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DERMAL DENDROCYTES IN DUPUYTREN'S DISEASE: A LINK BETWEEN THE SKIN AND PATHOGENESIS?

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The skin has previously been implicated in the process of Dupuytren's disease. The dermal dendrocyte is a factor XIIIa positive cell, which has been found in normal skin and some pathological conditions associated with fibrosis. In this study we examined the distribution of factor XIIIa positive cells in and around tissue from Dupuytren's disease. Immunohistochemistry was performed using a panel of antibodies for factor XIIIa, macrophages (CD68 and MAC387) and MHC II. Many factor XIIIa positive dendritic cells were present in and around Dupuytren's tissue; fewer CD68 and MHC II positive cells and very few MAC387 cells were seen. We propose that the factor XIIIa positive cells are dermal dendrocytes. This study may indicate an important link between the skin and pathogenesis of Dupuytren's disease.

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Dupuytren's disease is a common self-limiting fibro-contractile condition affecting the hand. Replacement of palmar skin with full thickness skin grafts is associated with low recurrence rates after surgery (Hueston, 1985), which suggests that the skin may be involved in the pathogenesis of the disease. Despite this, there have been few studies of the skin or skin-associated cells in Dupuytren's disease.

The dermal dendrocyte is a recently described cell that is factor XIIIa positive and is found in normal skin (Cerio et al, 1989a; Headington and Cerio, 1990). This cell is thought to be of macrophage lineage and has been implicated in some fibrotic conditions. Two previous studies of factor XIIIa positive cells have each included one specimen of Dupuytren's disease, with one showing presence of the cells (Ádány et al, 1988) and the other finding no evidence of them (Cerio et al, 1989a).

The pathogenesis of Dupuytren's disease is obscure but previous studies have suggested that macrophages may have a role to play (Vande Berg et al, 1982; Józsa et al, 1988; Andrew et al, 1991). The macrophage is involved in both normal and abnormal fibrotic conditions including wound healing, liver cirrhosis, scleroderma and lung fibrosis, probably due to its ability to produce fibrogenic cytokines (Nathan, 1987; Leroy et al, 1990). Because of this link and the known importance of the skin in Dupuytren's disease, we investigated macrophages and factor XIIIa positive cells in Dupuytren's disease.

MATERIALS AND METHODS

The tissues used in this study were from patients operated on for Dupuytren's disease of the hand. In total there were specimens from 49 patients, of which 12 included fragments of overlying skin. The specimens were fixed in buffered formal saline. Immunohistochemistry was performed using the primary and secondary antibodies detailed in Tables 1 and 2. Slides were first dewaxed in xylene followed by neat

industrial methylated spirits (IMS), and then endogenous peroxidase was blocked by immersing in a solution of 0.3% hydrogen peroxide in IMS for 20 minutes. Sections were trypsinized by incubating at 37°C in 0.01% chymotrypsin in TBS for 20 minutes (except for CR3/43). After washing in TBS, sections were incubated with blocking serum (see Table 1) for 15 minutes. This was then tipped off and the primary antibody (Table 1) applied for 1 hour. After copious washing, the secondary antibody was applied for 30 minutes. Following further washing, avidin biotin HRP complex (Dako) was applied for 30 minutes, washed and then developed in 0.01% solution of 3,3'-diaminobenzidine in TBS with hydrogen peroxide for 15 minutes. Slides were counterstained in haematoxylin.

Where cell counts are given, these were performed using a Graphic Information Systems image analyzer -IMAGAN 2 programme on a 286 personal computer (Dell) attached to a Leitz Ortholux 20ED microscope. Cells were counted per 20 high power fields.

RESULTS

The distribution of cells was assessed in three different areas of section—nodule, cord and subcutis. Subcutis was defined as the area outside the cord or nodule in which skin appendages could be identified; nearly all sections contained areas of this nature, not just those containing fragments of skin. When skin was present on the section, cells were also assessed in this tissue.

