Reactivity of Cells in Nodules of Dupuytren's Contracture with Monoclonal Antibodies Recognizing Leukocyte Antigens and von Willebrand's Factor

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Introduction

In populations of Scandinavian or Celtic origin Dupuytren's contracture belongs to the most frequent disorders of connective tissue (Hill 1985). The most significant symptoms of this disease are the development of nodules in the palmar aponeurosis and progressive irreversible contractures involving one or more fingers (Luck 1959: McFarlane 1983).

The etiology and pathogenesis of Dupuytren's contracture are still poorly understood. By ultrastructural studies a peculiar cell type has been identified in the nodules which are widely thought to represent the active phase of the disease. These cells, which were termed "myofibroblasts," posess characteristics of both fibroblasts and smooth muscle cells and seem to be responsible for the contractile forces leading to the clinical deformities (Gabbiani and Majno 1972; James and Odom 1980; Brickley-Parsons et al. 1981; McFarlane 1983; Tomasek et al. 1987). The cells of Dupuytren's contracture differ from normal palmar fibroblasts with respect to their morphologic, cytogenetic and metabolic features (Azzarone et al. 1983; Delbrück and Schröder 1983; Mohr and Vossbeck 1985; Wurster-Hill et al. 1988). Immunologic studies of the cytoskeleton and the extracellular matrix of myofibroblasts revealed that these cells are a nonmuscle type (Azzarone et al. 1983; Tomasek et al. 1986), but their exact origin remains unclear. They may develop from resident palmar fibroblasts or perivascular cells (James and Odom 1980; Kischer et al. 1982; Kischer and Speer 1984; Mohr and Vossbeck 1985), but even the possibility of them deriving from peripheral blood leukocytes or endothelial cells cannot fully be ruled out. Immunostaining with antibodies to connective tissue differentiation antigens showed a considerable variability of antigen expression in the cells of Dupuytren's contracture (Bartal et al. 1987).

Monoclonal antibodies have proven to be useful tools to identify different subsets of leukocytes and other cell types based on the presence of cell surface antigens. Therefore, the reactivity of cells in nodules of Dupuytren's contracture was examined with a panel of antibodies marking the cell types possibly playing a role in the pathogenesis of the disease.

Materials and Methods

Tissue

Twenty patients with Dupuytren's contracture underwent partial or total fasciectomy. The age of the patients (all male) was 32–69 years (median: 53.5 years) and the duration of the disease 1–20 years (median: 6 years). The palmar fascia was transported to the laboratory immediately after excision; there, a large nodule (defined as a hard fusiform thickening of the palmar fascia) was dissected from the surrounding tissue, which was then fixed in 5% formalin for routine hematoxylin-eosin staining to confirm the diagnosis. The nodule was cut into pieces 1–2 mm in diameter and frozen to −70°C. Later, the pieces were covered with OCT compound (Ames, Indiana, USA), and cryostat sections of 4–5 μm thickness were prepared and mounted on glass slides. The slides were stored at −70°C until the indirect immunofluorescence assay. Small strips of normal palmar fascia were obtained from five patients operated on for carpal tunnel release. They were treated and stored as described above to serve as controls.

Antibodies

The monoclonal antibodies for cell type characterization are listed in Table 1. In all immunofluorescence assays IgG-specific affinity-purified goat anti-mouse immunoglobulin, conjugated with dichlorotriazinylaminofluorescein (Dianova, Hamburg, FRG), served as secondary antibody.

Table 1. Primary monoclonal antibodies

Monoclonal antibody (clone)	CD designation	Major specifities	Working dilution	Source
Anti-LCA	CD 45	Peripheral blood cells, tissue macrophages, histiocytes	1:100	1
27 E 10	None	Granulocytes, monocytes, macrophages in acute inflammation	1:100	2
25 F 9	None	Macrophages of chronic stage inflammation	1:100	2
EBM 11	CD 68	Monocytes, wide range of macrophages	1:50	3
MT 310	CD 4	T helper lymphocytes, monocytes, some macrophages	1:100	3
DK 25	CD 8	Suppressor/cytotoxic T lymphocytes	1:100	3
F 8/86	None	Endothelial cells, megakaryocytes	1:50	3
V 3260	None	All mesenchymal cells including endothelium, fibroblasts, macrophages and histiocytes	1:100	1

1, Merck, Darmstadt, FRG; 2, Dianova, Hamburg, FRG; 3, Dakopatts, Glostrup, Denmark.

