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4 Cellular biology

THE MYOFIBROBLAST

Definition, ultrastructural features and role in wound contraction

The progressive contraction of the palmar aponeurosis is considered to be responsible for the retraction of one or more digits. The fibroblasts of the Dupuytren's nodule have been shown to be ultrastructurally similar to myofibroblasts of granulation tissue (Gabbiani & Majno 1972). Since granulation tissue myofibroblasts were thought to be responsible for wound contraction (Gabbiani et al 1971; Majno et al 1971), the presence of this cell type in Dupuytren's disease (DD) nodules suggested that it could well be responsible for contractile events. Indeed, in addition to packed cisternae of endoplasmic reticulum typical of fibroblasts, myofibroblasts exhibit ultrastructural features reminiscent of smooth muscle cells. These consist of microfilament bundles usually arranged parallel to the long axis of the cell, amongst which are interspersed electron-opaque areas similar to the dense bodies of smooth muscle cells. Myofibroblasts are partly covered by a well defined layer of material having the structural features of a basal lamina and are interconnected by gap junctions (Gabbiani et al 1978). Myofibroblasts are connected to the extracellular matrix by fibronexus, which are transmembrane complexes of intracellular microfilaments in apparent continuity with extracellular fibronectin fibres (Singer 1979; Singer et al 1984). Finally, the nucleus of myofibroblasts consistently shows indentations or deep folds, an ultrastructural feature that has been correlated with cellular contraction in several systems (Lane 1965; Bloom & Cancilla 1969; Franke & Schinko 1969; Majno et al 1969).

The in vitro contraction of myofibroblast-containing tissues in response to various drugs known to act on smooth muscle contraction strongly suggested that myofibroblasts were responsible for the contractile events of wound healing (Gabbiani et al 1971; Majno et al 1971; Ryan et al 1974) and of liver (Irlé et al 1980) and lung (Evans et al 1982) contracture. The ability of myofibroblasts to produce forces strong enough to generate tissue contraction has also been deduced from experiments carried out on in vitro fibroblasts, which are structurally similar to in vivo myofibroblasts (see below). This was well shown in an in vitro model of wound contraction in which fibroblasts cast into a collagen lattice caused its contraction; the rate of this phenomenon was directly dependent on the number of cells seeded within the gel (Bell et al 1979). During the active phase of contraction fibroblasts exhibited numerous stress fibres and gap junctions, but when the gel was fully compacted, the number of microfilament bundles and gap junctions was decreased while the number of synthetic organelles was increased (Bellows et al 1982).

The forces generated by the cultured fibroblasts are traction rather than contraction forces, as shown by experiments in which fibroblasts distorted a sheet of silicon on which they were grown (Harris et al 1981). Several observations suggested that stress fibres are probably the force generating elements in wound contraction since:

1. They contract upon addition of adenosine triphosphate on glycerinated fibroblasts (Hoffman-Berling 1954; Isenberg et al 1976; Kreis & Birchmeier 1980).
2. Microinjection experiments showed that they

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were functionally analogous to skeletal muscle fibrils (Kreis & Birchmeier 1980; Burridge 1981).

Fibronexus are probably the elements through which the forces generated by the actin filament bundles of myofibroblasts are transmitted to the extracellular matrix (Singer et al 1984) and the gap junctions found between these cells may synchronize their contractile action (Gabbiani et al 1978). To our knowledge the contractility of Dupuytren's nodules or of cells derived therefrom has not yet been examined; it remains to be shown whether the model of wound contraction by myofibroblasts also applies to Dupuytren's contracture.

THE MYOFIBROBLAST IN PATHOLOGICAL CONDITIONS

Following the recognition and characterization of the contractile myofibroblast in granulation tissue of healing wounds (Gabbiani et al 1971; Majno et al 1971), this cell was described in a wide assortment of normal and pathological conditions (for review on myofibroblasts in normal conditions see Skalli & Gabbiani 1988). Pathological settings in which myofibroblasts represent a principal cellular component fall into three groups (Seemayer et al 1980, 1981):

1. Response to injury and repair phenomena.
2. Quasineoplastic proliferative conditions.
3. Stromal response to neoplasia.

Response to injury and repair phenomena

These comprise the following:

1. Human and experimental liver cirrhosis (Bhatal 1972; Rudolph et al 1979; Irlé et al 1980).
2. Tenosynovitis (Madden 1973).
3. Radiation-induced pseudosarcoma of skin (Woyke et al 1974).
4. Burn contracture (Larson et al 1974).
5. Ischaemic contracture of intrinsic muscles of the hand (Madden et al 1975).
6. Renal interstitial fibrosis during obstructive nephropathy (Nagle et al 1973).
7. Pulmonary sarcoidosis (Judd et al 1975).
8. Giant cell granuloma of jaws (El-Labban & Lee 1983).
9. Schistosomal liver fibrosis (Grimaud & Borojevic 1977).
10. Regenerating tendon (Postacchini et al 1977).
11. Fibrous capsule around silicone mammary implants (Rudolph et al 1978; Zimman et al 1978).
12. Nodular hyperplasia of the liver (Callea et al 1982).
13. Ganglia of soft tissue (Ghadially & Mehta 1971).
14. Hypertrophic scars (Baur et al 1975).
15. Cataract (Novotny & Pau 1984).
16. Bleomycin-induced interstitial fibrosis of the lung in the rat (Woodcock-Mitchell et al 1984).

Quasineoplastic proliferative conditions

This group embodies the poorly understood, but very important and frequent fibrous tissue proliferations included under the broad heading of fibromatoses, as well as many other soft tissue proliferations, often mimicking sarcomas, which share their predominant myofibroblastic composition and which display a variable proliferative potential, yet do not disseminate or metastasize. (Seemayer et al 1980, 1981). Myofibroblasts constitute the principal cellular components of superficial and deep musculoaponeurotic fibromatoses (Enzinger & Weiss 1983). Superficial fibromatoses include palmar fibromatosis (Gabbiani & Majno 1972; Chiu & McFarlane 1978, Meister et al 1979; Navas-Palacios 1983; Ushijima et al 1984), plantar fibromatosis (Gabbiani & Majno 1972), penile fibromatosis (Ariyan et al 1978) and knuckle pads. Deep musculoaponeurotic fibromatoses comprise extra-abdominal, abdominal and intra-abdominal variants, collectively also called desmoid tumours. To this group also belong infantile fibromatoses, the childhood counterpart of adult musculoaponeurotic fibromatoses, infantile myofibromatosis (Chung & Enzinger 1981) and desmoplastic fibroma of bone (Lagacé et al 1979). Other soft tissue proliferations predominantly composed of myofibroblasts are:

1. Nodular fasciitis (Wirman 1976).
2. Proliferative fasciitis (Chung & Enzinger 1975).
3. Proliferative myositis (Povysil & Matejovsky 1979).
4. Giant cell fibroma of oral mucosa (Weathers & Campbell 1974).
5. Dermatofibroma (Stiller & Katenkamp 1975).
6. Elastofibroma (Ramos et al 1978).
7. Plasma cell granuloma of the lung (Buell et al 1976).
8. Juvenile nasal angiofibroma (Taxy 1977).

