Plantar Fibromatosis: An Immunohistochemical and Ultrastructural Study

Luigi de Palma, M.D., † Attilio Santucci, M.D., ‡ Antonio Gigante, M.D., * Alessandro Di Giulio, M.D., * and Sergio Carloni, M.D.*
Ancona and Rome, Italy

ABSTRACT
The analogies between plantar fibromatosis and Dupuytren's disease (palmar fibromatosis) are well known. The latter is clinically more frequent and has been the object of extensive immunohistochemical and ultrastructural studies, with a view to investigating its pathogenesis. By contrast, such data on plantar fibromatosis are quite scarce. A histochemical, immunohistochemical, and ultrastructural study was performed on nodule tissue from six patients who were subjected to total fasciectomy for plantar fibromatosis. The study of myofibroblasts revealed features suggestive of their fibroblastic origin and evidenced a cytoskeleton and an extracellular filamentous system that could enable myofibroblasts to generate and exert the intracellular forces that contribute to the contraction of the aponeurosis. These aspects are similar to those observed in Dupuytren's disease and seem to lend support to the theory that the two diseases are expressions of the same disorder.

INTRODUCTION
Plantar fibromatosis, also called Ledderhose's disease, is a benign proliferation of well differentiated fibroblasts in the plantar fascia or aponeurosis (Fig. 1). It is much less common than palmar fibromatosis (Dupuytren's contracture) and shares with it several features. It affects, preferentially, men of 50 to 60 years of age and is associated with diabetes, alcoholism, epilepsy, and other fibromatous diseases like Dupuytren's, Peyronie's, knuckle-pad thickening, and frozen shoulder.

Although Dupuytren's contracture has been widely investigated immunohistochemically and ultrastructurally, the literature on plantar fibromatosis is scarce.

MATERIALS AND METHODS
Patients
Between 1990 and 1995, six patients (four men and two women; mean age, 46 years) with plantar fibromatosis received surgical treatment. Two patients had bilateral disease but were operated on one limb. In no patient was an association with alcoholism, epilepsy, or diabetes found. In all cases, the clinical findings were nodules in the sole of the foot (Fig. 1) and pain with weightbearing.

To reduce the risk of recurrence associated with local excisions, total excision of the fascia was performed in all patients. Access to the sole was gained by a medial incision extending from the calcaneal tuberosity to the first metatarsal head (Fig. 2). Fourteen nodules were removed (Fig. 3). After adequate hemostasis, the wound was sutured over a drain, which was removed on the second postoperative day.
Compressive dressing was applied for 3 weeks, during which time, the foot was not loaded. There were no recurrences from 2 to 7 years follow-up. The patients with bilateral involvement are still asymptomatic on the untreated side at 2 years follow-up.

Histo- and Immunohistochemistry

Immediately after removal, specimens were fixed by immersion in 4% paraformaldehyde in 0.1 mol/L phosphate buffer, pH 7.4, at 4°C for 24 hr. Histochemistry and immunohistochemistry were performed on different sections of each sample. The specimens undergoing light microscopy were decalcified in 4 N formic acid and sodium citrate, embedded in paraffin, cut into transverse and longitudinal sections (3–5 micrometers thick), and stained with hematoxylin and eosin (H&E) and Gomori’s stain.

Paraffin sections were dewaxed in xylene and hydrated in descending series of ethanol. After washing in 0.01 mol/L phosphate-buffered saline (PBS) at pH 7.4, sections were incubated for 30 min at room temperature in PBS containing 3% normal goat serum (PBS-3% NGS). Samples were incubated with monoclonal antibody against anti-α-sarcomeric actin antibodies (monoclonal, clone No. 5C5, Sigma-Aldrich s.r.l., Milano, Italy) diluted 1:2000, anti-cellular fibronectin (monoclonal, clone No. FN-3E2, Sigma-Aldrich) and antilaminin (polyclonal, clone No. 4C12, Immunotech, Marseille, France) antibodies, in PBS-3% NGS overnight at 4°C. The avidin-biotin-peroxidase technique ( Vectastain ABC Elite, Vector Laboratories, Burlingame, CA) was used, and the reaction was developed with 3,3'-diaminobenzidine-tetrahydrochloride (Sigma-Aldrich) as chromogen. Sections were dehydrated and mounted in Eukitt resin (BDH, Milan, Italy). Control sections treated with mouse or rabbit normal immunoglobulins instead of specific antibodies exhibited no immunoreaction product.

