cells, vimentin in mesenchymal cells, desmin in myocytes, glial fibrillary acidic protein in glial cells, and neurofilaments are found in most neurons.[3, 5-7] Intermediate filaments are, therefore, specific tissue markers. As the five types of intermediate filaments are antigenically distinctive, monoclonal antibodies have been raised and used extensively in diagnostic surgical pathology to identify cell types and classify neoplasms.[8-13]

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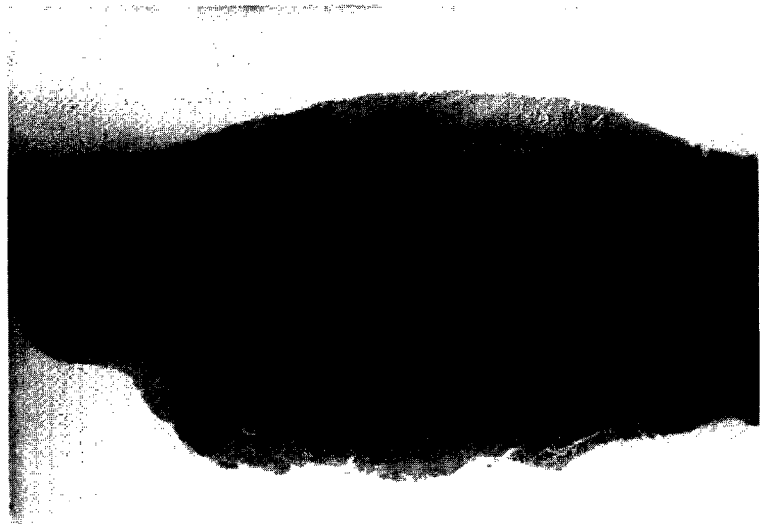

Fig. 1. A typical Dupuytren's nodule submitted for the study. Cell distribution is uneven. The less cellular, hence more fibrous and eosinophilic area appears more darkly stained *(solid arrow)* than the more cellular area *(open arrow). Thin arrows* show vessels in the nodule. (Hematoxylin and eosin stain. Original magnification ×25.)

The basic pathologic process in Dupuytren's disease is a proliferation of spindle-shaped cells that have been considered to be fibroblasts or perivascular smooth muscle cells (pericytes) or myofibroblasts based on light microscopic and electron microscopic examinations.[14-18] Intense morphologic scrutiny of these cells, however, failed to positvely identify the cell of origin.

With the rapid advances of immunohistochemical techniques, a positive identification of the intermediate filaments of the cells in Dupuytren's disease represents a logical step.

Schurch et al.[19] in a study of the origin of the myofibroblasts, included three cases of fibromatosis, one of which was a lesion from Dupuytren's disease. They reported that the cells were vimentin positive and desmin negative. The lack of desmin type intermediate filaments was cited as evidence against the pericytes being the cell of origin in Dupuytren's disease. However, the ability of pericytes to acquire the ultrastructural and functional characteristics of myofibroblasts has been well established in several experimental situations.[20, 21] Furthermore, investigations into microvascular changes in Dupuytren's disease by Kisher and Speer[22] have suggested that pericytes could differentiate into myofibroblasts from hypoxic stimulus. We decided that more cases of this disease should be studied for evidence of desmin in the proliferating cells.

We hypothesized that desmin-positive cells would be found in the cellular proliferating lesions of Dupuytren's disease, and that the pericytes lose the ability to express the desmin gene once transformed into myofibroblasts, which are more adapted for collagen production. Identification of tissue in the early and active phases of disease[16] would be of utmost importance in trying to understand the role of pericytes and myofibroblasts in the genesis of the lesion. It is not uncommon to see areas of varying degrees of cellular activity in the same specimen. Therefore, cell counts per unit area have been used as a more objective criterion of cellular activity in our study.