Myofibroblasts are also present, to a lesser extent, in the right heart plaque of carcinoid heart disease (Lagacé et al 1975), cardiac myxomas (Ferrans & Roberts 1973) and uterine plexiform tumours (Fisher et al 1978).

Stromal response to neoplasia

Many, invasive and metastatic carcinomas are characterized by hard consistency, retraction and are often fixed to adjacent tissues due to what is generally designated as a myofibroblastic stromal reaction. Myofibroblasts are particularly numerous within the stroma of desmoplastic carcinomas (Seemayer et al 1979a, 1979b; Tremblay 1979; Ohtani & Sasano 1980; Schürch et al 1981, 1982). The retraction phenomenon associated with such carcinomas is attributed to the contractile forces generated by stromal myofibroblasts. These stromal cells are not observed in in situ carcinomas (Seemayer et al 1980; Schürch et al 1982), suggesting that stromal invasion, beyond the epithelial basal lamina, is required to evoke a myofibroblastic stromal reaction. Myofibroblasts are notably absent or equivocally present within carcinomas lacking significant stromal desmoplasia (Schürch et al 1981). Myofibroblasts have been found in sarcomas where they generally constitute a small fraction of the cellular population (Gabbiani et al 1972, Lagacé et al 1980) and in nodular sclerosing Hodgkin's disease (Seemayer et al 1980).

Finally, a few reports describe myofibroblastic tumours, some considered as sarcomas (Churg & Kahn 1977; D'Andiran & Gabbiani 1980; Ghadially et al 1983), although the existence of such tumours as bona fide neoplasms has been questioned (Seemayer et al 1980).

CELLULAR AND EXTRACELLULAR COMPOSITION OF THE DIFFERENT STAGES OF DD

With the electron microscope, three main cell types are identified within the nodules of DD: — immature fibroblasts, myofibroblasts and mature fibroblasts or fibrocytes (Iwasaki et al 1984). Nodules of the proliferative phase (Luck 1959) are composed mainly of myofibroblasts admixed with a few immature fibroblasts (Gabbiani & Majno 1972; Enzinger & Weiss 1983; for review see Skalli & Gabbiani 1988). Lesions of the involutional phase consist of nodules and fibrous cords.

There is general agreement on the cell type composing nodules of DD in the involutional and residual phases (Meister et al 1979; Iwasaki et al 1984). However, according to Meister et al (1979), nodules of the proliferative phase are composed of cells displaying ultrastructural features of fibroblasts, thereby assigning a dominant cell to the proliferative, involutional and residual phases of Luck (1959), i.e. fibroblastic, myofibroblastic and fibrocytic, respectively.

Proliferative phase nodules

Ultrastructurally, the nodules are composed of large myofibroblasts with numerous long cytoplasmic extensions, joined by various gap junctions and relatively undifferentiated junctions (Fig. 4.1a–c). Their plasma membrane reveals focal deposition of basal lamina, some plasmalemmal attachment plaques and pinocytotic vesicles, as well as cell to stroma attachment sites in the form of fibronexus (Singer 1979; Fig. 4.1d). The cytoplasm reveals a well developed granular endoplasmic reticulum and Golgi apparatus and a number of bundles of microfilaments with dense bodies, often oriented parallel to the long axis of the cells. The nucleus is typically indented and often shows one or several nuclear bodies. Among these typical myofibroblasts, a few smaller cells are observed, corresponding to fibroblasts with a well developed granular endoplasmic reticulum and smooth contoured nucleus but lacking bundles of cytoplasmic microfilaments with dense bodies. The extracellular matrix in this phase contains a few mature collagen fibres (64 nm periodicity) ad-

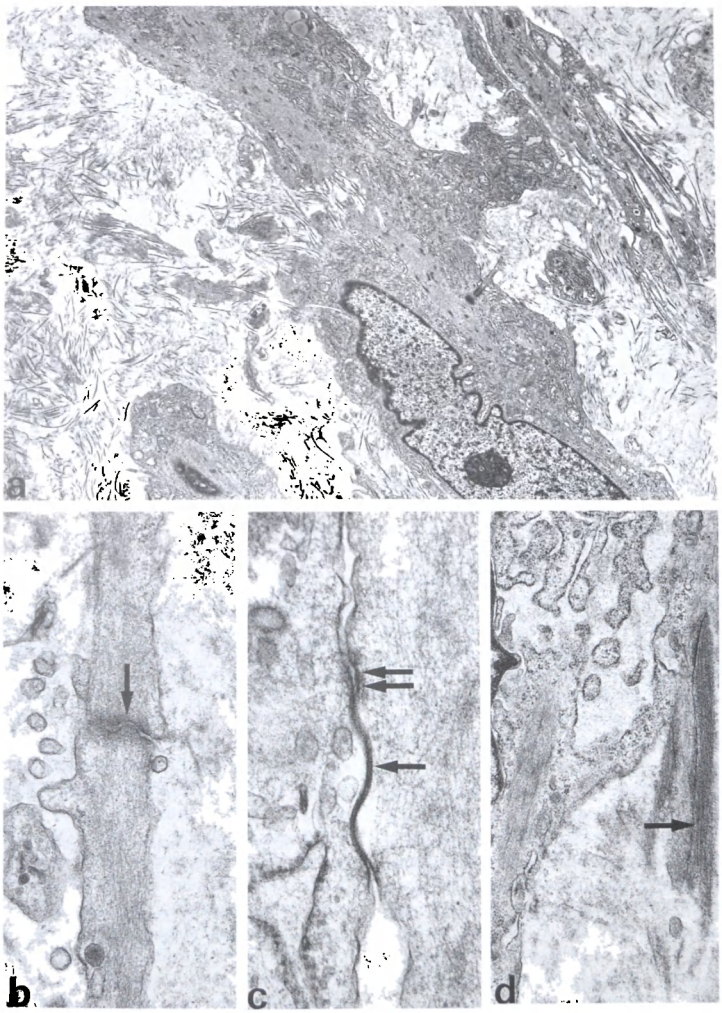

Fig. 4.1 Transmission electron micrographs of proliferative phase nodules. **a** Large typical myofibroblast with numerous cytoplasmic extensions, well developed granular endoplasmic reticulum, cytoplasmic bundles of microfilaments, oriented parallel to the long axis of the cell, cilium and indented nucleus. The extracellular matrix contains few mature collagen fibres. **b** Poorly differentiated junction between cytoplasmic extensions of two myofibroblasts (arrow). **c** Gap junction between two myofibroblasts (arrow) followed by poorly differentiated junction (double arrow). **d** Microtendon (fibronexus) establishing cell to stroma contact (arrow). Uranyl acetate and lead citrate (**a** × 4200; **b** × 30 000; **c** × 60 000; **d** × 20 000).