Immunofluorescence Staining

To run the indirect immunofluorescence assays the slides were thawed and air dried for 20 min. The sections were fixed for 10 min in acetone at room temperature; to control antigen degradation during fixation, for every primary antibody used some additional unfixed slides were also examined. A washing step was performed (15 min in phosphate-buffered saline, PBS, 0.01 *M* phosphate, 0.15 *M* NaCl, pH 7.2), and a monoclonal antibody in a dilution predetermined to be appropriate (Table 1) was placed on the slides. After incubation (1 h at room temperature) the slides were washed with PBS for 30 min, incubated (1 h) with the secondary antibody in a 1:20 dilution and washed again, this time for 90 min. Finally the sections were mounted in a buffered glycerol solution (nine parts glycerol, one part PBS). Routine controls for staining specifity were carried out in parallel sections by omitting the primary antibody and incubating with normal mouse serum. These control treatments consistently resulted in no detectable staining except for the autofluorescence of elastic fibers, which because of their yellowish color are easily distinguishable from the specific fluorescence. Additionally, for all antibodies to leukocyte and macrophage antigens, cytosmears of peripheral blood cells or cultured monocytes were used to control antibody reactivity.

Evaluation

Even within a single nodule of Dupuytren's contracture cell density may vary considerably. In this study preliminary experiments were performed to select areas with high cell densities, which are best suited for the examination of cell surface antigens. The grade of cellularity was measured by reaction with anti-vimentin antibody, which according to its known specifity immunostains all mesenchymal cells and thereby every cell type possibly present in Dupuytren's contracture. The reactivity of one of the other monoclonal antibodies to the cells was judged by comparison with the binding of anti-vimentin antibody. A reaction was determined to be strongly positive if the extent of immunostaining was comparable to the result with anti-vimentin. It was judged weakly positive if only scattered cell clusters or small parts of the tissue reacted leaving distinct areas within the section unstained. All nonstaining tissues were judged as negative.

Results

With anti-vimentin antibody it was possible to identify regions of high cellularity in every specimen examined. Anti-vimentin antibody was able to immunostain the cells of Dupuytren's contracture and of normal palmar fascia, while the extracellular matrix remained negative. As expected from light microscopy, the cell density generally was higher in Dupuytren's contracture

Table 2. Reactivity of interstitial cells in nodules of Dupuytren's contracture with monoclonal antibodies recognizing leukocyte antigens and von Willebrand's factor

Patient	Grade of reactivity with monoclonal antibody						
	Anti-LCA (CD 45)	27 E 10	25 F 9	EBM 11 (CD 68)	MT 310 (CD 4)	DK 25 (CD 8)	F 8/86
1	−	−	++	++	−	+	E
2	−	−	++	+	−	−	E
3	−	−	++	+	−	−	E
4	+	+	+	+	−	+	E
5	−	−	+	−	−	−	E
6	−	−	++	+	−	−	E
7	−	−	++	+	−	+	E
8	−	−	+	−	−	−	E
9	−	−	++	+	−	−	E
10	−	−	++	++	−	−	E
11	−	−	++	+	−	−	E
12	−	−	++	+	−	−	E
13	−	−	+	+	−	+	E
14	+	+	++	+	−	+	E
15	−	−	++	+	−	−	E
16	−	−	++	++	−	−	E
17	−	+	+	−	−	+	E
18	−	−	+	+	n.t.	−	E
19	−	−	++	−	−	−	E
20	−	−	++	+	−	−	E

Reactivity: ++, strongly positive reaction (comparable to anti-vimentin); +, weakly positive reaction; −, negative reaction; E, positive reaction restricted to endothelial cells; n.t., not tested.

than in normal palmar fascia. The reactions of the other monoclonal antibodies with preselected areas of high cell density are summarized in Table 2.

With antibody to leukocyte common antigen and with monoclonal antibody (MoAb) 27 E 10, in the majority of cases no immunostaining was observed. Isolated antibody binding cells could be seen in the tissue or occurring as small scattered clusters in 2 and 3 patients and no and one control, respectively.

