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What is This?

LANGERHANS CELLS IN DUPUYTREN'S CONTRACTURE

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We have examined biopsies of Dupuytren's contracture palmar fascia, overlying subcutis and skin, and have correlated the distribution of gross macroscopic changes in the hand, mapped pre- and intraoperatively, with light microscopic immunohistochemical findings. We report increased numbers of S100 positive Langerhans cells (an epidermal cell of dendritic lineage) and CD45 positive cells, both in "nodules" and at dermo-epidermal junctions, in the biopsied tissues. This suggests that Langerhans cells migrate from the epidermis into Dupuytren's contracture tissue, possibly in response to local changes in levels of inflammatory cytokines within the tissue. Our findings, together with other reports of increased numbers of dermal dendrocytes and inflammatory cells in Dupuytren's contracture tissue, lend circumstantial support to the "extrinsic theory" of the pathogenesis of Dupuytren's contracture. However, the earliest stages of the disease process have not been defined, and therefore the events which ultimately produce fibrosis in the palmar fascial complex in susceptible individuals could begin in the skin and/or within deeper tissues, especially where there is dysregulation of the immune system.
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INTRODUCTION

Dupuytren's contracture is a benign non-lethal disease, with a well characterized pathology but an unresolved pathogenesis (McFarlane, 1993; Fitzgerald et al., 1999). It most commonly occurs in Caucasians, with an increasing incidence in the elderly, associations with alcohol abuse, diabetes, smoking and epilepsy (Yi et al., 1999) and evidence of genetic predisposition (Burge, 1999).

Some of the earliest manifestations of Dupuytren's contracture are thickening and "tethering" of the palmar skin (Rayan, 1999b), which are followed by fibrotic changes in the palmar fascial complex, producing the characteristic digital flexion deformity (Salamon et al., 1997; Tomasek et al., 1999; Rayan, 1999a). In the absence of histopathological studies of the earliest stages of Dupuytren's contracture, or an animal model which mimics the condition, it is perhaps not surprising that the two theories which have dominated thinking about the pathogenesis of Dupuytren's contracture focus on changes which occur in these different sites. The "extrinsic" theory champions initiating changes within the overlying dermis (Hueston, 1963; 1985), whereas the "intrinsic" theory argues that the disease process originates within the palmar fascia (McFarlane, 1974). There have been numerous studies of the cellular and molecular changes which occur within the palmar fascial complex, whereas relatively little is known about the dermatopathology of Dupuytren's contracture (Vande Berg et al., 1982).

Skin has a complex defence function, acknowledged by a plethora of acronyms which reflect the relationships between skin cells and the immune and nervous systems. These include skin associated lymphoid tissue, (SALT); skin immune system (SIS); cutaneous associated functional immune system (CAFIS); dermal microvascular unit (DMU) (Bos, 1997) and neuroimmunocutaneous system (NICS) (Misery, 1997). Dendritic cells are prominent components of these systems, and two subsets have been described in the skin. These are Langerhans cells (epidermal dendritic cells) which are found predominantly in the strata basale and spinosum of the epidermis (Strobl et al., 1998), and dermal dendritic cells (myeloid dendritic cells) (Caux et al., 1996; McLellan et al., 1998).

Previous studies of Dupuytren's contracture have demonstrated increased numbers of Factor XIIIa positive dermal dendrocytes (Sugden et al., 1993) and inflammatory cells (Andrew et al., 1991) in biopsied tissue. We present a immunohistochemical report of the distribution of S100 positive Langerhans cells and CD45 positive cells in biopsies of Dupuytren's contracture and the overlying skin. We have correlated the distribution of gross macroscopic changes in the hand, mapped pre- and intra-operatively, with our light microscopic immunohistochemical findings.

PATIENTS AND METHODS

Tissue for analysis was obtained from twelve male patients undergoing fasciectomies for Dupuytren's contracture of the hand (age range, 49–72 years). Three of these samples included areas where there was clinical evidence of skin involvement (skin pitting). Control tissue was obtained from palmar fascia excised during carpal tunnel decompression ($n = 3$) and from cadaveric hands ($n = 5$). All subjects, both Dupuytren's contracture and control, were Caucasian. Orientation of each sample, from surgery through to tissue block, was maintained using a selection of India inks to mark the

superficial, deep, proximal and distal aspects of each biopsy. Specimens were fixed by immersion in 4% paraformaldehyde solution for 24 hours, washed in phosphate buffered saline (PBS) for 2 hours and then overnight at 4°C, dehydrated through increasing concentrations of industrial methylated spirits (IMS) and blocked out in $1 cm^3$ cubes in polyester wax at 40°C.

