

Dupuytren's contracture studied with monoclonal antibodies to connective tissue differentiation antigens

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SUMMARY

Seventeen patients with Dupuytren's contracture underwent partial fasciectomy, and frozen tissue sections from the involved palmar fascia were prepared for binding studies with hybridoma-derived murine monoclonal antibodies (MoAb) recognizing connective tissue differentiation antigens. The two MoAb used were both generated using human sarcomas as immunizing agents, 23H7 known to bind to an antigen shared by selected sarcomas and carcinomas but not normal adult tissues except a subset of granulocytes, and 12C9 shown to recognize a common fibroblastic marker. MoAb 23H7 was discovered to bind to a subset of fibroblasts within the lesions of six of 17 patients with Dupuytren's disease. Occasionally it immunostained a single cell population associated with tissue granulocytes dispersed in the surroundings of the lesions. MoAb 12C9 was found to be expressed in only 12 of 17 specimens prepared from involved lesions from Dupuytren's disease. It is suggested that fibroblasts from selected patients with Dupuytren's contracture express a novel antigen, defined by MoAb 23H7, previously shown to be associated with human sarcomas and other neoplasia. The other fibroblast marker which is defined by MoAb 12C9 and known to be a common connective tissue antigen, is only occasionally expressed in lesions involved with this disease. Though additional markers associated with Dupuytren's contracture remain to be defined, the MoAb, capable of defining connective tissue differentiation markers, reported in this study may serve as new immunological probes for immunodissecting this syndrome into subsets of diseases which may better define the variety of clinical patterns presented by patients.

Keywords Dupuytren's contracture hybridoma monoclonal antibodies fibroblast antigens

INTRODUCTION

Dupuytren's contracture is a disease clinically manifested by contractures involving the palmar aponeurosis (Bazin *et al.*, 1980; McGrouther, 1982). The exact pathogenesis is as yet unknown. Pathological studies demonstrate excessive fibroblastic proliferation associated with inflammatory cells (Luck, 1959; Hueston, Hurley & Whittingham, 1976). Hybridoma technology has opened to the clinical investigator new avenues using MoAb (Bartal & Hirshaut, 1987) in both basic and applied research. Such antibodies are currently used as reagents to identify functionally different subsets of lymphocytes based on the antigens expressed upon their surfaces (Reinherz *et al.*, 1980). In recent years we have generated MoAb recognizing human sarcomas and connective tissue antigens (Feit *et al.*, 1984; Bartal *et al.*, 1985a, b, c). Monoclonal antibody 12C9 has been shown to

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identify a common fibroblast marker associated with sarcomas, and MoAb 23H7 was shown to bind to a variety of sarcomas, other neoplastic tissues and neutrophils but not to normal connective tissues. Both these MoAb were originally generated from a fusion using fibrosarcoma cells as the immunizing antigen (Bartal *et al.*, 1985b, c). The purpose of this paper is to report the mode of interaction of these MoAb with frozen tissue sections prepared from the palmar fascia involved with Dupuytren's contracture.

MATERIALS AND METHODS

Patients. Partial fasciectomy was performed on 17 patients with Dupuytren's contracture. The age, sex and stage of disease of the patients are shown in Table 1. Clinical staging was determined by the following criteria (McFarlane, 1983): stage I was defined as localized nodule formation; stage II as cord formation; and stage III as overt contracture with functional disability of the hand. The lesion excised from each patient was referred for pathological evaluation and for frozen section studies with MoAb. Following the operation all the patients remained under follow-up and exhibited no signs of local recurrence up to 8 months after excision.

Tissue studies. Diseased palmar fascia from each patient was removed and part was fixed in formalin, and embedded in a paraffin block for routine haematoxylin-eosin and Van Gieson staining for orientation and diagnosis. Another part was immediately placed in normal saline and frozen to -20°C . One to three weeks later, the latter was thawed and frozen sections were prepared to a thickness of 3–4 μm . These sections were placed on slides and frozen at -20°C until the indirect immunofluorescence assay with MoAb to connective tissue markers. Normal tissues such as skin, liver, spleen and colon as well as human sarcomas were obtained from surgical pathology specimens; frozen sections were prepared and stored as described above to serve as controls.