In contrast the myofibroblasts of Dupuytren's contracture were stained strongly by MoAb 25 F 9, while normal palmar fascia fibroblasts did not react (Fig. 1). In 14 of the 20 tissue sections of Dupuytren's contracture nearly all of the cells present in the specimen bound MoAb 25 F 9, while in six cases a more regional pattern of immunostaining was observed, with some parts of the section remaining negative. MoAb 25 F 9 bound intensely to the cytoplasm of the cells leaving the cell nuclei and the surrounding extracellular matrix unstained. Most sections of Dupuytren's contracture bound MoAb EBM 11, too. Nevertheless, with this antibody focal or regional patterns of positive cells dominated, and diffuse immunostaining was restricted to three patients. Moreover, four patients' specimens did not react at all with MoAb EBM 11. Of the five sections of normal palmar fascia four were negative, while the fifth reacted weakly positive with widely scattered stained cells.

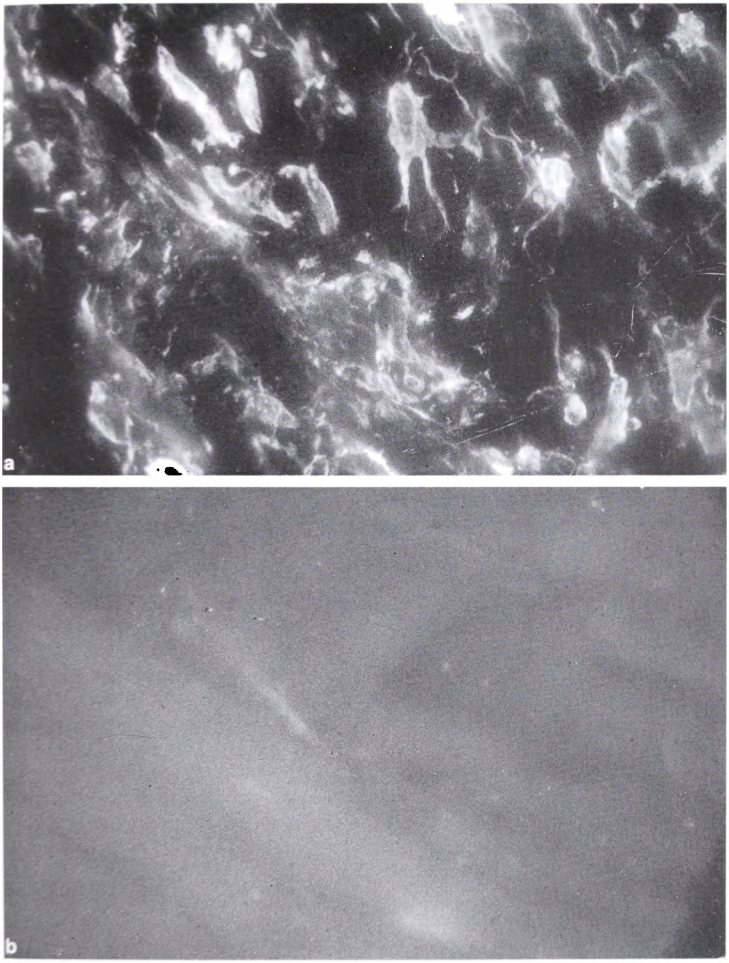

Fig. 1a,b. Photomicrographs of indirect immunofluorescence with MoAb 25 F 9. In nodules of Dupuytren's contracture (**a**) diffuse and intense immunostaining is observed, while in normal palmar fascia (**b**) there is nearly no reaction (patient 16; see Table 2). ×400