Seven μm transverse sections (cut using a Picro-cool microtome) were immunostained using the following primary antibodies and dilutions: anti-S-100 which labels Schwann cells, melanocytes and Langerhans cells depending upon cellular context (Dako Ltd., Ely, Cambridgeshire, UK, 1:200, IF; 1:5000 IP); anti-panaxonal antibody which labels axonal cytoskeletal elements (Affiniti Research Products Ltd., Exeter, UK, 1:1000, IF); anti-CD45 which labels T lymphocytes intensely, and decorates macrophages and polymorphonuclear leucocytes weakly (Dako Ltd., Ely, Cambridgeshire, UK, 1:800, IP); anti-Factor XIIIa which labels dermal dendritic cells (Cerio et al., 1989a,b), (Dako Ltd., Ely, Cambridgeshire, UK, 1:300, IP, according to the protocol described in Cerio et al., 1989b); anti-laminin which labels laminin, a component of all basal laminae (Sigma Biosciences, Dorset, UK, 1:100, IF); anti-α smooth muscle actin which labels an isoform of actin specific to smooth muscle (Dako Ltd., Ely, Cambridgeshire, UK, 1:200). For double-immunofluorescence (IF), sections were co-incubated with both anti-S100 and panaxonal antibodies for 24 hours, then washed in PBS, subsequently treated with a mixture of anti-rabbit FITC and anti-mouse TRITC (Dako Ltd., Ely, Cambridgeshire, UK) in a humidified chamber for 1 hour, washed with PBS, post-fixed in 2% paraformaldehyde solution for 5 minutes and finally mounted in Citifluor (to prevent quenching) and stored at 4°C. For immunoperoxidase (IP) immunostaining, sections were treated with 0.3% solution of hydrogen peroxide for 45 minutes, to block endogenous peroxidase, washed in PBS, incubated with blocking serum for 30 minutes and finally incubated overnight with the primary antibody. Secondary antibody was applied for 1 hour: biotinylated anti-mouse for anti-CD45, and biotinylated anti-rabbit for anti-S100. Sections were washed thoroughly in PBS, treated with avidin-biotin HRP complex (Dako Ltd., Ely, Cambridgeshire, UK) for 30 minutes, washed and then developed with 0.01% solution of 3′3′-diaminobenzidine in PBS with hydrogen peroxide for 2 to 10 minutes. Slides were counter-stained using Gurr's Haematoxylin. Control sections were incubated without the primary antibody. Sections were also stained using a picropolychrome technique to examine the distribution of collagens within the biopsies (Fitzgerald et al., 1995).

Sections were examined using a PROVIS 2000 microscope (IF) and a ZEISS microscope (IP) and counts of immunopositive cells per section were performed at × 40 magnification. Sections from each tissue block were examined, and a minimum of five non-overlapping fields were examined in each section. It quickly became apparent that the distribution of immunopositive cells within sections was non-uniform, thus in routine analyses many fields were devoid of cells, and a relatively small number of fields in each section contained variable numbers of "target" cells. For example, at the centre of a nodule, only a single immunopositive cell might be present, whereas localized foci of more than 50 cells were typically seen at dermo-epidermal junctions. Expressing immunopositive cells in terms of their density would therefore inevitably have given a skewed picture of their distribution within each biopsy. Equally, expressing numbers of cells per section, while often reported, is inappropriate, since sections from different tissue blocks are unlikely to share identical dimensions. The distribution of immunostaining within individual sections was correlated microscopically with the results of a concomitant histopathological assessment of disease activity within each block (based on conventional light microscope staining of adjacent sections) and macroscopically with the position of each block within the intra-operative clinical "maps" of cords and nodules.

Artefactual differences in levels of immunostaining (differences reflecting variations in histological technique rather than real differences between and within sections) were minimized since all sections for each antibody were processed together. Reliability was maintained during microscopical assessment by ensuring that sections were viewed in antibody-specific batches by two independent observers.

RESULTS

Control tissues

α smooth muscle actin

Blood vessel walls were strongly immunopositive for α-smooth muscle actin. There was no evidence that other cell types were labelled.

