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Cell Behavior and Cell-Matrix Interactions of Human Palmar Aponeurotic Cells In Vitro

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The present investigation has been performed to better characterize, in vitro, normal aponeurotic cells in comparison with dermal fibroblasts and with cells derived from Dupuytren's affected aponeuroses. Cells were cultured in monolayer and/or into three-dimensional collagen gels. Cell structure, adhesion, and spreading capability on different substrates, as well as integrin expression were investigated by light and electron microscopy and by flow cytometry. Cell-matrix interactions were also analyzed by gel retraction experiments in the presence, or absence, of RGD peptides and anti-integrin antibodies. Normal aponeurotic cells, compared with dermal fibroblasts, exhibited in vitro peculiar structural features, which were substantially maintained in Dupuytren's aponeurotic cells, irrespective of the substrate they were grown on. By contrast, the aponeurotic cell behavior was different in normal and diseased cells, these latter approaching that of dermal fibroblasts. Normal aponeurotic cells, in fact, were characterized by low efficiency in retracting the collagen gel, low α_2, α_1, and α_5 integrin subunit expression and low adhesion properties onto collagen and fibronectin, whereas cells isolated from the aponeuroses of Dupuytren's patients exhibited higher capability of retracting the collagen gel, increased adhesion properties toward collagen and fibronectin, and higher levels of integrin expression. No differences were observed between dermal fibroblasts from Dupuytren's patients or from normal subjects. These in vitro results are consistent with those previously obtained in situ, suggesting that palmar aponeurotic cells have a peculiar phenotype and that changes in cell-matrix interactions occur in Dupuytren's contracture. Moreover, by comparing data obtained from the retracted fibrotic cords and the still clinically unaffected aponeuroses of the same patients, it may be noted that Dupuytren's disease is not only confined to the clinically involved branches, but includes the whole aponeurosis of the affected hand. **J. Cell. Physiol. 173:415–422, 1997.** © 1997 Wiley-Liss, Inc.

In previous studies from this laboratory, it has been shown that normal aponeurotic tissue is characterized by polymorphic cells exhibiting features typical of myofibroblasts (i.e., positivity for smooth muscle cell alpha-actin, cytoplasmic bundles of filaments with periodical densities, pinocytotic vesicles, periplasmalemmal discontinuous basement membrane, Eyden et al., 1994) surrounded by a thick matrix coat (Baccarani Contri et al., 1994). Dupuytren's contracture is a fibromatous disorder affecting one or more branches of the palmar aponeurosis, causing a physical shortening of the affected branches and invalidity of the fingers involved (Dupuytren, 1831; Chiu and Mcfarlane, 1978; Hueston, 1985; McGrouther, 1985). Histologically, the disease is characterized by cellular nodules comprised of macrophages, fibroblasts, and myofibroblasts (Badalamente et al., 1983, Gabbiani and Montadon, 1985) and by an extended fibrotic process where the great majority of cells appear as fibroblast-like cells with none of the characteristics described for control cells (Pasquali Ronchetti et al., 1993). An increased deposition of fibrillar collagens, fibronectin, and glycosaminoglycans has been widely described in the diseased tissue (Menzel et al., 1979; Bazin et al., 1980; Brickley-Parson et al., 1981; Flint et al., 1982; Slack et al., 1982; Menzel, 1984) even though cellular and extracellular matrix alterations have been observed also in the apparently unaffected branches of the palmar aponeuroses of Dupuytren's patients (Pasquali Ronchetti et al., 1993), suggesting the involvement of the whole aponeurosis during the disease.

The present study investigates, in vitro, structural

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and behavioral features of palmar aponeurotic cells derived from normal subjects and from Dupuytren's affected patients, in comparison with dermal fibroblasts from healthy individuals and from the same patients. The aim of this work is to add information on the physiological role of normal aponeurotic cells and on the importance of cell-matrix interactions in Dupuytren's disease. Data indicate that normal human palmar aponeurotic cells have little interaction with collagen and fibronectin, compared with normal human dermal fibroblasts, and that Dupuytren's aponeurotic cells exhibit higher expression of some integrins and of adhesion properties to the same matrix ligands, compared with normal aponeurotic cells.

MATERIALS AND METHODS

Cells and tissues

Cells from palmar aponeuroses of eight control subjects, treated for trauma, and of 10 patients affected by Dupuytren's disease, who underwent surgical treatment, were grown in DMEM plus antibiotics in 25 cm^2 flasks (Nunc, Roskilde, Denmark) and were used between the third and the eight passage. Diseased cells obtained from still clinically normal aponeurotic branches and from fibrotic retracted cords of the same patients were kept separate and grown in parallel. Forearm dermal fibroblasts from six healthy individuals and from two Dupuytren's patients were obtained during surgical procedures, after informed consent, and cultured in parallel with the aponeurotic cells. Small samples from each biopsy were also fixed and embedded for electron microscopy in order to have always a morphologic comparison of the cellular and extracellular compartments in situ.