Production of hybridomas. To generate MoAb 23H7 and 12C9, BALB/c mice were weekly immunized with finely minced fresh human sarcoma tissues in complete Freund's adjuvant and

Table 1. Clinical characteristics of patients with Dupuytren's contracture and mode of interaction of frozen tissue sections prepared from involved lesions with MoAb 23H7 and 12C9, as studied by indirect immunofluorescence

Patient	Age	Sex	Stage	MoAb 23H7	PMN	MoAb 12C9
1 P.Y.	68	M	I	+	–	+++
2 K.M.	54	M	I	+	+	++
3 S.I.	45	M	I	+++	–	++++
4 Y.Y.	30	M	I	–	+	+++
5 K.R.	65	F	II	–	–	–
6 H.Z.	60	F	II	–	+	–
7 S.C.	49	M	II	+w	+w	++++
8 R.I.	65	M	II	–	–	++
9 Y.A.	24	M	II	–	+w	++
10 A.P.	50	M	II	–	–	–
11 C.M.	71	M	III	–	+w	–
12 O.A.	71	M	III	–	+	+++
13 H.H.	44	M	III	–	–	–
14 N.B.	54	F	III	–	–	++
15 M.E.	42	F	III	++	–	++++
16 Z.A.	61	M	III	+	+	+++
17 E.E.	46	M	III	–	w	++

M, male; F, female; PMN, polymorphonuclear cells; MoAb, monoclonal antibody; w, weak.

boosted before fusion with an additional inoculum of sarcoma cells. The fusion technique used for the generation of hybridomas has been previously described (Feit *et al.*, 1984). Twenty-one days after fusion, supernatants from wells containing hybridomas were screened for antibodies by means of an immunofluorescence assay (Feit *et al.*, 1984; Bartal *et al.*, 1985b, c). The target cells were cultures of sarcoma and carcinoma cell grown in the wells of plastic-coated multi-well slides (Hendley-Essex, Sussex, England). Selected hybridomas were cloned by the limiting dilution method.

Determination of Ig type. The immunoglobulin type of 23H7 and 12C9 was determined by using heavy-chain specific fluorescinated goat anti-mouse antisera (Meloy Laboratories, Springfield, MA) in an immunofluorescence assay and similar unlabelled goat antisera for testing by the Ouchterloney method (Cappel Laboratories, Doeringtown, PA).

Maintenace of hybridoma cell lines. Hybridoma clones 23H7 and 12C9 are maintained in a tissue culture medium composed of RPMI 1640 (Biological Industries, Israel), supplemented with 10% fetal calf serum (FCS) (Biolab, Jerusalem), and generally passed weekly in a routine fashion. Supernatants are collected from the flasks, checked for activity and frozen at -20°C until used.

Indirect immunofluorescence. The functional activity of supernatants obtained from clones 23H7 and 12C9 was routinely determined by MoAb binding to human sarcoma frozen tissue sections previously shown to interact strongly. To run the indirect immunofluorescence test each of the MoAb was placed on the slides which were then incubated for 1 h at 37°C . After incubation the slides were washed twice for 5 min with phosphate buffered saline (PBS) and then briefly immersed in distilled water. After complete drying, 1 : 20 diluted IgG and IgM specific affinity purified goat anti-mouse immunoglobulin, conjugated with fluorescein isothiocyanate (Meloy Labs, Springfield, MA), was placed on the slides. The slides were again incubated for 1 h at 37°C . Following the second incubation, the wash cycle was repeated and the slides, still wet, were placed in Evan's blue (0.005%) for 5 min. This step counterstains those portions of the cells which have no antigens and diminishes nonspecific autofluorescence. Finally the slides were washed again, dried, covered with 50% glycerol and examined using a Leitz Ortholux 11 microscope equipped with Ploem optics.

