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# AN INVESTIGATION INTO THE ROLE OF INFLAMMATORY CELLS IN DUPUYTREN'S DISEASE

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**An immunohistochemical study was performed on nodules excised from the palmar fascia of patients with Dupuytren's contracture. In cellular nodules, antibodies to actin (used as a marker for myofibroblasts), desmin, vimentin, Mac 387 (a macrophage marker) and leucocyte common antigen were used. A correlation was demonstrated between the numbers of macrophages and the presence of myofibroblasts. The presence of myofibroblasts is generally considered to indicate the active stage of the disease. Inflammatory cells other than macrophages were largely absent from the nodules, although lymphocytes were frequent in the tissue around the nodules. Microvascular changes were prominent in the nodules and pericyte proliferation was observed around occluded capillaries. Release of growth factors from macrophages may be important in Dupuytren's contracture, as is the case in other fibrotic diseases. The possible role of macrophages in the aetiology of Dupuytren's disease is discussed.**

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The causes and pathogenesis of Dupuytren's disease are a source of continuing fascination to hand surgeons. Luck (1959) described three stages of the condition: proliferative, involucional and residual. MacCallum and Hueston (1962) observed vascular proliferation and perivascular cuffing of small round cells in the first stage of the disease. They thought that, although there was no definite inflammatory process, the general pattern was more in keeping with a local allergic response than any other type of change, and that these initial changes in the prefascial tissues were the "clue to the production of Dupuytren's tissue". Other workers (Nezelof, 1974; Vande Berg et al., 1982) have stated that the "small round cells" are probably macrophages. We report an immunohistochemical study of the role of these cells. This aspect of Dupuytren's disease has not previously been specifically studied.

There has been much recent interest in the vascular changes within Dupuytren's nodules. Kischer and Speer (1984) reported capillary occlusion due to endothelial cell proliferation with basement membrane duplication. It is possible that these changes are important in the pathogenesis of the disease as hypoxia is known to be a stimulus to fibroblasts in tissue culture (Hunt et al., 1985). We also investigated the vascular changes within nodules.

## Material and methods

Material (nodule and cord) was obtained from 40 cases of Dupuytren's disease, operated on for digital contracture and not for painful nodules without contracture. The specimens were fixed in buffered formol saline. The nodules were dissected and sectioned transversely as microvessels lie longitudinally in the cord structure (Kischer and Speer, 1984), so that transverse sectioning demonstrates them to best advantage. H.&E. stained

sections were examined and 14 specimens selected for further study. These were those with high cellularity (> 200 nuclei per high-power field), which suggested that they were in the active stage of the disease. Three specimens included pieces of skin from adjacent to the nodule. The remaining specimens, which varied to some degree in cellularity but which were all mostly fibrous tissue, were discarded.

Sections of the selected specimens were examined under light microscopy. A battery of monoclonal primary antibodies (Table 1) was used for immunohistochemistry. A Fab2 secondary antibody was used as this was found markedly to reduce non-specific background staining. An avidin-biotin/ peroxidase technique of staining was used. Appropriate negative controls were employed. Positive controls were employed for macrophage (Mac387) and Leucocyte Common Antigen (using tonsillar tissue from routine tonsillectomies) and desmin (using myometrium). Sections were also stained with Masson's trichrome and P.A.S. methods as Kischer and Speer (1984) found that these methods demonstrated capillary basement membrane changes more readily than H.&E. staining.

Table 1

Antibody	Significance
Actin	Used as marker for myofibroblasts.
Desmin	Marker for smooth muscle cells.
Vimentin	Present in fibroblasts, smooth muscle.
Mac 387	Surface marker for macrophages.
Leucocyte common antigen	From lymphocytes.

Note: Trypsinisation of sections for Mac387 only (10 minutes)  
: Desmin antibody from I.C.N. Biomedicals, Ohio, U.S.A. All other antibodies, FAB2 secondary antibody and avidin/biotin staining kit from Dako Pats A.G., Glostrup, Denmark.