Materials and methods

Thirty-seven specimens from 30 consecutive cases of Dupuytren's disease were used in the study. There were 29 men and one woman. Their ages ranged from 30 to 86 years at the time of operation. The interval between time at onset of the disease to the time of operation varied from months to 34 years.

The specimens were obtained by one of us (R. M. M.) at the time of operation. Great care was taken to obtain nodular tissue that was free of surrounding fat and fascia and, therefore, represented the area of greatest cellular activity. Guided by loupe magnification, a nodule was cut so that only the central portion was submitted for examination.

Immunohistochemistry

The tissue was snap frozen in liquid isopentane at −130° C to −140° C and then embedded in OCT medium. The 5 μm sections were then prepared for

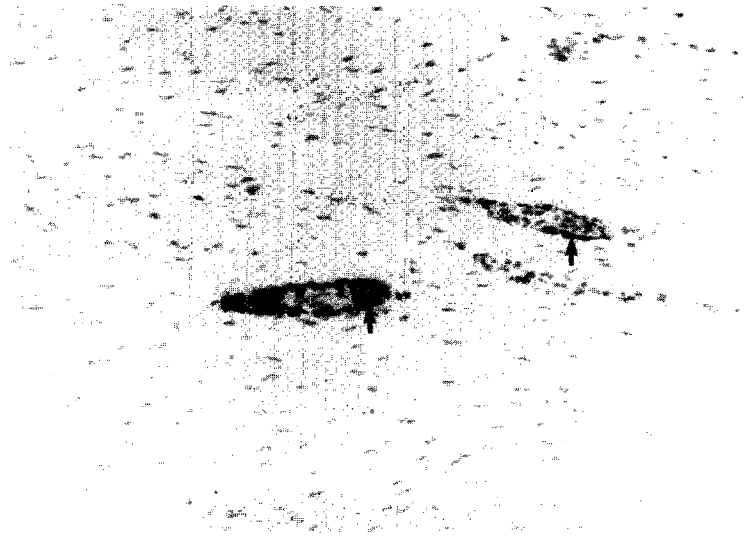

Fig. 2. Normal location of desmin-positive cells *(arrows)* in vessel wall. (Anti-desmin antibody peroxidase counterstained with Myer's hematoxylin. Original magnification × 100.)

incubation with various antisera by air-drying at room temperature for 30 minutes, followed by fixation in acetone at 4° C for 10 minutes, then immersed in tris buffer for 20 minutes. Monoclonal antibodies against the intermediate filaments desmin (monoclonal antibody to desmin from mouse-mouse-hybrid cells [clone DE-B-5], Boehringer Mannheim Biochemica, Dorval, Quebec; monoclonal anti-desmin antibody code RPN 1101, Amersham Corp., Arlington Heights, Ill.) were used. The avidin-biotin staining technique described by Hsu, Raine, and Fanger[23] was followed and amnio-ethyl-carbazole was employed as the substrate. All slides were counterstained with Myer's hematoxylin. The slides were then examined and the morphology of the cell nuclei was recorded as predominantly round, oval, or elongated. The presence or absence of desmin-positive cells and the pattern of their distribution were noted. In addition, the areas of highest cell density of each lesion was determined by first screening the entire nodule at 250 times magnification. Areas of higher cell density were then reexamined with a 40× objective and 10× eyepieces, which covered an area of 0.16 mm^2. The number of cell nuclei within this 0.16 mm^2 high-power field (HPF) was manually counted and an average of 4 to 5 HPF were counted for each specimen. The highest count was used to arbitrarily classify a nodule into one of three groups: (1) lesions with more than 400 nuclei per HPF (2) lesions with between 200 to 400 nuclei per HPF, and (3) lesions with 200 or less nuclei per HPF.

In the same HPF, the number of desmin-positive cells were also manually counted, and the percentage of desmin-positive cells were calculated by dividing the number of desmin-positive cells with the total number of nuclei within the same HPF in each case.