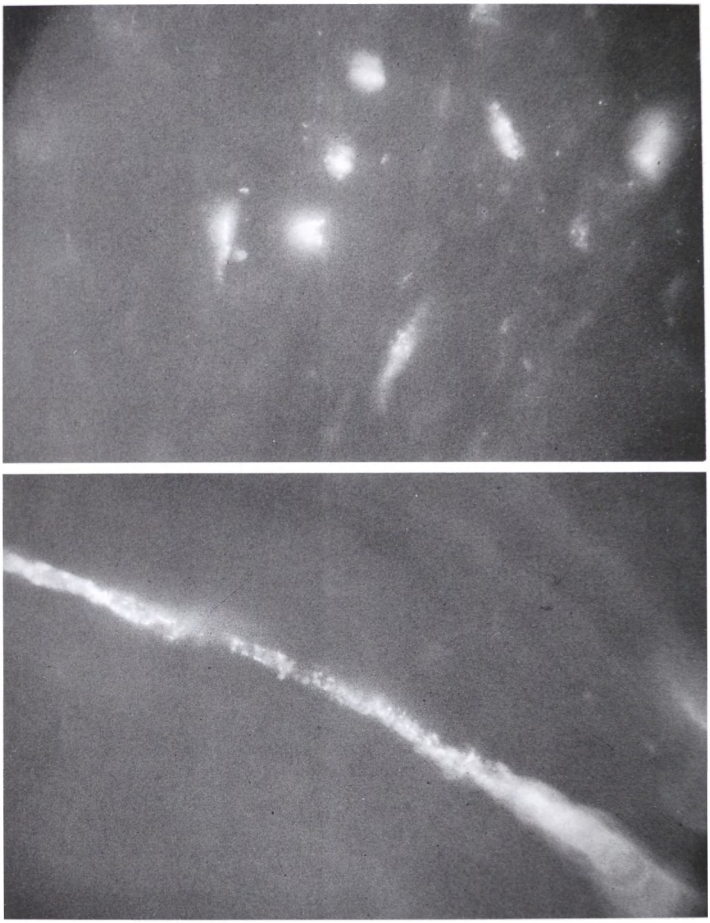

Fig. 2 *(above)*. Photomicrograph of a cluster of cells stained by MoAb DK 25 (anti-CD 8) in a nodule of Dupuytren's contracture (patient 7; see Table 2). ×400

Fig. 3 *(below)*. Photomicrograph of indirect immunofluorescence produced by MoAb F 8/86 (anti-von Willebrand's factor). The course of a small vessel is marked with all other parts of the nodule remaining negative (patient 2; see Table 2). ×400

Neither patient nor control sections showed any binding of MoAb MT 310 (anti-CD 4). In contrast, with MoAb DK 25 (anti-CD 8) weakly positive reactions with isolated clusters of immunostained cells were observed in six of 20 patient samples examined (Fig. 2). On retesting with parallel sections this finding could be reproduced in five of the six cases, while the majority of patients remained consistently negative. None of the normal palmar fascia sections bound MoAb DK 25.

MoAb F 8/86 (anti-von Willebrand's factor) was found to bind strongly to the vessel endothelium marking the course of small vessels throughout the normal or diseased palmar fascia (Fig. 3). Nevertheless, the reactivity to MoAb F 8/86 was strictly confined to endothelial cells, since this antibody does not immunostain the other cell types of the fascia or any part of the extracellular matrix.

Discussion

Dupuytren's contracture is characterized by the development of nodular structures in the palmar fascia and consecutive contractures of one or more fingers (Luck 1959; McGrouther 1982; McFarlane 1983). The highly cellular nodules appearing early in the disease process seem to play an important role in the pathogenesis of the contracture. In the nodules most cells have been converted into myofibroblasts, which are widely thought to be responsible for the contractile forces resulting in the clinical deformations (James and Odom 1980; Brickley-Parsons et al. 1981; Hill 1985). Myofibroblasts are not specific to Dupuytren's contracture, but can be found in various other conditions, many of them involving some kind of contraction (Bhatal 1972; Ryan et al. 1974; Baur et al. 1975, Churg and Kahn 1977). Ultrastructural studies revealed the morphologic features of myofibroblasts, but their origin remains unknown. The immunological properties of these cells have been examined with respect to their cytoskeleton, their attachment to the extracellular matrix and their expression of connective tissue differentiation antigens (Azzarone et al. 1983; Tomasek et al. 1986; Bartal et al. 1987).

In the last few years there has been increasing evidence that, under certain circumstances, leukocyte differentiation antigens are shared by connective tissue cells generally not considered to be directly involved in immunological processes (Malejczyk and Romaniuk 1989; Skjodt et al. 1989; Castagnoli et al. 1990). Although autoimmunological and immunopathological mechanisms in the pathogenesis of Dupuytren's contracture have repeatedly been discussed (Millesi 1959; Gay and Gay 1972; Busse et al. 1983; Jozsa et al. 1988), little is known about the possible role of myofibroblasts in such processes. The monoclonal antibody technique represents an important tool both for recognizing different cell types and for analyzing functionally different subsets of morphologically identical cells. Therefore the reactivity of cells in Dupuytren's contracture to a panel of monoclonal antibodies – recognizing different markers of blood leukocytes, tissue macrophages or endothelium – was examined.