Factor XIIIa positive cells

In the subcutis many cells were stained (50–600 per section). These cells were spread throughout the tissue, but there were large numbers around blood vessels, especially capillaries. These cells were also prominent around sweat glands. When skin was present in the section cells were present in the dermal papillae. In the dermis and subcutis the cells had a marked dendritic appearance,

Table 1—Primary antibodies used

Specificity	Clone	Ligand	Concentration	Source
Macrophages	MAC387	Myeloid/histiocytic antigen	1/200	Dako
Macrophages	KP1	CD68	1/40	Dako
MHC II	CR3/43	HLA-DR	1/20	Dako
Factor XIIIa	N/A	Factor XIIIa	1/200	Behring

Table 2—Secondary antibodies used

Antibody	Raised in	Used with	Source
F(ab') ₂ anti-mouse	Rabbit	CR3/43, MAC387, CD68	Dako
Anti-rabbit	Swine	FXIIIa	Dako

with a single lobed centrally placed nucleus (Fig 1). Within nodules there were again large numbers of cells (50–600 per section). The cells did not have a consistent relationship to the surrounding tissue, but there tended to be greater numbers near blood vessels towards the periphery of the nodule (Fig 2). In the nodule the cells were dendritic or fusiform. In the cord there were significantly fewer positive cells than in either the nodule or subcutis. These cells were generally spindle shaped, much less dendritic than in the nodule and lying parallel to the collagen bands.

MAC387

There were very few cells stained in any of the sections, with no apparent difference in distribution in the various stages (0–40 per whole section). The few cells present did not show any association with other structures such as blood vessels and occurred singly in the tissue. The typical cell was oval in shape with a single lobed nucleus that was often indented to give a kidney shaped appearance. These cells were not dendritic.

CD68

The subcutis contained significantly more cells (0–300 per section) than either the nodule or cord. Cells were spread throughout the subcutis and were prominent around blood vessels. In the skin CD68 positive cells were present in the dermal papillae. The nodules generally had greater numbers of positive cells than the cord. In most areas the cells had a dendritic appearance with a single lobed nucleus, but there were some that had an oval shape with no dendritic processes.

HLA-DR (MHC II)

Three different types of cells appeared to be stained by this antibody. First, there was a dendritic cell that was predominantly in the nodule and subcutis. Second, there were groups of cells numbering up to 30, often situated

at the periphery of a nodule. The cells in these groups had a single lobed, deeply basophilic nucleus and little cytoplasm. Finally, the endothelium in small vessels was positive in some sections. The vessels that stained were often grouped together but represented a small proportion of the total number of vessels in the section. Sweat glands were invariably positive for MHC class II.

DISCUSSION

Several studies have indicated a role for the skin in Dupuytren's disease. Replacement of the palmar skin by full thickness skin grafts prevents recurrence (Hueston, 1985), and McGrouther (1985) found that early fibrotic changes in Dupuytren's disease affected fibres running vertically from the skin. However, no aetiological link has been established and mechanisms by which the skin can affect underlying fibrosis are obscure.

The method of immunocytochemistry has the advantage that the cells involved in a pathological process can be identified in the sites in which they were acting at the time of biopsy. A limitation of the method is that antigens may not be limited to the cells being examined and that the cells in question may not express the antigen under all circumstances. CD68 is a lysosomal protein of unknown function present in the cytoplasmic granules of macrophages, neutrophils, basophils and renal tubules. It is expressed by 80 to 100% of cells in the macrophage lineage and is therefore a good marker of the presence of macrophages (Stockinger, 1989). MAC387 recognizes the antigen L1 which consists of two calcium binding proteins called calgranulin. It occurs in cells of the macrophage lineage and in some epithelial cells (Brandtzaeg et al, 1988). The numbers of MAC387 cells seen in this study are consistent with a previous publication on the same subject (Andrew et al, 1991). However, the observations of CD68 positive cells indicate that the previous study underestimated the number of macrophages present. Macrophages are a heterologous population of cells with many subsets. No one marker is present on all subsets and so it is to be expected that different antibodies will stain different populations, albeit with some overlap. These results show the value of using multiple markers in immunocytochemical studies.