S-100

Three types of S-100-positive cells, namely Langerhans cells, melanocytes and Schwann cells, were observed within the sections of control tissues. Langerhans cells were process-bearing, S-100-positive (Cocchia et al., 1981; Shrestha et al., 1998), laminin-negative cells, and were never associated with panaxonal positive profiles. Within the epidermis, Langerhans cells were located preferentially within the stratum spinosum, where they extended cytoplasmic processes between keratinocytes. Langerhans cells were occasionally seen as isolated cells in the papillary layers of the dermis, just below the epidermal rete pegs, but were never observed within the palmar fascia. Melanocytes were infrequent constituents of the stratum basale. Although they were also S-100 positive and laminin-negative, they could be

distinguished easily from Langerhans cells on the basis of their content of pigment granules (confirmed in skip-serial toluidine blue stained sections). Intensely immunopositive Schwann cells were widely distributed throughout the dermis, hypodermis and connective tissue of all control specimens, always lying in close association with bundles of pan-axonal positive axons, often in perivascular locations (Terenghi et al., 1998). S-100-positive Schwann cells were invariably invested with laminin positive basal laminae, which further distinguished them from both Langerhans cells and melanocytes.

Factor XIIIa

Labelled cells were seen throughout the dermis, subcutis and fascia, and were most numerous in the high dermis: no labelled cells were seen within the epidermis. Spindle-shaped fibroblasts were consistently immuno-negative.

CD45

Intra-luminal and perivascular CD45-positive cells were seen occasionally either within or near blood vessels in the dermis and underlying tissues, but were not otherwise present within the samples.

Dupuytren's contracture biopsies – general features

Each biopsy was at least 5 cm in length, with a maximum diameter of 1 cm. Routine screening confirmed that all but one contained highly cellular (>250 nuclei per $0.25\,mm^2$), well vascularized nodules, composed of masses of α-smooth muscle actin positive myofibroblasts (McCann et al., 1993) (Fig 1). Perinodular tissue exhibited varying degrees of cellularity and vascularity and contained dispersed α-smooth muscle actin-positive myofibroblasts. Cords consisted predominantly of bundles of collagen, were relatively avascular and rarely contained α-smooth muscle actin-positive cells. Smooth muscle cells within blood vessel walls were consistently strongly immunopositive. Herovici's picropolychrome technique revealed non-uniform staining of connective tissue within each biopsy. In general, the sparse collagen within nodules stained blue, indicating type III collagen fibres, whereas the collagen in the cords stained purple/red, indicating type I collagen (Fitzgerald et al., 1995). In marked contrast, all of the connective tissue in the control specimens stained purple/red using Herovici's picropolychrome technique.
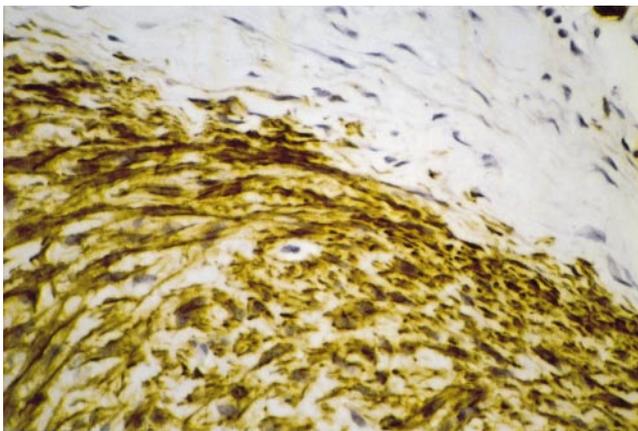
There was no discernible proximo-distal pattern in terms of the arrangement of cords and nodules. Thus, in some biopsies, nodules were present only in distal segments, with the more proximal portions containing cords and loose connective tissue; in other biopsies, nodules and cords were interspersed throughout the sample. In one of the three cases of Dupuytren's contracture with skin tethering, focal aggregations of α-smooth muscle actin-positive cells were seen close to the dermo-epidermal junctions.