Actin staining was used as to demonstrate the presence of myofibroblasts. The presence or absence of actin staining was generally clear cut. Internal controls for actin were present on the sections with pericytes and/or arteriolar smooth muscle cells staining intensely for actin in all sections (Fig. 2). Myofibroblasts were judged to be present if actin staining of fibroblast cells was as intense as that of these controls. The number of myofibroblasts present was estimated as a percentage of total fibroblasts, and lesions placed into one of five groups: 0, <20%, <40%, <60%, >60%.

In analysing the results, the number of Mac387 and Leucocyte Common Antigen positive cells was counted. Between six and eight high-power fields were counted. The average number of macrophages and lymphocytes per high-power field was thus estimated. Statistics were performed using Spearman rank correlation with correction for ties.

**Results**

The results are presented in Table 2. Seven nodules contained significant numbers of macrophages (more than one or two on the whole section) as shown in Figure 1. These generally occurred around linear structures, which were thought to be vessels running parallel to the line of the section. Some areas of perivascular cuffing around capillaries were also seen. Skin specimens from three cases were also examined. One of these contained large numbers of macrophages in the vertical collagen fibres attaching the nodule to the dermis. None of the nodules contained significant numbers of lymphocytes, although many sections showed perivascular cuffing of

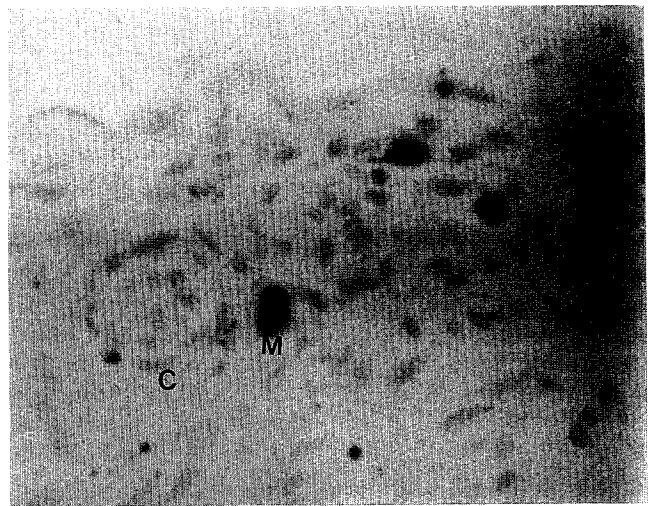


Fig. 1 Macrophages (M) adjacent to occluded capillary (C) (Mac 387, ×400).

lymphocytes around small vessels at the edge of the nodules.

Five sections were considered positive for myofibroblasts (Fig. 2). This proportion of nodules containing myofibroblasts is similar to that found by other workers (Tomasek et al., 1987). A positive correlation was noted between the numbers of macrophages and myofibroblasts in nodules. Spearman rank correlation gave coefficient of correlation  $RS=0.58$ ,  $0.01 < p < 0.05$ .

All the sections showed the expected actin staining in arterioles. More surprising was the number of heavily-staining actin "rings" which were present in all but two

Table 2—Results of sections of nodules

Specimen	Macrophages	Myofibroblasts	Leucocyte Common Antigen	Desmin	Vimentin
1	2	0	—	—	+
2	1	1	—	—	+
3	2	2	—	—	+
4	2	0	—	—	+
5	1	0	—	—	+
6	0	1	—	—	+
7	0	0	—	—	+
8	3	3	—	—	+
9	0	0	—	—	+(weak)
10	0	0	—	—	+
11	0	0	—	—	+
12	0	0	—	—	+(weak)
13	6	4	—	+	+
14	1	0	—	—	+

Numbers of macrophages per high-power field (mean of 8) to nearest whole number. Myofibroblast grouping explained in text. Correlation of macrophages and myofibroblasts: Spearman rank correlation  $RS=0.58$ ,  $0.01 < p < 0.05$



Fig. 2 Occluded capillaries (C) adjacent to area of myofibroblasts (M). The dark staining lines are actin bundles. (Anti-actin  $\times 400$ ).

of the sections. Comparison with the desmin-stained sections indicated that these "rings" were not arterioles as they contained no smooth muscle. Closer examination of the rings showed that they occurred around microvessels which were either completely or partly occluded due to endothelial cell hyperplasia. Apart from these apparently occluded vessels, capillaries and venules were not observed within the nodules.