The principal finding in this study is that factor XIIIa positive cells are present in the skin, subcutis, nodule and cord. These appear to be a single population of

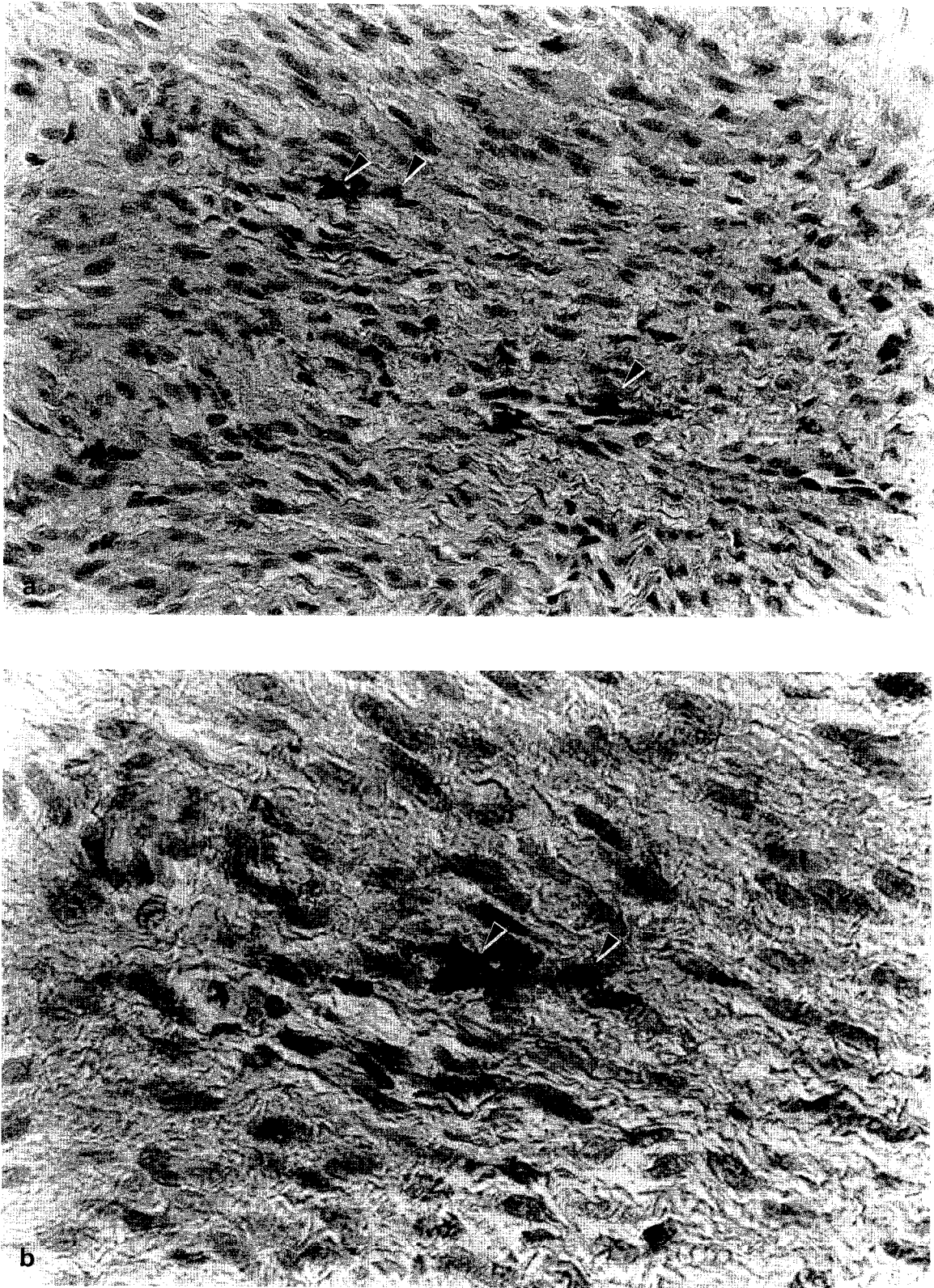


Fig 1 (a) Low power view of factor XIIIa positive cells. These cells have a dendritic morphology ($\times 100$). (b) High power view of factor XIIIa positive cells showing their dendritic processes ($\times 400$).