S-100

The distribution of S-100 positive Schwann cells and melanocytes was indistinguishable from that seen in control tissues. However, many more Langerhans cells were seen in Dupuytren's contracture tissue than were seen in any of the controls, typically near capillaries at the edges of nodules (Fig 2) and at dermo-epidermal junctions (Fig 3). Isolated Langerhans cells were an occasional finding, either deep within nodules, or at the

Fig 1   SMA positive myofibroblasts within a nodule. Note the distinct interface between the highly cellular nodule and the adjacent unlabelled hypocellular connective tissue. Immunoperoxidase labelling × 500.
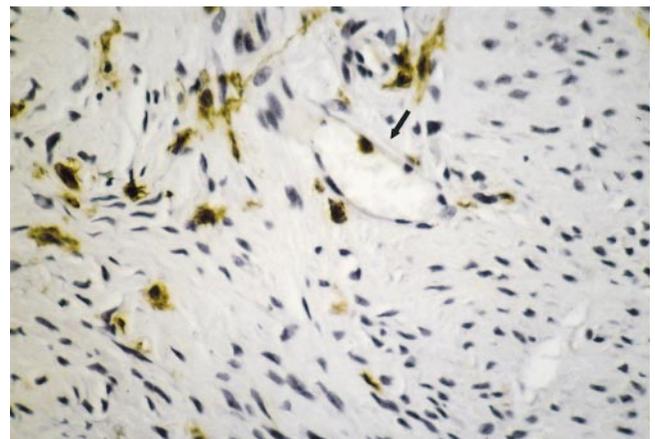
Fig 2   S 100 positive Langerhans cells lying near a capillary (arrow) at the edge of a nodule. Immunoperoxidase labelling × 500.
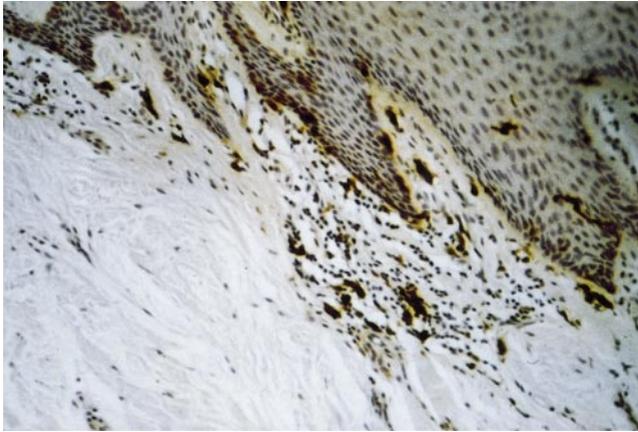
Fig 3 S 100 positive Langerhans cells lying in a dermal papilla. Immunoperoxidase labelling ×200.
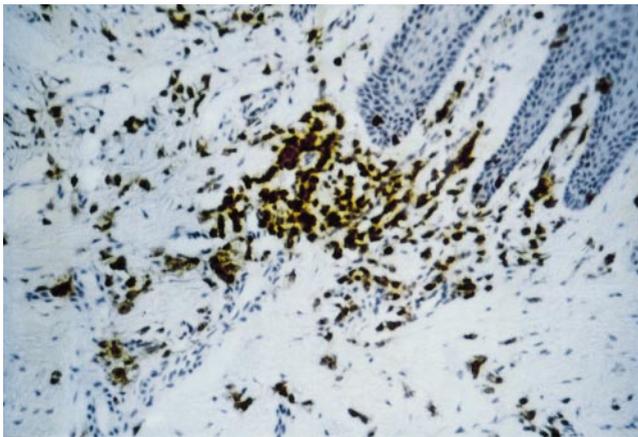
Fig 4 CD45 positive cells lying in the dermis near the dermo-epidermal junction. Compare the distribution of these cells with that of the S100 positive Langerhans cells in Figure 3. Immunoperoxidase labelling ×200.

periphery of nodules. Langerhans cells were never observed in cords.

Factor XIIIa

The distribution of immunopositive cells within the dermis was essentially similar to that seen in control tissue. Within the palmar fascia, Factor XIIIa positive cells were present within nodules and near blood vessels: they were rarely seen in cords.

CD45

CD45 positive cells were a prominent feature of Dupuytren's contracture tissue. Immunopositive cells were seen within nodules, often occupying peri-vascular positions; within foci of Langerhans cells near dermo-epidermal junctions (Fig 4); and in less cellular peri-nodular tissue. No immunopositive cells were found in acellular cords.