Table I. Significant decrease of the percentage of desmin-positive cells as the Dupuytren's lesion became less cellular

Total cells per HPF 0.16 mm^2	*Number of samples*	*Mean percentage of desmin-positive cells*
Over 400	12	19.8
200 to 400	16	8.3
200 or less	9	1.2

There is a significant difference between each of the three groups. $P = 0.001$.

Fig. 1 shows a typical nodule submitted for the study. The size of the nodules ranged from 0.3 cm to 1 cm and when examined microscopically a nodule showed cells irregularly distributed within a fibrous stroma. An HPF of 0.16 mm^2 with the highest cell density is chosen in each specimen for comparative analysis.

Results

Results with the two different monoclonal anti-desmin antibodies were identical. Fig. 2 shows the normal location of desmin-positive cells. These are smooth muscle cells as pericytes in the wall of vessels of various caliber. Desmin-positive cells are not seen in any location other than the vessel wall.

Table I shows the relationship of the cellular activity to the desmin content of the lesions.

Fig. 3. Lesion with more than 400 cell nuclei per HPF. The superior boundary of this lesion reflects its nodular architecture. Cells positive for desmin have darkly stained cytoplasm. (Anti-desmin antibody peroxidase counterstained with Myer's hematoxylin. Original magnification $\times 100$.)

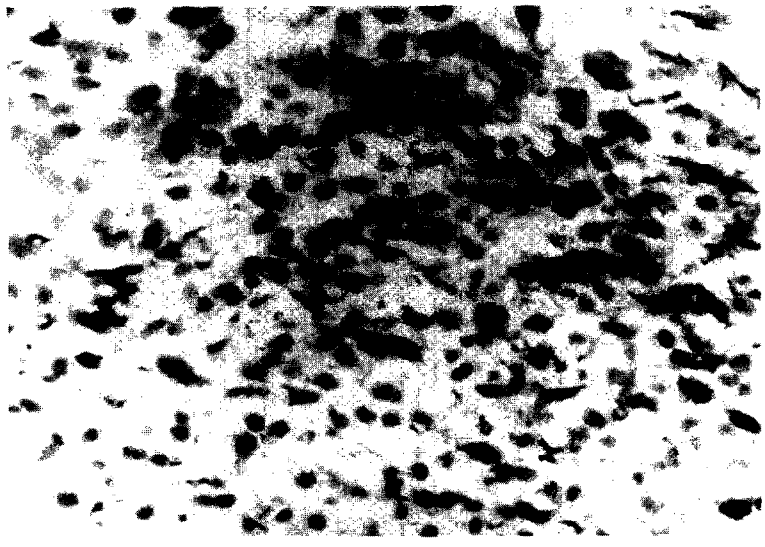

Fig. 4. Desmin-positive cells *(solid arrow)* intermingle with non-desmin positive *(open arrow)* in a cellular lesion of Dupuytren's disease. (Anti-desmin antibody peroxidase counterstained with Myer's hematoxylin. Original magnification $\times 400$.)

Twelve of the 37 lesions had over 400 cell nuclei per HPF. These lesions tended to be vascular, with centrally and peripherally located vessels. The majority of the cells had rounded nuclei, and there was a small amount of intercellular stroma (Fig. 3). An average of 19.8% of cells present were stained positive for desmin. These were cells with darkly stained cytoplasm present around vessels and at varying distances from vessels among non-desmin-positive cells in the lesion (Fig. 4). Fig. 5 illustrates the "stellate" or "radiating" pattern of the desmin-positive cells. Vessels are observed in the centers of some of these cell clusters.