Transmission Electron Microscopy

For transmission electron microscopy (TEM), a small portion of each sample was treated separately. It was fixed by immersion in 4% paraformaldehyde/2.5% glutaraldehyde/0.1% tannic acid in 0.1 mol/L cacodylate buffer, pH 7.4. Tissue blocks (1-mm) were postfixed in 1% osmium tetroxide (Merk, Bracco, Milan, Italy), dehydrated in ascending alcohol series, and embedded in MY753 araldite resin (BDH). Ultrathin 60 to 90-nm sections were stained with uranyl acetate and lead citrate and examined with a transmission electron microscope (Zeiss EM 902).

RESULTS

At gross examination, all aponeuroses appeared thickened (Fig. 2). They contained nodules located medially in the intermediate third that differed in number (2 to 4) and diameter (0.5 to 3 cm) (Fig. 3).

Histo- and immunohistochemistry

In all sections, H&E staining revealed a connective tissue particularly rich in spindle fibroblast-like cells that were arranged in randomly oriented fascicles and were uniform in size and morphology (Fig. 4). In the nodules, a microvascular proliferation could be observed (Fig. 4). The matrix was very poor. In the internodular tissue and the cords, extracellular matrix prevailed over scarce fibroblasts of uniform size and morphology (Fig. 5).

Samples processed for immunohistochemistry and tested with anti-α-sarcomeric actin antibodies revealed, in 4 of 14 nodules, the presence of myofibroblasts, whose cytoplasm stained intensely, owing to
Fig. 4. Plantar fibromatosis. In the nodules, the fibroblasts are arranged in randomly oriented large fascicles. Microvascular proliferation is observed (H&E × 20).

Fig. 5. Plantar fibromatosis. In the internodular tissues, extracellular matrix prevails over scarce fibroblasts of uniform size and morphology. Inflammatory infiltration is observed (H&E × 20).

Fig. 6. The cytoplasm of fibroblasts stains strongly for α-actin. (Immunohistochemistry: anti-α-actin antibodies × 20).

Fig. 7. Ladderhose’s nodules contain extracellular matrix which stains for fibronectin. (Immunohistochemistry: antibodies anti-fibronectin × 20).

The presence of considerable amounts of cytoskeletal α-actin (Fig. 6).

In all samples, the extracellular matrix was strongly positive for the anticalcellular fibronectin antibodies, indicating an abundance of this protein (Fig. 7). No extracytoplasmic reaction to the antilaminin antibodies was observed in any sample, except in vascular structures.

Transmission Electron Microscopy

Ultrastructural analysis showed myofibroblasts containing abundant rough endoplasmic reticulum (RER) and a well developed Golgi apparatus; both are indices of intense protein synthesis (Fig. 8). Within the cytoplasm, aggregates of microfilaments approximately 4 nm in diameter were consistent with actin, although others of microfilaments of greater diameter were probably myosin (Figs. 8 and 9).

In the extracellular space, aggregates of microfilaments 3 to 5 nm in diameter were observed close to some areas of the surface of myofibroblasts (Fig. 9);
sometimes microfilaments 10 to 13 nm in diameter were also evident. These extracellular filament aggregates seemed to be juxtaposed to the cytoplasmic membrane. The cytoskeletal filament bundles seemed to terminate on the inner side of this membrane (Figs. 8 and 9). Away from the cytoplasmic membrane, the extracellular filamentous aggregates seemed to merge with the collagen fibrils of the extracellular matrix or to extend to the surface of the cytoplasmic membrane of other myofibroblasts.

**DISCUSSION**

Actin is a cytoskeletal protein which, by interacting with myosin, produces contractile forces both in smooth-muscle and non-muscle cells. Fibronectin is an extracellular glycoprotein which binds fibroblasts to type I and type III collagen of the extracellular matrix. Laminin is a glycoprotein of the extracellular matrix which anchors smooth-muscle cells to type IV collagen of the extracellular matrix.