DISCUSSION

We have described the distribution of Langerhans cells and CD45 positive cells in extensive samples of Dupuytren's contracture tissue. We confirm previous reports that such tissue is infiltrated with inflammatory cells (Baird et al., 1993a; Gudmundsson et al., 1998) and demonstrate Langerhans cells clustered around dermo-epidermal junctions and capillaries, and isolated in nodules, in biopsies of Dupuytren's contracture palmar fascia and the overlying subcutis and skin. Since the size of the epidermal Langerhans cell population decreases with age, as part of a generalised phenomenon of "immunosenescence" (Sunderkotter et al., 1997; Cantrell & Norman, 1998), it is unlikely that this is an incidental finding. However, neither is it direct evidence that Langerhans cells play a role in the pathogenesis of Dupuytren's contracture.

Epidermal Langerhans cells constitute a network of antigen-trapping "sentinel" cells, which ingest and process antigens but are weak stimulators of naïve T cells (Udey, 1997; Austyn, 1998; Banchereau and Steinman, 1998). Once activated, by external or internal stimuli such as the pro-inflammatory cytokines IL-1, TGF-β and TNF-α (Steinman, 1991; Cumberbatch et al., 1997), some Langerhans cells leave the epidermis, cross the dermo-epidermal junction and migrate to T cell-dependent regions of local draining lymph nodes: these mature "licenced" Langerhans cells are potent stimulators of naïve T cells.

The presence of foci of Langerhans cells at the dermo-epidermal junctions and close to capillaries in deeper diseased tissue, usually co-localized with CD45 positive T cells, is consistent with current thinking about interactions between dendritic cells and T cells (Katz et al., 1985; Banchereau and Steinman, 1998), and provides strong circumstantial evidence that the Langerhans cells in the Dupuytren's contracture biopsies were migrating. Although it has been suggested that Dupuytren's contracture is an immune-mediated or even atypical auto-immune disease (Baird et al., 1993b; Neumüller et al., 1994; Gudmundsson et al., 1998), the significance of this observation must be interpreted cautiously, since it appears that Langerhans cells "do not need to be fully mature in phenotype or function before they leave the skin" (Geissman et al., 1999).

The triggers which stimulate the proliferation of fibroblasts within the palmar fascial complex and overlying skin, and their transformation into myofibroblasts in Dupuytren's contracture have not been identified. Elevated levels of fibrogenic cytokines and growth factors (derived from recruited macrophages, or

inflammatory cells or mast cells) and/or reactive oxygen species (derived from reactive endothelial cells as a result of microvascular damage and local ischaemia and hypoxia) are frequently cited as the most probable mediators of the disease process (e.g., Bower et al., 1990; Murrell, 1992; Baird et al., 1993a; Alioto et al., 1994; Meek et al., 1999) in Dupuytren's contracture. Although most attention has centred on the responses of the fibroblast population in the palmar fascial complex in Dupuytren's contracture, local changes in the cytokine milieu, evoked by a variety of pathogens and traumatic events, would inevitably influence the functions of dendritic cells and other cells within the skin (Jaksits et al., 1999). Thus, increased levels of TNF-α and Il-1 could provoke Langerhans cell migration, whereas increased levels of TGF-β1 would prevent the non-cognate maturation of human Langerhans cells via bystander inflammatory cytokines (Geissmann et al., 1999). Moreover, recent studies have demonstrated the existence of the nitric oxide pathway in a number of cell types within the skin, including Langerhans cells: inducible nitric oxide synthase is expressed in keratinocytes and Langerhans cells after cytokine challenge (Bruch-Gerharz et al., 1998).

Biopsies of Dupuytren's contracture tissue provide a snapshot of well-established disease, but inevitably give little indication of where or how the disease process began. Previous studies have demonstrated increased numbers of Factor XIIIa positive dermal dendrocytes (Sugden et al., 1993) and inflammatory cells and their products (Andrew et al., 1991, Meek et al., 1999) in Dupuytren's contracture tissue. We have now shown that increased numbers of Langerhans cells are present in Dupuytren's contracture tissue, in association with T cells. Bearing in mind the acknowledged association between inflammation, cytokines and fibrosis (Kovacs, 1991), these findings appear collectively to support the extrinsic theory of the pathogenesis of Dupuytren's contracture (Hueston, 1963, 1985). However, it may be unwise to regard the "intrinsic" and "extrinsic" theories as being mutually exclusive: events which ultimately produce fibrosis in the palmar fascial complex in susceptible individuals may begin in the skin and/or within deeper tissues, especially where there is dys-regulation of the immune system (Bower et al., 1990).

Acknowledgements

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