Sixteen of the lesions had between 200 to 400 cell nuclei per HPF. Like the previously described group, capillaries were frequently observed with cell masses. However, the cell nuclei were more oval in shape and

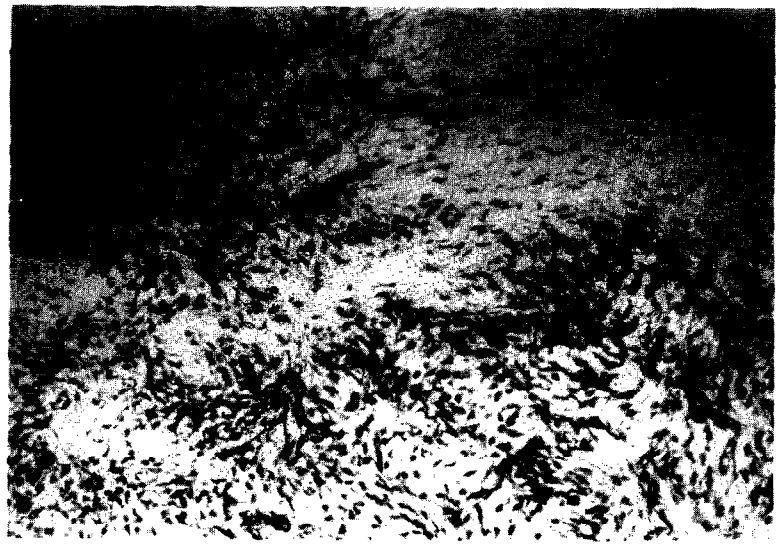

Fig. 5. The "stellate" or "radiating" pattern of desmin-positive cells suggesting cellular migration from the vessel wall as normally, desmin-positive cells are observed only in vessel wall and not found in surrounding fibrous tissue. *Solid arrows* show vessels in the centers of cell clusters. (Anti-desmin antibody peroxidase counterstained with Myer's hematoxylin. Original magnification ×100.)

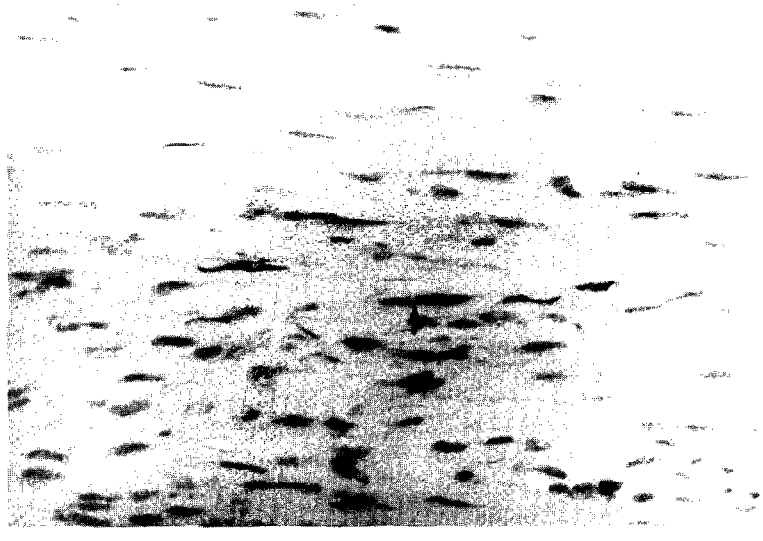

Fig. 6. Infrequent presence of desmin-positive cells *(arrow)* in fibrous lesions of Dupuytren's disease. (Anti-desmin antibody peroxidase counterstained with Myer's hematoxylin. Original magnification ×400.)

less closely packed. An average of 8.3% of cells present were desmin positive.

In the remaining nine lesions, the cellularity was less than 200 cell nuclei per square millimeter. The nuclei were wavy, spindled, and elongated, and there was abundant intercellular collagenous stroma. Desmin-positive cells were infrequent and accounted for an average of only 1.2% of total cell population (Fig. 6).

Discussion

A previous study by Schürch et al.[19] of a single case of Dupuytren's disease using monoclonal antibodies

and the immunofluorescence technique was unable to demonstrate desmin within cells in the diseased tissue, and this finding was interpreted as evidence against the myofibroblasts originating from the perivascular smooth muscle cells.