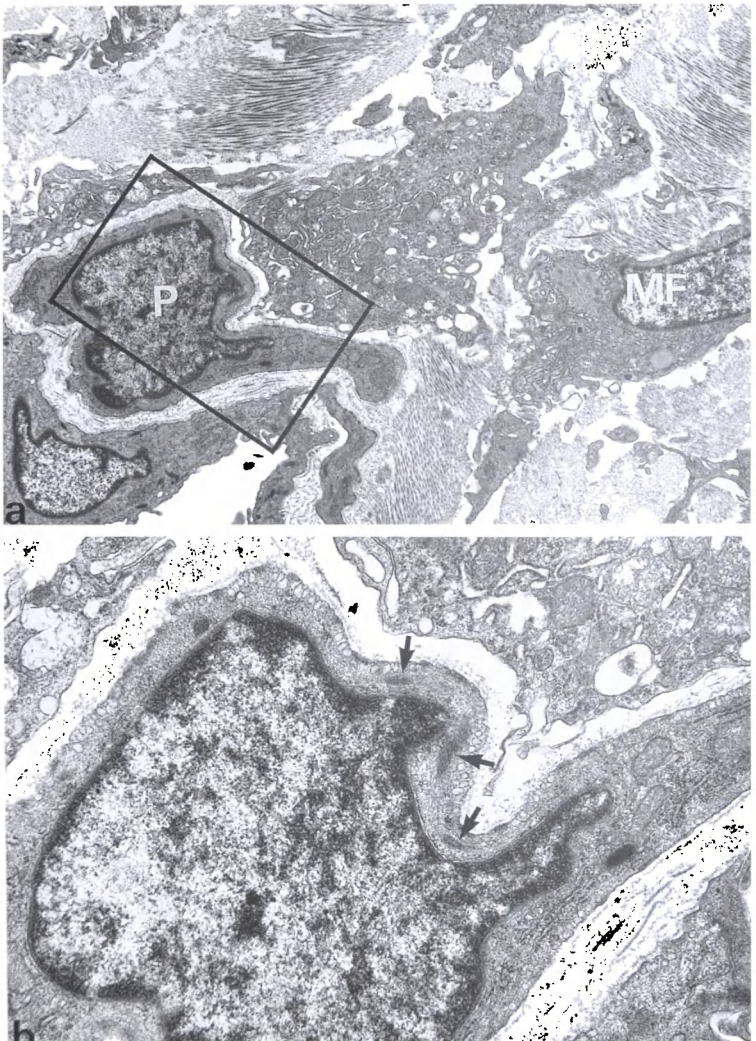

Fig. 4.2 a Transmission electron micrograph illustrating capillary of proliferative phase nodule with large pericyte (P) enveloped by a continuous basal lamina and in the close vicinity of a large myofibroblast (MF) revealing aggregate of microfilaments with dense bodies. b Detail of pericyte illustrated in a showing bundles of microfilaments with dense bodies (arrows).

mixed with granular, non-distinct fibrillar and basal lamina-like material (Fig. 4.1a). The capillaries reveal large prominent pericytes often displaying bundles or aggregates of cytoplasmic microfilaments with irregular densities. These large pericytes are often in the close vicinity of typical myofibroblasts (Fig. 4.2).

Involutional phase nodules

These regions also feature high cellularity but individual cells are smaller than in proliferative phase nodules and tend to be aligned in the same direction (Fig. 4.3). Ultrastructurally, these nodules are essentially composed of myofibroblasts which are also connected by gap junctions and poorly differentiated junctions. Intercellular junctions, however, seem to be less numerous than in proliferative phase nodules. The most striking difference compared to proliferative phase nodules is the amount of collagen. Individual myofibroblasts are enveloped by thick bundles of mature collagen fibres (Fig. 4.4). The myofibroblasts are somewhat smaller than in proliferative nodules and their cytoplasmic extensions are less numerous and appear shorter. Otherwise, the myofibroblasts composing involutional phase nodules are essentially similar to those observed in proliferative nodules and display indented nuclei (Fig. 4.4a). Capillaries are numerous and reveal prominent large pericytes.

Residual phase nodules

Such regions are hypocellular and the slender and aligned cells are surrounded by thick bands of collagen, giving them a tendon-like appearance on semi-thin sections (Fig. 4.3). Ultrastructurally, they are composed of mature fibroblasts (fibrocytes), some containing small aggregates of cytoplasmic microfilaments. The fibrocytes are connected by a few poorly differentiated junctions. Gap junctions, however, are no longer observed.

The slender fibrocytes also show smooth contoured nuclei and are embedded in a dense collagenous matrix formed by thick bands of mature collagen fibres (Fig. 4.5). The poorly vascularized nodules contain small pericytes with little cytoplasm devoid of a well developed microfilamentous apparatus with irregular densities, as observed in proliferative and involutional phase nodules (Fig. 4.6).

In summary, significant ultrastructural differences exist between proliferative, involutional and residual phase nodules in DD in relation to the cells, intercellular junctions, extracellular matrix and capillaries. In the proliferative phase, nodules are composed of typical large myofibroblasts connected by numerous poorly differentiated junctions as well as gap junctions, the latter considered as low resistance pathways for intercellular communication (Gabbiani et al 1978). The extracellular matrix is scant, but capillaries are numerous and feature large prominent pericytes displaying smooth muscle features. The involutional phase nodules, still composed of typical myofibroblasts, reveal fewer gap junctions and poorly differentiated junctions and the extracellular matrix is more abundant and composed of large amounts of mature collagen. Capillaries are numerous and reveal prominent large pericytes. In the residual phase, nodules are composed of slender fibrocytes embedded in a dense collagenous matrix. Gap junctions are not observed and fibrocytes are connected by occasional poorly differentiated junctions. Capillaries, few in number, show small inconspicuous pericytes devoid of a well developed microfilamentous apparatus.

CYTOSKELETAL COMPOSITION OF MYOFIBROBLASTS IN NON-TUMOURAL CONDITIONS

Intense efforts have been directed to establish whether myofibroblasts are derived from smooth muscle cells or fibroblasts. The resemblance in the organization of actin filament between myofibroblasts and smooth muscle cells obviously does not establish that myofibroblasts are of smooth muscle origin. Indeed, cultured fibroblasts acquire ultrastructural features which closely resemble that of myofibroblasts (Buckley & Porter 1967; Gabbiani et al 1973; Bellows et al 1982); moreover, other cell types, such as endothelial cells, develop under various circumstances bundles of actin filaments similar to those observed in