The natural history of plantar fibromatosis has been described as consisting of three histological phases: proliferative, active, and maturation phase. In the first phase, fibroblasts proliferate in a scanty intercellular substance, resulting in the formation of the nodule. In the active phase, the nodule has developed, and the number of fibroblasts in the extracellular matrix diminishes. The histological picture of the third phase, that of the inveterate nodule, is characterized by the presence of scarce fibroblasts in abundant matrix, constituted by thick bundles of collagen fibers. Among the nodules and in continuity with them, the tissue exhibits a prevalence of collagen fibers over fibroblasts.

The present study shows that, in the active phase, the nodules contained cells characterized by some typical features of smooth-muscle cells (large intracytoplasmic bundles of actin and myosin microfilaments) and of fibroblasts (numerous mitochondria, abundant RER, and well-developed Golgi complex); in 1972 Gabbiani and Majno called these cells "myofibroblasts." Because they have not been visualized in any other phase, they could either be briefly present in the nodule during the active phase or acquire a different role later, thereby losing the features that had previously enabled them to be identified by routine histological methods.

It has been shown by immunohistochemistry that the myofibroblasts of Dupuytren’s contracture contain non-muscle myosin and \( \alpha \)-actin, although the surrounding extracellular matrix contains abundant fibronectin (typical of fibroblasts) but none of the glycoproteins associated with smooth-muscle cells, such as laminin. It has, therefore, been hypothesized that myofibroblasts are either altered fibroblasts of the normal aponeurosis or belong to a subpopulation of mesenchymal cells, with no relation to smooth-muscle cells.

Based on the observations made in the present study, plantar fibromatosis seems, histologically, very similar to Dupuytren’s disease for the proliferation of well-differentiated fibroblasts immersed in a collagen stroma and the presence of myofibroblasts, as reported by the authors in a previous study. The observation of myofibroblasts in only four of fourteen nodules examined leads us to concur with the predominant literature in hypothesising their presence in only some phases of the disease or, alternatively, their manifestation with these features only at certain stages, with a subsequent return to latency and non-detection by routine histological methods.

In the present cases, as in the nodules from Dupuytren patients, immunohistochemistry showed, in the cytoskeleton of myofibroblasts, the presence of large amounts of \( \alpha \)-actin and, in the extracellular matrix, it showed large amounts of fibronectin and the absence of laminin. These features seem to exclude the smooth-muscle cell nature of myofibroblasts. These data seem to indicate that in plantar fibromatosis, myofibroblasts are in fact fibroblastic cells capable of contractile activity and central to the pathogenesis of the contraction of the plantar aponeurosis.

This hypothesis is also supported by the electron-microscopic data. Together with the absence of the basal lamina that covers the surface of smooth-muscle cells, the abundant RER and the well-developed Golgi apparatus observed in myofibroblasts closely resemble fibroblasts. The characteristically large
amounts of cytoskeletal actin observed by immunohistochemistry seem to be paralleled by the electron-microscopic findings of abundance of microfilaments with features similar to those of actin and myosin, which could endow myofibroblasts with the ability to generate intracellular contractile forces. Some cells are surrounded by aggregates of extracellular filaments, consistent with fibronectin. On one side, these are connected to the cytoskeleton and, on the other, they merge with the collagen fibrils of the extracellular matrix or terminate on the surface of other fibroblasts. These connections could represent the transmission structure of the intracellular contractile forces to the extracellular environment.

These cells could transmit intracellular forces to the extracellular environment via the microfilaments observed at the electron microscope, which could in turn connect the myofibroblasts to other myofibroblasts and to the collagen of the extracellular matrix, thus playing an important role in the pathogenesis of the contracture of the aponeurosis. These features are similar to those observed in Dupuytren's disease and, together with the epidemiological, clinical, and histological features shared by the two diseases, lend further support to the hypothesis that they are clinical expressions of the same disorder.

ACKNOWLEDGMENTS

The authors wish to thank Silvia Modena for reviewing the English.

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