All the nodules contained vimentin positive fibroblasts. Only one specimen showed desmin-positive cells around microvessels.

### Discussion

The myofibroblast is probably the cell responsible for contracture in Dupuytren's disease (Gokel and Hübner, 1977; Tomasek et al., 1987). The most distinctive feature of the cell is the presence of large numbers of actin bundles in the cytoplasm. Gelberman et al. (1980) found that recurrence following surgery was related to the presence of myofibroblasts in excised tissue. It has been shown that immunofluorescence using an anti-actin antibody is an accurate predictor of the presence of myofibroblasts on electron microscopy (Tomasek et al., 1986). We have demonstrated, using an immunoperoxidase technique, that the presence of cells staining strongly for actin is associated with macrophages in Dupuytren's nodules.

Macrophages have been shown to be important in other fibrotic diseases, such as cirrhosis, fibrosing alveolitis and scleroderma (Leroy, 1985; Rojkind and Valadez, 1985). Why should this be so? Many cells have been found to release small peptides which are extremely potent modulators of cell function. These are known as growth factors or cytokines. The macrophage, in addition to its phagocytic properties, is a prolific source of growth factors. Nathan (1987) lists 19 growth factors amongst

about 100 substances which have been shown to be secreted by macrophages. These growth factors act on many cell types, stimulating chemotaxis, cell proliferation, collagen deposition and cell transformation (i.e. a shift towards neoplastic behaviour).

There is a little independent evidence which indicates that growth factors may be important in Dupuytren's disease. First, Azzarone et al. (1983) found that cells from Dupuytren's nodules were partly transformed and noted a high level of tissue plasminogen activator release from nodule cells. Merlo et al. (1986 and 1987) also noted a high level of tissue plasminogen activator in Dupuytren's nodules and subsequently showed that a high level at primary surgery correlated with recurrence of the disease. It is now recognised that growth factors modulate tissue plasminogen activator levels in many normal tissues (Bunning et al., 1987) and tumours (Laiho, 1988). Barsky and Gopalakrishna (1987) found that myofibroblast proliferation in breast cancer is caused by a growth factor released by the tumour cells.

The number of macrophages seen in sections in our study was rather small and there were few lymphocytes and other inflammatory cells within the nodules. This does not agree with the findings of Jozsa et al. (1988) who, in an electron microscopic study, found that 26% of the cells in nodules were of an "inflammatory nature". It seems unlikely from our results that macrophage-derived growth factors are the sole stimulus to proliferation of fibroblasts in this disease.

We found large numbers of macrophages in one skin specimen out of three. Vande Berg et al. (1982) studied the relationship of skin and nodule systematically, and found that the dermis and subdermis overlying Dupuytren's nodules contain large numbers of macrophages. It is possible that the removal of these cells accounts for the favourable effects of dermofasciectomy and grafting on preventing recurrence of disease (Tonkin et al., 1984).

The second striking feature of our results was the presence of occluded capillaries. Kischer and Speer (1984) observed capillary occlusion due to proliferation of endothelial cells with basement membrane duplication in Dupuytren's nodules. Similar changes had previously been noted in hypertrophic wounds (Kischer et al., 1982). Murrell et al. (1987 and 1989) confirmed these findings and suggested that fibroblast proliferation may be induced by local hypoxia, with associated release of oxygen free radicals. Hypoxia is a stimulus to fibroblasts in tissue culture (Hunt et al., 1985). Kischer and Speer (1984) proposed that the microvascular changes, which have also been noted in hypertrophic wounds and diabetic microangiopathy, may be a common pathway in the development of fibrotic lesions.

The occluded capillaries are surrounded by cells which stain strongly for actin. These cells are almost certainly pericytes which have proliferated. In sections of normal tissues only one pericyte is found around each capillary.

Kischer et al. (1982), in an investigation of hypertrophic scars (which bear a remarkable resemblance to Dupuytren's contracture) found that occluded capillaries are surrounded by several pericytes, which in many cases had all the characteristics of myofibroblasts, including actin bundles.