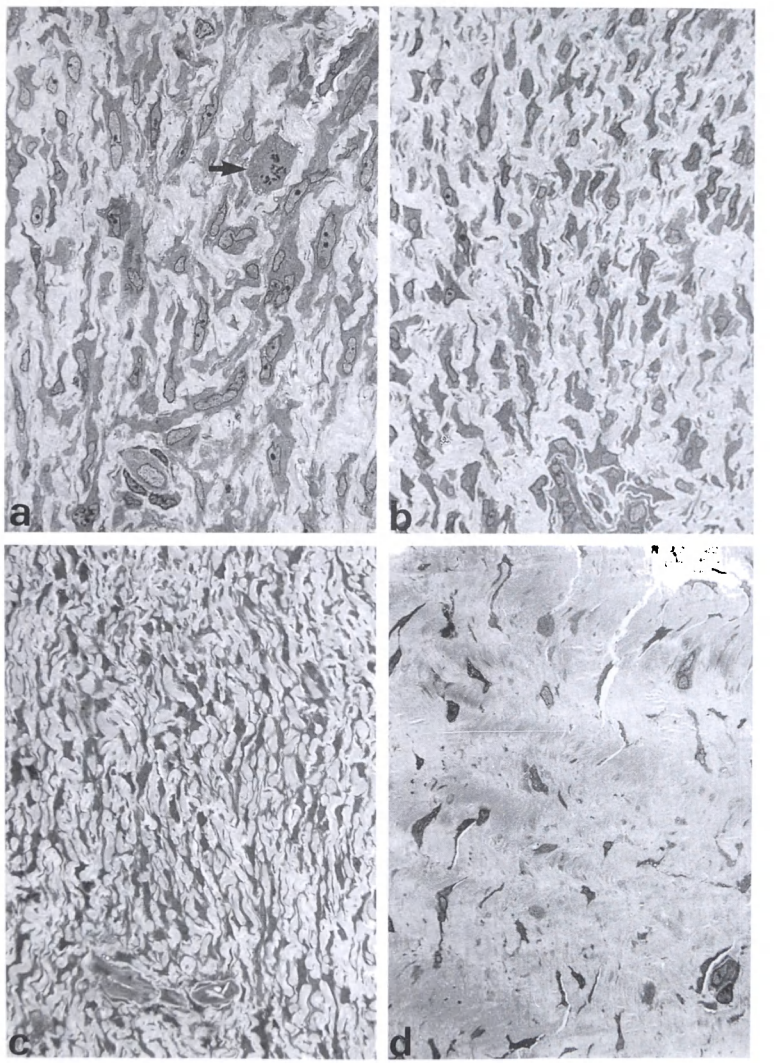

Fig. 4.3 Toluidine blue stained semi-thin sections. a Proliferative phase nodule illustrating large elongated cells with numerous cytoplasmic extensions and indented nuclei, some of them in division (arrow). b and c Involutional phase nodule composed of aligned spindle cells which display fewer and shorter cytoplasmic extensions than in a and which are also smaller in size. d Residual phase nodule showing slender spindle cells in a poorly vascularized and intensely collagenous matrix (× 500).

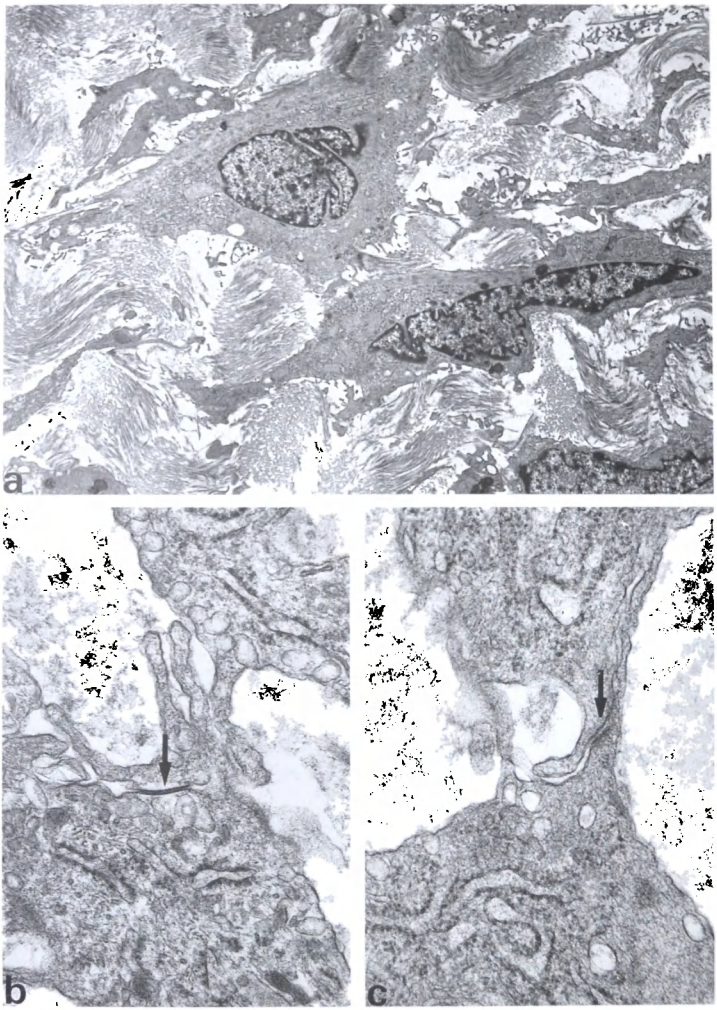

Fig. 4.4 Transmission electron micrographs from involutional phase nodule. a Two typical myofibroblasts with cytoplasmic extensions, bundles of cytoplasmic microfilaments, indented nuclei, surrounded by bands of mature collagen fibres. b Gap junction between two myofibroblasts (arrow). c Poorly differentiated junction connecting two myofibroblasts (arrow). Uranyl and lead citrate (a × 4200; b × 28 000; c × 42 000).

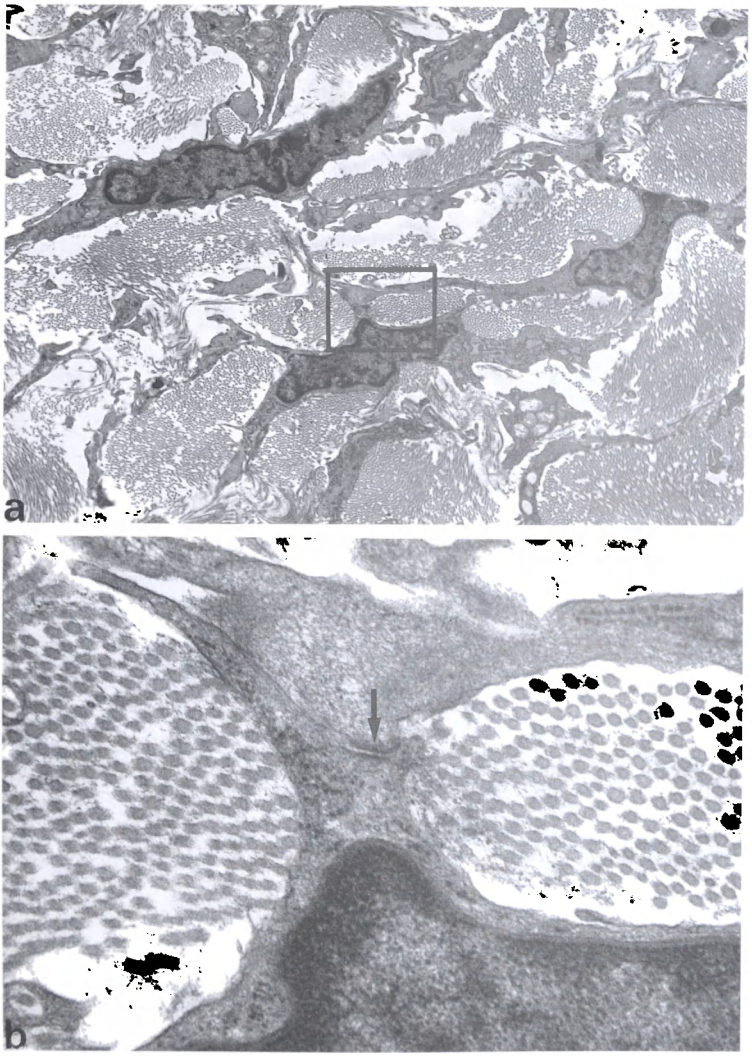

Fig. 4.5 Transmission electron micrographs from residual phase nodule illustrating a slender fibrocytes with smooth contoured nuclei embedded in a dense collageneous matrix, and b joined by few poorly differentiated junctions (arrow). Uranyl acetate and lead citrate (a × 6300; b × 48 000).