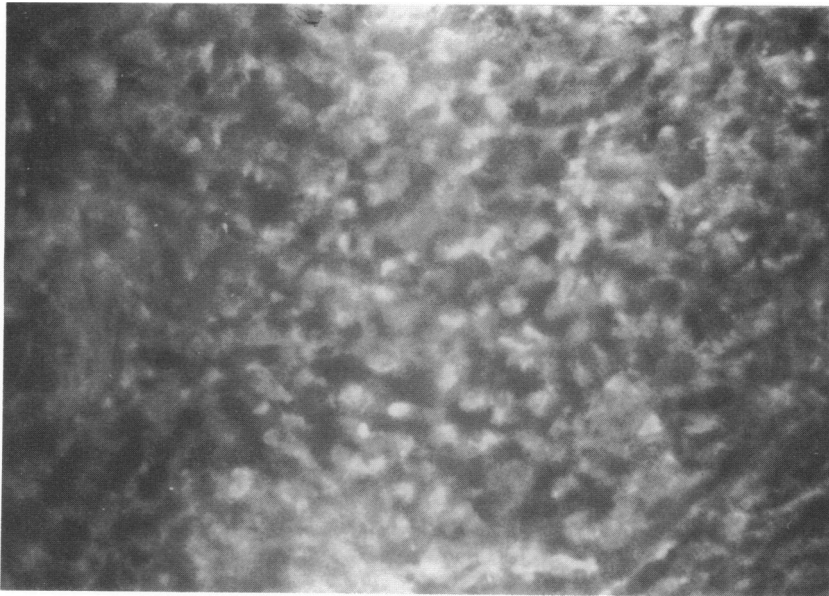


Fig. 1. Photomicrograph of indirect immunofluorescence produced by MoAb 23H7 on tissue section prepared from Dupuytren lesion (Patient no. 3). Note the intense binding of the antibody to the cells throughout the lesion. $\times 400$.

RESULTS

As shown in Table 1, MoAb 23H7 failed to immunostain fibroblasts or other components of connective tissues within the tissue sections prepared from 11 of the 17 patients included in this investigation. Of the six patients who showed positive binding (Fig. 1), the pattern of staining observed was of focal binding and cluster formation of cells binding this MoAb, leaving distinct areas within the section unstained. Furthermore, while specimens of tissue involved from three of four patients with stage I disease were positive with MoAb 23H7, sections from only one of six patients in stage II and two of seven sections in stage III were positive. In addition, as shown in Table 1, MoAb 23H7 was able to immunostain a subset of granulocytes dispersed within the connective tissue surrounding the lesions and occasionally also the few cells invading the cords (Fig. 2). The pattern of binding of the MoAb to this class of cells was morphologically distinct from that obtained when fibroblasts were stained. There was also no significant relation between the detection of fibroblast-associated 23H7-positive clusters and the cells identified as granulocyte-related (Table 1). Control tissue sections from normal adults including skin, fascias and visceral organs typically remained negative except occasionally granulocyte-related-cells dispersed within these tissues identified by this MoAb. On the other hand, human sarcoma-derived tissue sections as well as peripheral blood neutrophils strongly stained with the same 23H7 supernates.

Table 1 also illustrates the mode of interaction observed when the above tissue sections were studied with MoAb 12C9. In contrast to the narrow spectrum of binding of MoAb 23H7, MoAb 12C9 was found to bind strongly and diffusely to 12 of the 17 tissue sections prepared from the patients above (Figs 3 & 4). This antibody bound diffusely to the cytoplasm of fibroblasts making up these sections, leaving the nuclei unstained. The ground substance and matrix surrounding the immunostained fibroblasts remained negative. Various other patterns could be observed, from diffuse to focal mode of immunostaining. Since MoAb 12C9 is known to detect a common connective tissue specific marker broadly distributed in normal adult tissues, it is worthwhile emphasizing the five patients who revealed no binding within the involved lesions. Three of the five specimens were of disease stage II and two were from patients with stage III. Control tissue sections

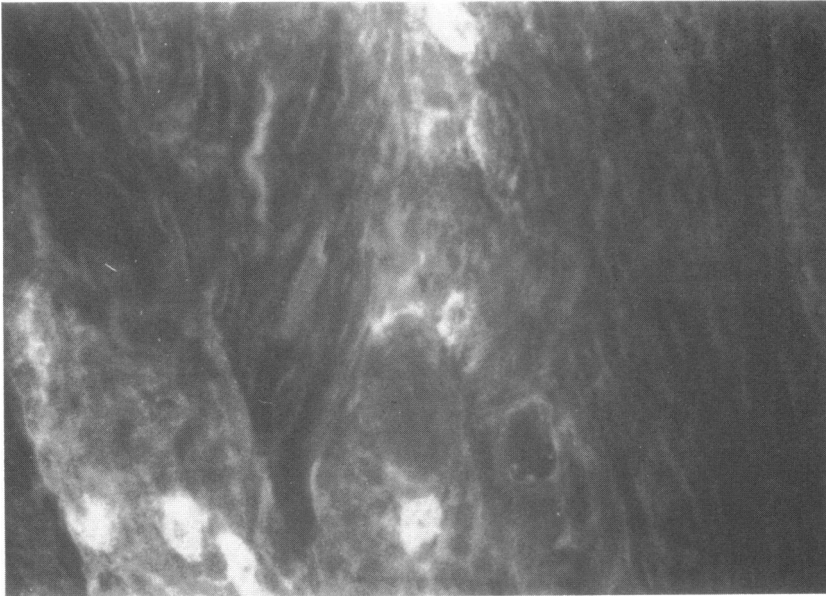


Fig. 2. Photomicrograph of indirect immunofluorescence produced by MoAb 23H7 on frozen tissue section prepared from Dupuytren lesion (Patient no. 6). Note the intense binding of the MoAb to granulocytes whereas the surrounding tissue remains negative. $\times 400$.