We, however, support the views of Murray, Schrodt, and Berg,[20] Esterly, Glagov, and Ferguson[21] and Kischer and Speer[22] that perivascular smooth muscle cells (which we have shown to be desmin positive) could be the cell of origin and that these cells differentiate into the so-called *myofibroblast* under a yet undefined stimulus. We further hypothesize that these perivascular smooth muscle cells could lose their expression of the desmin gene during the differentiation and be transformed into collagen-producing myofibroblasts. According to this thesis, desmin-positive cells would only be observed in the active proliferating cellular lesions of Dupuytren's disease.

The variable activities and different phases of lesions of Dupuytren's disease were first described by MacCallum and Hueston[15] when, microscopically, they divided the lesions into the fibroblastic, proliferative phase and the collagen forming phase. Our present study used the number of cell nuclei per unit area to introduce an objective criterion for characterization of these phases of the disease. By dividing the lesions into three groups according to their cellularity, we have demonstrated a higher percentage of desmin-positive cells present in the more cellular lesions and that the absolute number and the percentage of desmin-positive cells decreased significantly in the less cellular lesions of Dupuytren's disease. Moreover, desmin-positive cells were especially abundant around vessels of some cellular lesions. In fact, an array of desmin-positive cells radiating from a centrally located capillary (as illustrated in Fig. 5) was a rather constant feature in most proliferating Dupuytren's nodules. By examination of the 37 Dupuytren's nodules of different cellularity and comparison of the distribution of the desmin-positive cells, with respect to the vessels, we gained the impression that these were probably migrating cells from the vessel wall to the peripheral connective tissue. It is also logical to conclude that the most likely progenitor for the migrating desmin-positive cells is the perivascular smooth muscle cell, which is normally desmin positive.

As yet, the significance of this pericytic migration in Dupuytren's disease remains unknown. It is quite possible that the proliferation of the perivascular smooth muscle cells is just an epiphenomenon and is part of the vascular change caused by the surrounding cellular activity of Dupuytren's disease. However, on the basis of the demonstrated close relationship between the desmin-positive cells and the other proliferating cells in Dupuytren's lesions, we choose to believe that the pericytes are the cells of origin of the myofibroblast, which as "transformed" smooth muscle cells, are capable of collagen formation but have lost the ability to express the desmin gene. Our hypothesis is based on purely morphologic interpretation of a histologic change, and we recognize that further studies of the Dupuytren's lesions, with the same and different techniques, are necessary for the confirmation of our observations.

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Lipofibromatous hamartoma of nerve

Seventeen cases of lipofibromatous hamartoma of nerve (14 with histologic confirmation) were treated between 1935 and 1985. One patient had bilateral involvement. Fourteen lesions were located in the upper extremity and four in the lower extremity. Twelve of the 18 lesions were associated with combined soft tissue and skeletal enlargement, or true macrodactyly. Three other lesions had soft tissue enlargement other than that noted intraneurally. Three patients were treated with carpal tunnel release alone and 14 with combined procedures to decrease the size of the affected part. Sensibility in the affected part often appeared unrelated to removal or preservation of hamartomatous nerve tissue. Complications related to nerve surgery included recurrence of carpal tunnel syndrome in one patient, recurrent soft tissue mass in one patient, and painful calcification of the involved tissues 20 years postoperatively in one patient. (J HAND SURG 1988;13A:67-75.)

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Lipofibromatous hamartoma is a rare neoplasm that most commonly involves the median nerve.[1-3] Involvement of the ulnar,[4] radial,[5] and plantar nerves[6] has also been described. The tumor is usually present at birth[7, 8] and may be associated with digital enlargement.[6, 9] Treatment of this neoplasm has been controversial: some authors recommend excision of the involved nerve[2, 4, 10, 11]; some, microsurgical intraneural