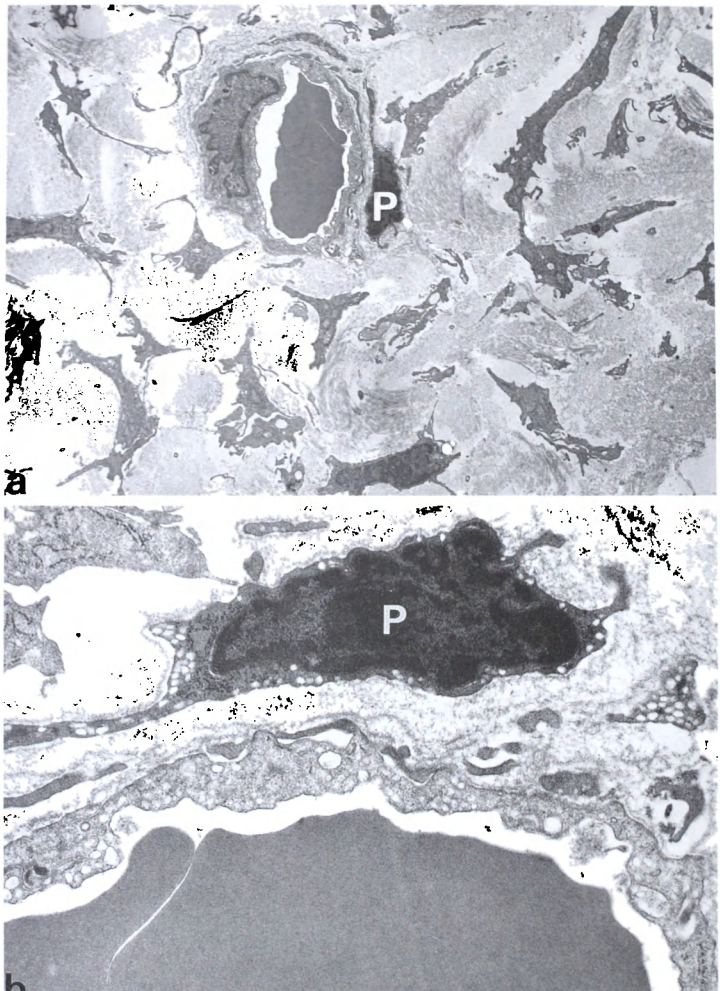

Fig. 4.6 Transmission electron micrographs from residual phase nodule illustrating: a small capillary with pericyte (P); **b** this pericyte is small with little cytoplasm and is devoid of bundles of microfilaments. For comparison, see Figure 4.2b. Uranyl acetate and lead citrate (**a** × 4200; **b** × 14 000).

myofibroblasts (Giacomelli et al 1970; Gabbiani et al 1979, 1983, White et al 1983; Wong et al 1983; Kocher et al 1985). On the other hand, proliferating smooth muscle cells from different pathological conditions and in vitro exhibit ultrastuctural features very similar to those of myofibroblasts (Poole et al 1971; Chamley-Campbell et al 1979; Olivetti et al 1980; Kocher et al 1984; 1985). From these considerations, it can be concluded that ultrastructural studies do not furnish any proof about the cellular derivation of myofibroblasts. Different authors have thus attempted to address this question by examining the cytoskeletal composition of myofibroblasts. Cytoskeletal proteins are of particular interest for this purpose since they display multiple variants that are encoded by multigene families or are the result of differential mRNA splicing (Caplan et al 1983). Intermediate filament proteins are good markers of cellular origin since they have a tissue-specific distribution which is retained during neoplastic conditions (Osborn & Weber 1983; Rungger-Brändle & Gabbiani 1983). Other cytoskeletal proteins, such as myosin isoforms also have a tissue-specific distribution but their expression changes promptly and reversibly in response to many physiological and pathological stimuli; such proteins are useful to evaluate the degree of cellular differentiation.

The determination of the intermediate filament protein content of myofibroblasts is of limited value for solving the riddle of their origin. Since myofibroblasts are of mesenchymal lineage (MacDonald 1959; Grillo 1963; Ross et al 1970) they may express vimentin and/or desmin. Vimentin is the unique protein subunit composing the intermediate filaments of most non-muscle mesenchymal cells such as fibroblasts, histiocytes, endothelial cells and white blood cells; desmin is the unique protein subunit composing the intermediate filaments of striated muscle cells and parenchymal smooth muscle cells. The two limitations for the use of these proteins as markers of myofibroblast origin are:

1. A large proportion of vascular smooth muscle cells express vimentin only, while others express vimentin and desmin (Berner et al 1981; Frank & Warren 1981; Gabbiani et al 1981; Schmid et al 1982).
2. Desmin has been increasingly found in an number of non-muscle mesenchymal cells such as endothelial cells (Fujimoto & Singer 1986; Toccanier-Pelte et al 1987), podocytes (Stamenkovic et al 1986) and stromal cells from various locations (Glasser & Julian 1986; Skalli et al 1986a; Franke & Moll 1987; Toccanier-Pelte et al 1987).

Other cytoskeletal proteins which may provide useful information about the histogenesis of myofibroblasts are actin isoforms, since the six actin isoforms expressed in mammals show a tissue-specific distribution (Vandekerckhove & Weber 1978a, 1978b, 1981). Thus, striated muscle cells coexpress various proportions of alpha-skeletal and alpha-cardiac actin, whereas smooth muscle cells coexpress various proportions of alpha- and gamma-smooth muscle actin. The two other actin isoforms are called beta- and gamma-cytoplasmic and are found in the cytoplasm of every cell. Alpha, beta and gamma refer to the electrophoretic mobility of these isoforms, when separated by isoelectric focusing or two-dimensional gel electrophoresis (Fig. 4.7). Further separation of the three isoforms with an alpha-electrophoretic mobility and of the two with a gamma-electrophoretic mobility requires electrophoretic analysis of the NH_2-terminal peptide (Vandekerckhove & Weber 1981). Two-dimensional gel electrophoresis of the actin isoform pattern of different mesenchymal tissues demonstrated that the presence of an alpha-actin spot allows the distinction between smooth muscle and fibroblastic tissues (Skalli et al 1987). This was confirmed at the histological level with a monoclonal antibody raised against the NH_2-terminal decapeptide of alpha-smooth muscle actin which recognizes exclusively this isoform (Skalli et al 1986a); this antibody did not stain normal fibroblasts but constantly decorated smooth muscle cells. Examination of actin isoform expression in various soft tissue tumours by means of immunofluorescence and two-dimensional gel electrophoresis showed that alpha-smooth muscle actin was present in well differentiated smooth muscle tumours, including leiomyomas and well differentiated leiomyosarcomas, but absent in poorly differentiated leiomyosarcomas (Schürch et