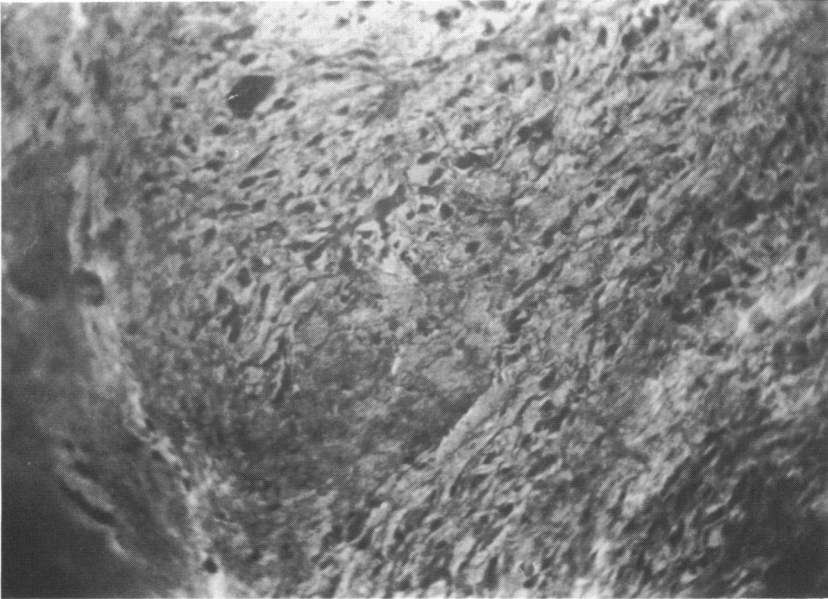


Fig. 3. Photomicrograph of indirect immunofluorescence produced by MoAb 12C9 on tissue section prepared from Dupuytren lesion (Patient no. 3) showing diffuse and intense binding of the MoAb to the fibroblasts composing the lesion. $\times 250$.

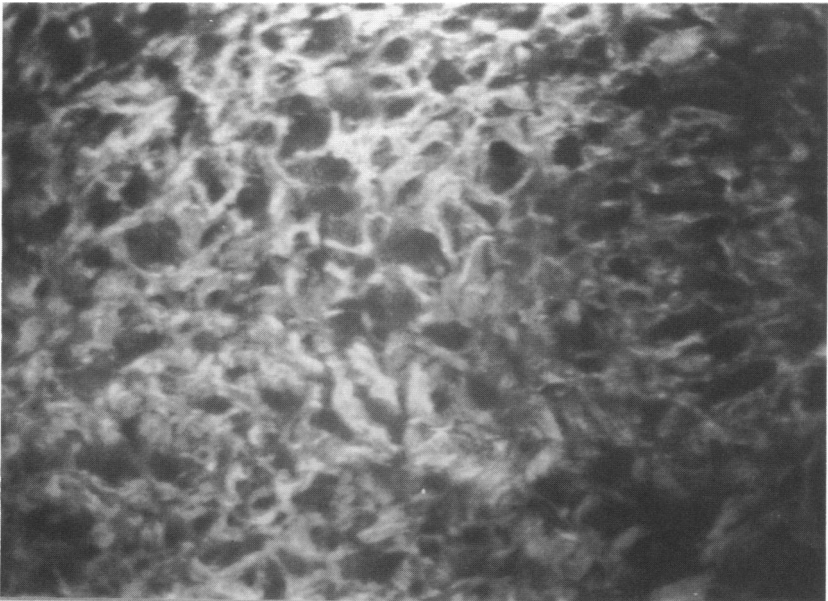


Fig. 4. Photomicrograph as in Fig. 3. $\times 400$.

were from multiple surgical pathology specimens obtained from normal adult as well as neoplastic tissues, all typically showing strong expression of this marker with the connective tissue compartment of the related biopsy. This included normal skin, liver, spleen and colon that bound MoAb 12C9, as well as human sarcoma and carcinoma tissue sections, allowing the epithelial compartment of this tissue to remain unstained.