The antibody to leukocyte common antigen (CD 45) was named as such because it immunostains all cells of myeloid or lymphoid origin with the exception of plasma cells, but including tissue macrophages. MoAb 27 E 10 reacts with more than 20% of freshly isolated monocytes and granulocytes. The corresponding antigen is strongly expressed on macrophages in acutely inflamed tissues (Zwadlo et al. 1986; Topoll et al. 1987), but is absent on nonmacrophage tissue cells and on normal resident mononuclear phagocytes in healthy tissues. Cells binding anti-CD45 or MoAb 27 E 10 were found very rarely in either Dupuytren's contracture or normal palmar fascia. Thus, in the nodules of Dupuytren's contracture there is no evidence of a major invasion of blood cells into the tissue or of an acute inflammatory process involving macrophages. These results correspond well with the known light microscopy findings in Dupuytren's contracture.

MoAbs 25 F 9 and EBM 11 (anti-CD 68) are able to stain mature tissue macrophages. The cells of Dupuytren's contracture, but not of normal palmar fascia, reacted strongly with both antibodies. Generally, using MoAb 25 F 9 (positive on resting mature macrophages and in many models of chronic stage inflammation, negative on blood monocytes; Hume et al. 1985; Zwadlo et al. 1985) the specimens were stained more thoroughly than with MoAb EBM 11 (a general monocyte and macrophage marker; Esiri and McGee 1986; Franklin et al. 1986). Nevertheless, with both antibodies there was considerable variation of immunostaining within the patient group. It is not clear why some markers (as those detected by MoAbs 25 F 9 and EBM 11) typical for macrophages should be expressed on the myofibroblasts of Dupuytren's contracture. Other antigens present on most tissue macrophages, such as leukocyte common antigen (CD 45) or CD 4, were not found on the cells of the nodules. Thus, these cells represent a cell type immunologically distinct both from normal resident palmar fibroblasts and from typical tissue macrophages but sharing some antigens with both cell species. The origin of the myofibroblasts remains unknown; they could originate from normal fibroblasts or be descendants (perhaps via pericytes) of blood monocytes, having invaded the palmar fascia at the very beginning of the disease. Nonetheless, since myofibroblasts bear antigens typical for macrophages, the possibility of them being able to produce or react to mediators of immune response, e.g., interleukins, should be considered. If myofibroblasts are sensitive to immunological signals or interactions, this could be of major importance for our understanding of the pathogenesis of Dupuytren's contracture.

In about a third of the examined nodules small clusters of CD 8-positive cells (probably lymphocytes) were seen. These cannot just represent unselective invasion of blood cells, since CD 4-positive cells – which are more frequent in peripheral blood – were not detected in any of the specimens of Dupuytren's contracture. The significance of this finding remains unclear.

With several antibodies, especially with those to the macrophage antigens, the intensity of reaction varied considerably in the patient collective. In different patients, but sometimes even within a single nodule, there seemed to exist

several functional states of the cells recognized by immunological methods. This finding may be important with respect to the clinical course of Dupuytren's contracture, but it may explain, too, the difficulties encountered in investigation of the pathogenesis.

References

Azzarone B, Failly-Crepin C, Daya-Grosjean L, Chaponnier C, Gabbiani G (1983) Abnormal behavior of cultured fibroblasts from nodule and nonaffected aponeurosis of Dupuytren's disease. J Cell Physiol 117:353

Bartal AH, Stahl S, Karev A, Lichtig C (1987) Dupuytren's contracture studied with monoclonal antibodies to connective tissue differentiation antigens. Clin Exp Immunol 68:457

Baur PS, Larson DL, Stacey TR (1975) The observation of myofibroblasts in hypertrophic scars. Surg Gynecol Obstet 141:22

Bhatal PS (1972) Presence of modified fibroblasts in cirrhotic livers in man. Pathology 4:139

Brickley-Parsons D, Glimcher, MJ, Smith RJ, Albin R, Adams JP (1981) Biochemical changes in the collagen of the palmar fascia in patients with Dupuytren's disease. J Bone Joint Surg [Am] 63:787

Busse E, Nilius R, Busse HJ (1983) Immunologische Aspekte der Pathogenese der Dupuytrenschen Kontraktur. Zentralbl Chir 108:855.