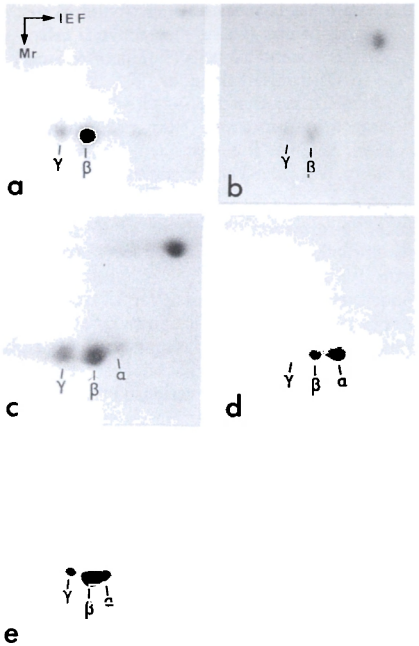

Fig. 4.7 Two-dimensional gel electrophoresis of actin isoforms in a dermis, b normal scar c, d hypertrophic scars and e palmar fibromatosis (e). Dermis and normal scar contain beta- and gamma-actins, whereas hypertrophic scars and palmar fibromatosis contain alpha-, beta- and gamma-actins. Note the alpha-spot is prominent in the hypertrophic scar occurring at the site of a smallpox vaccination (d). IEF, Mr, and the corresponding arrows indicate the direction of migration of isoelectric focusing and second dimension, respectively. Reproduced from Skalli et al (1989a), with permission.

al 1987). In benign smooth muscle proliferations alpha-smooth muscle actin was always present but at levels lower than in quiescent smooth muscle cells (Schürch et al 1987; Skalli et al 1987). This was also shown in human and experimental atheroma (Gabbiani et al 1984; Kocher et al 1984; Kocher & Gabbiani 1986) and for cultured aortic smooth muscle cells (Gabbiani et al 1984; Owens et al 1986; Skalli et al 1986b). Thus, the presence of alpha-smooth muscle actin in a cell can be taken as a good indication of a smooth muscle origin, as far as benign mesenchymal proliferations (such as those involving myofibroblasts) are concerned, and might indicate the state of differentiation in smooth muscle neoplasms.

Like actin, myosin is a ubiquitous cytoskeletal protein displaying a tissue-specific micro-heterogeneity (for review see Bandman 1985) which makes it potentially useful for the determination of myofibroblast origin. Smooth muscle cells express two myosin heavy chains, not normally found in other muscle or non-muscle cell types (Beckers-Bleukx & Maréchal 1985; Rovner et al 1986a; Kawamoto & Adelstein 1987). However, in proliferating smooth muscle cells the expression of smooth muscle myosin is replaced by that of non-muscle myosin, as has been established for benign and malignant smooth muscle proliferations (Donner et al 1983) and for cultured aortic smooth muscle cells (Chamley et al 1977; Larson et al 1984; Rovner et al 1986b; Kawamoto & Adelstein 1987; Benzonana et al 1988). Interestingly, in confluent aortic smooth muscle cell cultures, smooth muscle myosin is re-expressed (Chamley et al 1977, Larson et al 1984), indicating that the expression of myosin isoforms can be reversibly altered in these cells. Thus, myosin isoforms appear more useful for evaluating the degree of smooth muscle differentiation of cells or tissues than for determining their origin.

The determination of the cytoskeletal composition of myofibroblasts led some authors to postulate a fibroblastic origin whereas others favoured a smooth muscle derivation. Granulation tissue myofibroblasts were proposed to be of fibroblastic derivation since they are stained by vimentin and not by desmin antibodies (Skalli & Gabbiani 1988) and because two-dimensional gel electrophoresis failed to reveal the presence of an alpha-actin spot (Skalli et al 1987); (Fig. 4.7). A similar conclusion was reached for myofibroblasts from the stroma of invasive breast carcinoma (Shürch et al 1984) and pulmonary fibrosis (Woodcock-Mitchell et al 1984). However, myofibroblasts from these conditions reacted with a monoclonal antibody specific for alpha-smooth muscle actin, showing that they have smooth muscle cytoskeletal features (Skalli et al 1986a; Sappino et al 1988; Mitchell et al in press)

A positive reaction for desmin antibodies also suggested a smooth muscle origin for myofibroblasts of DD (Shum & McFarlane 1988) and infantile myofibromatosis (Fletcher et al 1987). Interestingly, desmin positivity was not found in all cases examined (Schürch et al 1984; Fletcher et al 1987). Staining with an antibody recognizing the four muscle actin isoforms further suggested a smooth muscle derivation for myofibroblasts from DD (Tsukuda et al 1987) and for inclusion body fibromatosis of adulthood (Viale et al 1988). Taken together, these studies suggested that myofibroblasts from different tissues disclose cytoskeletal heterogeneity. This point was systematically investigated by double immunofluorescence with antibodies against vimentin, desmin, alpha-smooth muscle and alpha-sarcomeric actin in diverse settings where myofibroblasts are present (Skalli et al 1989a). By this means it was possible to define four cytoskeletal phenotypes among myofibroblasts (Skalli et al 1989a):

1. Phenotype V, represented by myofibroblasts positive for vimentin only.
2. Phenotype VAD, represented by myofibroblasts positive for vimentin, alpha-smooth muscle actin and desmin.
3. Phenotype VA, represented by myofibroblasts positive for vimentin and alpha-smooth muscle actin.
4. Phenotype VD, represented by myofibroblasts positive for vimentin and desmin.

Myofibroblasts from all conditions examined were negative for alpha-sarcomeric actin. The recognition of these different cytoskeletal phenotypes allowed the distinction of two kinds of myofibroblastic proliferations: the first contains only V-cells and comprises normally healing granulation tissue, eschars and normally healed scars; the second embodies hypertrophic scars and fibromatoses, including Dupuytren's. In these settings V-cells were mixed with various proportions of cells expressing cytoskeletal markers of myogenic differentiation, i.e. VAD-, VA- and VD-cells. In most Dupuytren's nodules and other fibromatoses the number of VA-cells exceeded the number of VAD-cells (Fig. 4.8).

The tissue distribution of VA- and VAD-cells was homogeneous in some cases but in others it was focal. Whether these differences in the regional distribution of cells with various phenotypes affect the course of DD is unknown. Within the same nodule, cells with cytoskeletal smooth muscle features were present within the proliferative, cellular regions, and their number decreased progressively at the interface between these areas and sclerotic areas (Shum & McFarlane 1988; Skalli et al 1989a). The same observation was made for musculoaponeurotic fibromatoses (Skalli et al 1989a). Despite their heterogeneity in intermediate filament proteins and actin isoforms, myofibroblasts from different settings, including granulation tissue (Benzonana et al 1988; Eddy et al 1988), Dupuytren's nodules (Tomasek et al 1986; Benzonana et al 1986), and hypertrophic scars (Eddy et al 1988) express only non-muscle myosin. In these tissues, the extracellular matrix around myofibroblasts is strongly stained for anti-fibronectin but not for anti-laminin (Tomasek et al 1986; Eddy et al 1988).