DISCUSSION

Dupuytren's contracture is characterized by abnormal proliferation of fibroblasts with the excessive production of collagen (McGrouther, 1982; McFarlane, 1983; Chin & McFarlane, 1984). The extent and type of this proliferation varies according to the stage of the disease. While in the earlier stages fibroblasts are the prominent cells within the lesions involving the palmar aponeuroses, later stages are characterized by the frequent appearance of myofibroblasts. It is these myofibroblasts that are subsequently responsible for the development of the contractures and are usually typical and prominent in the cord formation. The late and advanced stage also known as the residual stage are characterized by mature fibrocytes surrounded by collagen and forming the contractures (Luck, 1959; Hueston *et al.*, 1976; Badalamantre, Stern & Hurst, 1983).

Monoclonal antibodies have provided in recent years a major breakthrough in defining subsets of cell populations in surgical pathology specimens based on the variety of antigens expressed on the cell surface (Bartal & Hirshaut, 1987). Thus, lymphocytes appearing microscopically identical can be subdivided using monoclonal antibodies into different functional subpopulations of cells, either helper or suppressor cells (Reinherz *et al.*, 1980). Our group has been involved in recent years in defining connective tissue differentiation antigens by novel monoclonal antibodies using human fresh sarcoma tissues as immunizing agents to sensitive BALB/c mice (Feit *et al.*, 1984; Bartal *et al.*, 1982). Hybridoma clone VIF3 was previously discovered to secrete a monoclonal antibody capable of identifying fetal fibroblasts and fibroblasts associated with neoplasms but not normal adult fibroblasts (Bartal *et al.*, 1985a; 1986). MoAb 12C9 was shown to bind only to sarcoma and normal fibroblasts cell lines and did not bind to cultured carcinoma cells (Bartal *et al.*, 1985c). Using fresh tissue sections from a large collection of normal fetal adult and malignant specimens, MoAb 12C9 was shown to identify a marker restricted only to connective tissues but not to epithelial or carcinoma cells (Bartal *et al.*, 1985c).

MoAb 23H7 has been previously shown in our laboratory to bind strongly to fresh frozen sections prepared from Kaposi sarcoma lesions, but very weakly or not at all to normal skin sections from the same patients (Bartal *et al.*, 1985b). Tissue distribution analysis demonstrated that this antibody binds to human sarcomas, and carcinomas, but not to normal adult tissues except a subset of neutrophils (Bartal *et al.*, 1984). Since the latter two MoAb identify distinct connective tissue markers, we hoped that they will distinctively interact with fibroblasts occurring in Dupuytren's disease.

MoAb 23H7 was revealed in the present study to be able to identify a subgroup of patients with Dupuytren's contracture whose lesion expressed the antigen defined by this MoAb. Most of these patients were allocated to those with stage I clinical disease. Dupuytren's contracture is considered a disorder involving an abnormality in fibroblast differentiation. It is far from clear why a sarcoma associated marker should appear upon fibroblasts and mostly in early Dupuytren's disorder cases. Since the 23H7 related antigen is considered a connective tissue differentiation antigen, it is suggested that this marker is occasionally shared by the fibroblasts involved in this disorder. One of the other compartments known to share this marker are a subject of myeloid cells (Bartal *et al.*, 1984). Therefore, it was not surprising when this MoAb bound to a subset of granulocyte-associated cells dispersed in the surroundings of the lesions and occasionally invading the cords and nodules. The additional role of MoAb 23H7 in this disease may be in evaluating the neutrophil cells associated, and aspects of the host inflammatory response occurring within the lesions.

MoAb 12C9 was shown in this study to bind to fibroblasts present within active Dupuytren's disease lesions. The ground substance appeared unreactive with MoAb 12C9. MoAb 12C9 is known from previous studies to define a common connective tissue specific marker broadly distributed in normal adult tissues and neoplasia-associated connective tissues but not binding to cells of epithelial origin (Bartal *et al.*, 1985b). Therefore it was quite surprising when active lesions from five patients did not bind this antibody whereas in the remaining, the binding was strong. All these patients had clinically advanced lesions. While in most cases, fibroblasts of Dupuytren's contracture do retain this common fibroblastic marker, it is suggested that this MoAb identifies a subgroup due to lack of its expression. The importance of this MoAb is suggested to be in its capacity to distinguish between these categories and as a research tool for further large prospective clinical investigations involving this disorder.