Castagnoli C, Stella M, Magliacani G, Teich-Alasia S, Richiardi P (1990) Anomalous expression of HLA class II molecules on keratinocytes and fibroblasts in hypertrophic scars consequent to thermal injury. Clin Exp Immunol 82:350

Churg A, Kahn LB (1977) Myofibroblasts and related cells in malignant fibrous and fibrohistiocytic tumors. Hum Pathol 8:205

Delbrück A, Schröder H (1983) Metabolism and proliferation of cultured fibroblasts from specimens of human palmar fascia and Dupuytren's contracture. J Clin Chem Clin Biochem 21:11

Esiri MM, McGee, J O'D (1986) Monoclonal antibody to macrophages (EBM/11) labels macrophages and microglial cells in human brain. J Clin Pathol 39:615

Franklin WA, Mason DY, Pulford K, Falini B, Bliss E, Gatter KC, Stein H, Clarke LC, McGee J O'D (1986) Immunohistological analysis of human mononuclear phagocytes and dendritic cells by using monoclonal antibodies. Lab Invest 54:322

Gabbiani G, Majno G (1972) Dupuytren's contracture: fibroblast contraction? An ultrastructural study. Am J Pathol 66:131

Gay S, Gay B (1972) Ist die Dupuytrensche Kontraktur eine Autoimmunerkrankung? Zentralbl Chir 97:728

Hill A (1985) Dupuytren's contracture. J Bone Joint Surg [Am] 67:1439

Hume DA, Allan W, Golder J, Stephens RW, Doe WF, Warren HS (1985) Preparation and characterization of human bone marrow-derived macrophages. J Leucocyte Biol 38:541

James WD, Odom RB (1980) The role of the myofibroblast in Dupuytren's contrcture. Arch Dermatol 116:807

Jozsa L, Demel S, Pinter T, Renner A, Reffy A, Santha A, Salamon A (1988) Immunopathological study on palmar aponeurosis in Dupuytren's disease. Acta Histochem (Jena) 83:153

Kischer CW, Speer, DP (1984) Microvascular changes in Dupuytren's contracture. J Hand Surg 9:58

Kischer CW, Thies AC, Chvapil M (1982) Perivascular myofibroblasts and microvascular occlusion in hypertrophic scars and keloids. Hum Pathol 13:819

Luck JV (1959) Dupuytren's contracture. A new concept of the pathogenesis correlated with surgical management. J Bone Joint Surg [Am] 41:635

Malejczyk J, Romaniuk A (1989) Reactivity of normal rat epiphyseal chondrocytes with monoclonal antibodies recognizing different leucocyte markers. Clin Exp Immunol 75:477

McFarlane RM (1983) The current status of Dupuytren's disease. J Hand Surg 8:703

McGrouther DA (1982) The microanatomy of Dupuytren's contracture. Hand Surg 14:215
Millesi H (1959) Neue Gesichtspunkte in der Pathogenese der Dupuytrenschen Kontraktur. Bruns Beitr Klin Chir 198:1
Mohr W, Vossbeck G (1985) Untersuchungen zur Proliferation und ^{3}H-Prolin-Inkorporation von Zellen der Palmarfibromatose (Morbus Dupuytren). Z Rheumatol 44:226
Ryan GB, Cliff WJ, Gabbiani G, Irle C, Montandon D, Statkov PR, Majno G (1974) Myofibroblasts in human granulation tissue. Hum Pathol 5:55
Skjodt H, Moller T, Freiesleben SF (1989) Human osteoblastlike cells expressing MHC class II determinants stimulate allogeneic and autologous peripheral blood mononuclear cells and function as antigen-presenting cells. Immunology 68:416
Tomasek JJ, Schultz RJ, Episalla CW, Newmann SA (1986) The cytoskeleton and extracellular matrix of the Dupuytren's disease "myofibroblast": an immunofluorescence study of a nonmuscle cell type. J Hand Surg [Am] 11:365
Tomasek JJ, Schultz RJ, Haaksma CJ (1987) Extracellular natrix-cytoskeletal connections at the surface of the specialized contractile fibroblast (myofibroblast) in Dupuytren's disease. J Bone Joint Surg [Am] 69:1400
Topoll HH, Zwadlo G, Lange DE, Sorg C (1987) Analyse des entzündlichen Infiltrates verschiedener Entzündungsstadien der marginalen Gingiva mittels monoklonaler Antikörper. Zahnärztl Z 42:467
Wurster-Hill DH, Brown F, Park JP, Gibson SH (1988) Cytogenetic studies in Dupuytren's contracture. Am J Hum Genet 43:285
Zwadlo G, Bröcker E, von Bassewitz D, Feige U, Sorg C (1985) A monoclonal antibody to a differentiation antigen present on mature human macrophages and absent from monocytes. J Immunol 134:1487
Zwadlo G, Schlegel R, Sorg C (1986) A monoclonal antibody to a subset of human monocytes found only in the peripheral blood and inflammatory tissues. J Immunol 137:512