The heterogeneous cytoskeletal composition of myofibroblasts raises questions as to their origin. Ultrastructural data provide evidence that during pathological and/or culture conditions fibroblasts and smooth muscle cells acquire morphological features similar to those of myofibroblasts (Moss & Benditt 1970; Poole et al 1971; Chamley-Campbell et al 1979, Olivetti et al 1980; Kocher et al 1984, 1985; Mosse et al 1985), thereby suggesting that both cell types may be the progenitor of myofibroblasts. The heterogeneous cytoskeletal composition of myofibroblasts may be in agreement with that theory since V-cells could be derived from fibroblasts, and VAD- and VA-cells could be derived from smooth muscle cells and/or pericytes, since these latter cells have cytoskeletal features similar to smooth muscle cells (Joyce et al 1984; Herman & D'Amore 1985; Fujimoto & Singer 1987, Toccanier-Pelte et al 1987; Skalli et al 1989b).

A vascular origin of myofibroblasts was also proposed on the basis of morphological observations, suggesting that desmin-positive cells were migrating from vessel walls to the tissue (Shum & McFarlane 1988); this suggestion is consistent with earlier studies showing an intimate relationship be-

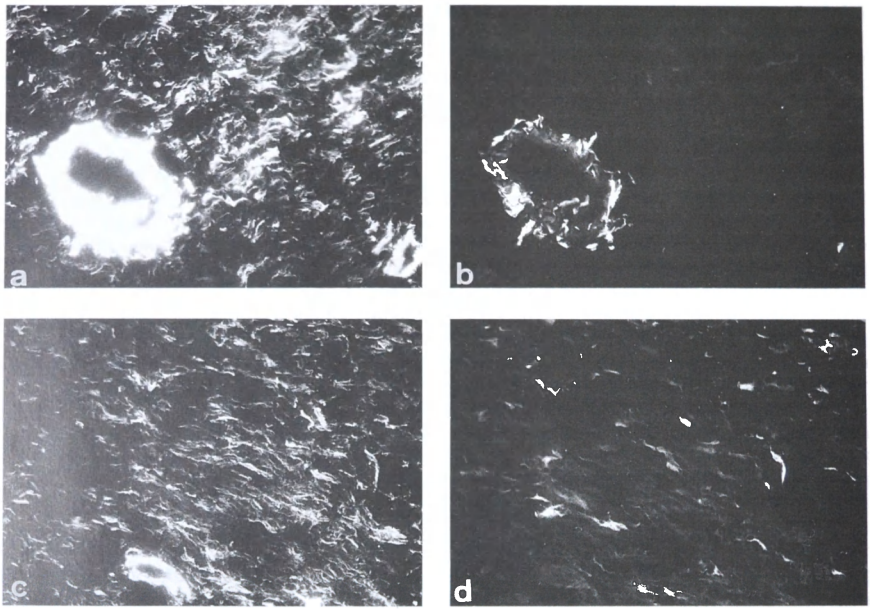

Fig. 4.8 Double immunofluorescent staining of a Dupuytren's nodule with anti-alpha-smooth muscle actin (a,c) and anti-desmin (b,d) disclosing areas of cells with different cytoskeletal phenotypes. a and c: area where many cells are positive for anti-alpha-smooth muscle actin and negative for anti-desmin; c and d: area where cells positive for anti-alpha-smooth muscle actin and anti-desmin are admixed with cells positive only for anti-alpha-smooth muscle actin (× 400).

tween proliferating cells of DD and blood vessels (Janssen 1902; Larsen & Posch 1958). One possible origin of VD-cells is the stromal cells positive for anti-desmin and negative for alpha-smooth muscle actin that are present in the stroma of various organs (Glasser & Julian 1986; Skalli et al 1986a; Franke & Moll 1987; Toccanier-Pelte et al 1987). The negative staining of DD myofibroblasts for a monoclonal antibody specific of alpha-sarcomeric actin (Skalli et al 1986a) strongly argues against an early theory which proposed that Dupuytren's nodules arise from residues of embryonic muscle in the palm (Krogius 1920; Stein et al 1960) or from local striated muscle (MacCallen & Hueston 1962).

In addition to an origin from different cell types, the cytoskeletal heterogeneity of myofibroblasts may also be accounted for by two alternative explanations. First, myofibroblasts may stem from fibroblasts which are induced to express smooth muscle cytoskeletal proteins upon some yet unknown stimuli. Secondly, myofibroblasts may originate from smooth muscle cells and/or pericytes, which in some instances, would 'switch off' the expression of alpha-smooth muscle actin and/or desmin. From these considerations it is clear that the data presently available on the cytoskeletal composition of myofibroblast do not allow a definitive conclusion of their cellular derivation; however, they demonstrate that subsets of myofibroblasts possess smooth muscle phenotypic features, as shown by their expression of alpha-smooth muscle and/or desmin. Interestingly, this smooth muscle differentiation is never fully expressed since smooth muscle myosin and laminin are not expressed by myofibroblasts from various

tissues (Tomasek et al 1986; Benzonana et al 1988; Eddy et al 1988).

PATHOGENESIS OF DD

The pathogenesis of DD remains obscure. The finding that the major cellular component involved in this disease is also found in granulation tissue and tumour stroma is of interest for this issue. Indeed, at the cellular level, a number of similarities exists between the process of wound healing, certain quasineoplastic proliferative conditions, especially fibromatoses, and the desmoplastic stromal reaction to invasive carcinomas (Schürch et al 1981). In such a conceptualization, myofibroblast induction may be viewed as a fundamental pathobiological process which, at one end of the spectrum, effects the closure of wounds or, in a pathologically excessive reparative reaction, is responsible for the reaction of the palmar fascia in DD. At the other extreme it represents a cellular response to invasive neoplasia (Seemayer et al 1982). Myofibroblasts possess not only contractile forces, but also synthetic properties for type III collagen (Gabbiani et al 1976). In granulation tissue of healing wounds myofibroblasts are especially numerous and the collagen produced is principally type III. When granulation tissue is resorbed following wound closure, myofibroblasts disappear (Rudolph et al 1977) and the more rigid type I collagen is identified (Gabbiani et al 1976). In like fashion, the proliferative cellular phase of DD is characterized by numerous myofibroblasts and contains a predominance of type III collagen, whereas in the residual fibrocytic phase, type I collagen predominates (Meister et al 1979). Finally, types pro-III and III collagen are present in increased amounts in the 'young' oedematous mesenchyme (Lagacé et al 1985), areas laden with myofibroblasts, corresponding to zones of early stromal invasion of breast carcinomas (Schürch et al 1981, 1982); contrariwise, type I collagen is most prominent within the central sclerotic zone of breast carcinomas (Lagacé et al 1985), areas where myofibroblasts are replaced by fibroblasts (Schürch et al 1981, 1982).

Substantial evidence has been provided about analogies between wound healing and the generation of tumour stroma at the level of spillage of plasma proteins including fibrinogen, fibronectin and plasminogen (Dvorak 1986). These plasma proteins form an extravascular clot serving as provisional stroma which provides a matrix for the immigration of fibroblasts, macrophages and new capillaries, i.e. granulation tissue, which is eventually resorbed and undergoes retraction and scarring.