It is expected that with the generation of additional connective tissue MoAb, more information will accumulate regarding the type of antigens expressed on fibroblasts typical to this condition compared with other fibroblast disorders, and will thus shed further light on its pathogenesis.

REFERENCES

BADALAMENTRE, M.A., STERN L. & HURST, L.C. (1983) The pathogenesis of Dupuytren's contracture: constructive mechanisms of the myofibroblasts. *J. Hand Surg.* **8**, 235.

BARTAL, A.H., FEIT, C., FASS, B., CHESS, Q., MCKENZIE, S. & HIRSHAUT, Y. (1982) The detection of a heterophile tumor associated tissue differentiation antigen by monoclonal antibody. *The 8th International Convocation on Immunology*. 9 June. Buffalo, NY.

BARTAL, A.H., LICHTIG, C.H., FEIT, C., BUSHKIN, Y., SHANDOR, G., RUDICK, L., VERMEULEN, B., SEGMAN, S., FASS, B., ROBINSON, E. & HIRSHAUT, Y. (1984) Murine monoclonal antibody detecting human granulocytes, platelets and neoplastic cells. *Second International Workshop and Conference on Human Leukocyte Differentiation Antigens*. 19 September. Boston.

BARTAL, A.H., LICHTIG, C., ROBINSON, E., FEIT, C., CORDON CARDO, C. & HIRSHAUT, Y. (1985a) Subset of fibroblasts defined by monoclonal antibody. *Arch Pathol. Lab. Med.* **109**, 212.

BARTAL, A.H., LICHTIG, C., FRIEDMAN-BIRNBAUM, R., AVRAHAM, Z., SPIVAK, N., FASS, B., FEIT, C., ROBINSON, E. & HIRSHAUT, Y. (1985b) The interaction of Kaposi's sarcoma with monoclonal antibodies to human sarcoma and connective tissue differentiation antigens. *Cancer* **56**, 1071.

BARTAL, A.H., FEIT, C., CORDON CARDO, C., LICHTIG, C., FASS, B., BUSHKIN, Y., ROBINSON, E. & HIRSHAUT, Y. (1985c) Monoclonal antibodies to human sarcoma and connective tissue differentiation antigens. In: *Conference on Immunity to Cancer*. (ed. A.E. Reif & M.S. Mitchel) Academic Press, London.

BARTAL, A.H., LICHTIG, C., CORDON CARDO, C., FEIT, C., ROBINSON, E. & HIRSHAUT, Y. (1986) Monoclonal antibody defining fibroblasts appearing in fetal and neoplastic tissues. *J. natn Inst. Cancer.* **76**, 415.

BARTAL, A.H. & HIRSHAUT, Y. (1987) *Hybridoma Formation*. Humana Press, New Jersey.

BAZIN, S., LE LOUIS, M., DUANCE, V.C., SIMS, T.J., BAILEY, A.J., GABBIANI, C., D'ANDRIAN, G., PIZZOLATO, C., BROWSKI, A., NICOLETIS C. & DELAUMAY, A. (1980) Biochemistry and histology of the connective tissue of Dupuytren's disease. *Eur. J. clin. Invest.* **10**, 9.

CHIN, H.F. & MCFARLANE, R.M. (1984) Pathogenesis of Dupuytren's contracture: a correlative clinical-pathological study. *J. Hand Surg.* **3**, 1.

FEIT, C., BARTAL, A.H., FASS, B., BUSHKIN, Y., CORDON CARDO, C. & HIRSHAUT, Y. (1984) Monoclonal antibodies to human sarcoma and connective tissue differentiation antigens. *Cancer Res.* **44**, 5752.

HUESTON, J.T., HURLEY, J.V. & WHITTINGHAM, S. (1976) The contracting fibroblast as a clue to Dupuytren's contracture. *Hand* **8**, 10.

LUCK, S.V. (1959) Dupuytren's contracture. A new concept of the pathogenesis correlated with surgical management. *J. Bone Joint Surg.* **41-A**, 635.

MCFARLANE, R.M. (1983) The current status of Dupuytren's disease. *J. Hand Surg.* **8**, 703.

MCGROUTHER, D.A. (1982) The microanatomy of Dupuytren's contracture. *Hand Surg.* **14**, 215.

REINHERZ, E.L., KUNG, P.C., GOLDSTEIN, G., LEVEY, R.H. & SCHLOSSMAN, S.F. (1980) Discrete stages of human intrathymic differentiation: analysis of normal thymocytes and leukemic lymphoblasts of T cell lineage. *Proc. natn Acad. Sci. USA.* **77**, 1588.