An hypothesis is suggested which appears to fit most of the known facts (Fig. 3). Perivascular macrophage cuffs occur in the initial stage of the disease (MacCallum and Hueston, 1962). This may follow acute trauma, which is known to be a precipitating cause for this condition (Clarkson, 1961). This stage of the disease is difficult to study as contracture, and hence the availability of surgical specimens, does not usually occur until later. However, the macrophages persist and growth factors are released which cause endothelial proliferation and also local proliferation of fibroblasts. The endothelial proliferation leads to microvascular occlusion. The decreased oxygen supply due to capillary changes is exacerbated by the high metabolic demand of the proliferating cells. As hypoxia is a stimulus to further fibroblast proliferation, a positive feedback loop is entered. As noted by Hunt et al. (1985), "oxygen is carried via vessels at high concentrations, about 100 mmHg. partial pressure, but by the time it reaches the mitochondria of a metabolically active cell . . . the pO<sub>2</sub> is reduced to about 0.5 to 3.0 mmHg. Addition of new cells such as inflammatory cells or injury to normal vasculature, makes major focal changes in the oxygen economy. So a few macrophages . . . could achieve very

low oxygen tensions." The mechanism proposed is essentially that proposed by Murrell et al. (1989), with the important addition that macrophage released growth factors are proposed as the initiating stimulus.

The suggestion that macrophage-released growth factors may be responsible for initiating Dupuytren's disease may indicate ways to investigate the genetic basis of the condition. Many growth factors are associated with so-called oncogenes, which are known to cause cell transformation (Marks, 1987). An abnormal single gene encoding for a growth factor might be enough to cause the development of Dupuytren's lesions. Possible growth factors responsible for Dupuytren's disease include platelet-derived growth factor, transforming growth factor beta and fibroblast growth factor, which is known to be a potent stimulus to angiogenesis and endothelial and fibroblast hyperplasia.

Dupuytren's disease remains a condition with many "mysterious aspects" (Iselin, 1974). However, recent developments in molecular biology have provided methods of investigation which may allow us to uncover the basis, both cellular and genetic, of this disease. Further investigation into the role of inflammatory cells and growth factors would be worthwhile.

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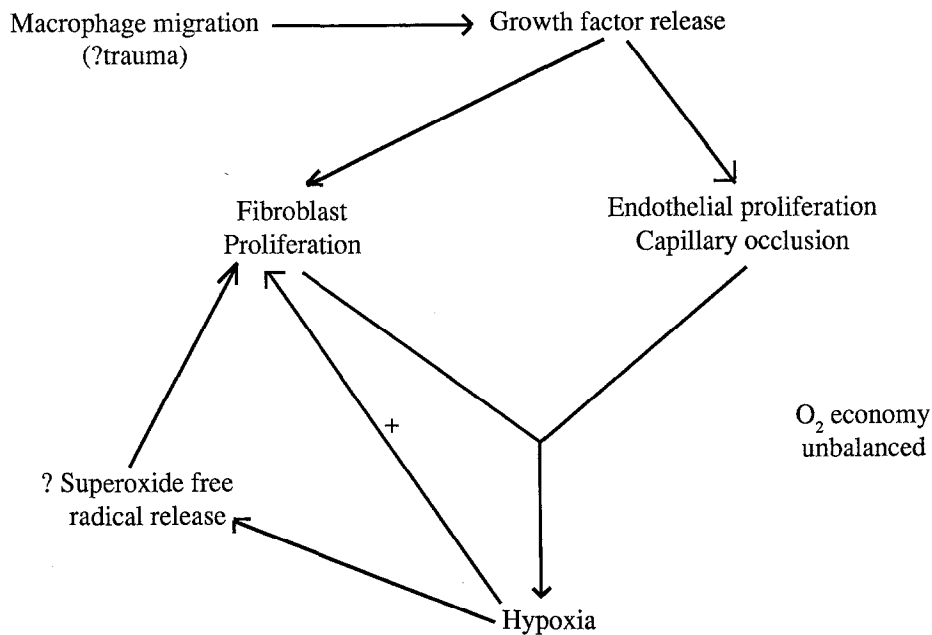


Fig. 3 Suggested pathogenesis of Dupuytren's lesions.

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