Some important topographical differences, however, exist between the desmoplastic stromal reaction in invasive carcinomas and the resorption of granulation tissue. The organization of granulation tissue during would healing is centripetal, whereas in carcinomatous invasion, the stroma is organized from within (point of departure of the carcinoma) outward and amidst the neoplastic elements. Organization in the desmoplastic stromal reaction of invasive carcinomas is therefore centrifugal and might limit (in theory) neoplastic extension and access of neoplastic cell to lymphatic and vascular channels, especially when the stromal reaction is precocious, that is, myofibroblasts precede the carcinoma cells by some distance into the adjacent tissue (Schürch et al 1982, Seemayer et al 1982).

The organization of granulation tissue and probably, Dupuytren's nodules is centripetal. The maturation of the principal cellular elements proceeds the opposite way, — blastic cells (myofibroblasts) are in the poorly collagenized centre and mature fibroblasts (fibrocytes) in the intensely collagenized periphery.

Although a number of similarities exists between the process of wound healing, quasineoplastic proliferative conditions and the desmoplastic stromal reaction of invasive carcinomas at the cellular and extracellular level, several fundamental differences exist concerning the maturation of the cellular elements. Myofibroblasts, during normal wound healing, disappear (Rudolph et al 1977), whereas in the stromal reaction to invasive carcinomas they persist. Thus, the stroma of invasive carcinomas resembles the non-healing wound (Dvorak 1986). In fibromatoses and also in DD myofibroblasts seem to persist more than in granulation tissue, therefore these quasineoplastic proliferations probably occupy a position intermediate between

granulation tissue in normal wound healing and the desmoplastic stromal reaction of invasive carcinomas.

It has been suggested long ago that DD may be related to the repair of minor trauma of the palmar aponeurosis. This hypothesis has gained some support from experiments on monkeys showing that a pathological response similar to that of DD was induced by stretching the palmar fascia (Larsen et al 1960). Moreover, the proliferative nodule of DD often contains small foci of perivascular haemorrhage and haemosiderin deposition accompanied by the accumulation of macrophages and lymphocytes (Iwasaki et al 1984). Although these findings represent weak evidence for trauma as a cause of DD, in humans the presence of macrophages in this disease is of interest, in view of the central role played by these cells in wound healing and in the stimulation of fibroblast and smooth muscle cell proliferation (for review see Riches 1988).

Experimental evidence for DD as a benign tumour of mesenchymal origin (Enzinger et al 1970) has come from culture of cells derived from nodules and palmar aponeurosis of patients affected by DD (Azzarone et al 1983). The growth properties of Dupuytren's nodule myofibroblasts were intermediate between those of normal fibroblasts and embryonic or virus-transformed fibroblasts.

As with normal cells, Dupuytren's nodule myofibroblasts displayed contact inhibition at the plateau phase and had a limited life span. But, like transformed cells, Dupuytren's nodule myofibroblasts formed colonies in soft agar, grew in the presence of reduced amounts of fetal calf serum and secreted high levels of the urokinase-like species of plasminogen activator. Karyotypic abnormalities were also found in these cells (Bowser-Riley et al 1975; Azzarone et al 1983; Sergovich et al 1983). Interestingly, cultures derived from the apparently normal palmar aponeurosis of patients affected by DD showed some, but not all, of the abnormal properties of cells derived from nodules. In situ, myofibroblasts and type III collagen have been found in the 'normal' palmar aponeurosis of patients affected by DD (Bazin et al 1980), suggesting that the syndrome is not strictly focal. These findings may provide a possible explanation for the local recurrences observed in DD.

The concept of a neoplastic transformation of Dupuytren's myofibroblasts is not incompatible with trauma as an aetiological factor. It could be hypothesized that trauma occurring in the palmar aponeurosis will heal normally in most instances but that in some cases the myofibroblasts involved in repair become transformed. Diseases associated with DD and/or certain hereditary settings could explain why this transformation occurs only in certain cases.

CONCLUSIONS

Ultrastructural, immunohistochemical, biochemical and cell culture experiments have revealed important information about the cellular biology of DD. These studies have established that the main cell type present within Dupuytren's nodules has contractile and synthetic features similar to those of myofibroblasts present in granulation tissue, where they are thought to generate contractile forces responsible for wound closure. Thus, myofibroblasts present within Dupuytren's nodules might also be responsible for the contractile events observed in this disease.

These observations reveal a number of analogies between the process of wound healing, Dupuytren's nodules and the generation of tumour stroma. These analogies concern primarily the formation of a vascularized 'young' mesenchyme rich in type III collagen, in which the myofibroblast represents the predominant cell. The analogies and discrepancies between wound healing, DD and tumour stroma formation are to a large extent unexplored, but represent an attractive area of research. Their detailed analysis could well encourage the development of new therapeutic strategies, modulating stroma formation to limit neoplastic growth.

Immunohistochemical studies have shown that Dupuytren's nodules always contain a subset of myofibroblasts exhibiting smooth muscle cytoskeletal markers — alpha-smooth muscle actin and/or desmin. Moreover, within the same nodules, cells with cytoskeletal smooth muscle features revealed zonal differences. They were

present within the cellular centre of proliferative nodules and decreased progressively at the interphase between these areas and the sclerotic zones. The presence of myofibroblasts in Dupuytren's nodules reinforces the notion that these cells are responsible for the contractile events in DD but does not provide any conclusive answer about their origin. Ultrastructural examination of Dupuytren's nodules reveals differences in proliferative, involutional and residual phase nodules concerning the cells, intercellular junctions, capillary pericytes and extracellular matrix. While myofibroblasts progressively disappear during evolution of the nodules, they are replaced by fibrocytes. The large and prominent pericytes of proliferative phase nodules, displaying smooth muscle features, are replaced by small inconspicuous pericytes in the poorly vascularized residual nodules. At the same time, gap junctions between myofibroblasts of proliferative and involutional phase nodules also disappear, and fibrocytes of residual nodules are connected by a few poorly differentiated junctions. Gap junctions are considered as low resistance pathways for intercellular communication. Parallel to these cellular events, a progressive collagenization takes place and the nodules are transformed into a scar-like tissue.

Ultrastructural examination of Dupuytren's nodules provides no information concerning the origin of myofibroblasts, even though morphological similarities exist between them and pericytes. Since Dupuytren's nodules are composed of cells with similar ultrastructural features but different cytoskeletal composition, the question has to be raised how these cells can be present within the same tissue. The phenotypic differences of myofibroblasts could be explained by the concept of isoformic transitions according to differences in cell function. For Caplan et al (1983), the concept of cellular isoforms is defined as 'the replacement of individual molecules and cells by molecular and cellular variants called isoforms because they are both similar and distinctly different and arise during embryonic development and later life'. According to this concept, the phenotypic modulation of myofibroblasts in DD might be viewed as isoformic transitions of a common ancestor cell which is still not defined. Future effort should be directed to determine which factors regulate the expression of smooth muscle proteins in myofibroblasts of DD and other pathological settings composed of myofibroblasts.

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