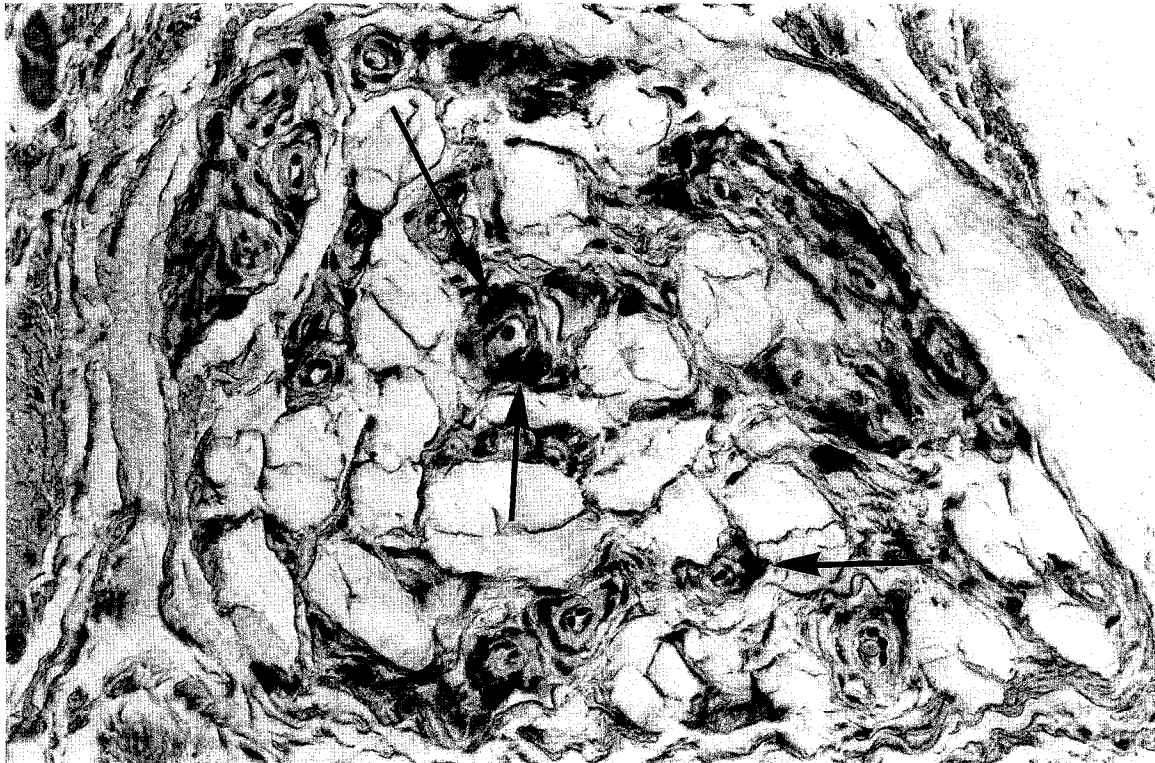


Fig 2 Perivascular factor XIIIa positive cells in subcutaneous fibrous septum adjacent to Dupuytren's tissue nodule ($\times 100$).

cells; if this is the case, then dermal dendrocytes are present in Dupuytren's tissue. These cells are probably of macrophage lineage and it has been proposed that they are aetiologically important in certain fibrotic conditions (Penneys, 1990). Diseases in which they are found in increased numbers include inflamed skin (Cerio et al, 1989a), histiocytoma cutis (Cerio et al, 1989b), fibrous papules and scars (Estrada and Piérard, 1990), in association with malignant neoplasms such as malignant melanoma (Estrada and Piérard, 1990) and nodular fasciitis (Penneys, 1990). These cells appear to be a possible link between the skin and the pathogenesis of Dupuytren's disease. If this could be conclusively established it would be the first association between a skin-derived cell and the pathogenesis of the disease.

HLA-DR is part of the major histocompatibility complex class II and is usually expressed by cells with an immunological function. Expression of this is known to be induced by a range of cytokines such as GM-CSF and TNF α . This was seen to be expressed in cells with the same morphology and distribution as dermal dendrocytes, and we believe that this indicates that the factor XIIIa positive cells are activated cells of macrophage lineage. Such cells may be important as a source of cytokines in initiating fibroblast proliferation (Nathan, 1986).

Further investigation will be required to establish that the factor XIIIa positive cells represent a single population, to show that the palmar skin and subcutis in

Dupuytren's disease contain abnormal amounts of them and to determine the cytokine expression of the cells. In conclusion we found that factor XIIIa positive dendritic cells, HLA-DR positive cells and CD68 positive cells are present in all stages of Dupuytren's disease as well as the surrounding tissue. These cells appear identical to the recently described dermal dendrocyte and may therefore represent an important link between the skin and pathogenesis of Dupuytren's disease.

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