Adhesion and spreading assay

Petri dishes (35 mm in diameter) were coated with the following matrix molecules: Vitrogen (10 $\mu g/cm^2$, Celtrix, Palo Alto, CA), collagen type III (10 $\mu g/cm^2$, Sigma, St. Louis, MO), fibronectin (3 $\mu g/cm^2$, Telios Pharmaceuticals, San Diego, CA), and gelatin (0.2 $\mu g/cm^2$, BDH, Poole England). Coating was performed with 1 ml of the solution that was allowed to dry in sterile conditions. Dishes were then washed with PBS before seeding the cells. Trypsinized cells were washed in serum-free DMEM, centrifuged, and 100,000 cells in 1 ml suspension were added to each dish and allowed to attach by setting for 15′, 30′, 60′, 90′, 120′, and 240′ at 37°C in 5% CO_2/95% air. Unattached cells were removed by gentle washes with PBS, whereas attached cells were fixed in 3% paraformaldehyde in PBS for 10′, washed in PBS, stained with crystal violet (0.5%, in 20% methanol), and extensively washed with water. The color retained by cells was eluted with 1ml of sodium citrate (0.1M in 50% ethanol, pH 4.2) and read in a spectrophotometer at 540 nm for cell adhesion evaluation. All experiments were performed in triplicate. Cell spreading was investigated by phase contrast light microscopy (Zeiss axiophot) (Santoro et al., 1994).

Collagen gels

Collagen gels were prepared by using both Vitrogen collagen solution (Celtrix) or collagen prepared by extraction from rat tail tendons in 0.1% acetic acid. In this latter case, the preparation was tested by Western blot: collagen type I represented 90–93% of the solution, fibronectin accounted for a further 1–2%, whereas the remaining 5–9% was comprised by other molecules, i.e., collagen type III and glycoproteins. Cells were added to the neutralized collagen solutions (2 mg protein/ml) at a concentration of 1×10^5 cells/ml. Aliquots (2 ml) of the cell/collagen mixture were placed in 35mm-diameter Petri dishes and allowed to polymerize at 37°C for 15–20 min. To create floating collagen gels, the edge of lattices was rimmed with a sterile pipette, the dish was gently shaken, and the gel was covered with 2 ml of DMEM with 10% fetal calf serum. Experiments were done in triplicate and retraction was determined every 12 hr during a 3-day period by measuring the gel area on a millimetric scale (Santoro et al., 1994).

Cell-matrix inhibitors

Cells were grown up to 48 hr into collagen gels in the presence of synthetic peptides (GRGDSP, GRGDTP, GRADSO, GRGESP) (Calbiochem, La Jolla, CA, and Telios) or in the presence of antibodies against α_2, α_5; or β_1 chains of the integrins (Telios). Peptides or antibodies were added to the medium, after gel polymerization, every 24 hr, at the concentration of 1 μg/ml.

Morphology

Cells, grown on glass or on plastic surfaces, coated or not with connective tissue molecules, were: a) fixed with 3% paraformaldehyde in PBS for 30′ at 4°C and observed, either unstained or stained with hematoxylin eosin or crystal violet, in a Zeiss axiophot optical microscope in a phase contrast mode; b) fixed with 2.5% glutaraldehyde in Tyrode's solution, dehydrated, processed for critical point drying, coated with platinum-coal, and observed with a Philips 400S scanning electron microscope; c) fixed with 2.5% glutaraldehyde in Tyrode's solution, dehydrated, embedded in Spurr resin, sectioned, and observed in a Siemens 1A and a Jeol 1200EXII transmission electron microscope.

Cells, grown into collagen gels, were a) fixed with 3% paraformaldehyde in PBS for 30′ at 4°C, stained with 1% Toluidine blue and observed with an Olympus inverted microscope; b) fixed in 2.5% glutaraldehyde in Tyrode's solution, dehydrated, embedded in Spurr, sectioned, and observed with a Siemens 1A and a Jeol 1200EXII transmission electron microscope.

FACS analysis

Before confluence, cells were removed from tissue culture flasks with 10 ml EDTA (10 mM in PBS, without calcium and magnesium) at 37°C. EDTA was blocked by addition of the same amount of PBS with calcium and magnesium. Cells were washed twice with PBS and resuspended to 5×10^5 cells in 200 μl PBS^- and incubated for 30′ with 5 μl of undiluted monoclonal antibodies against the α_2 and α_5 chains of human integrins (Telios) and the HaR (anti-human heterotypic adhesion receptor, Telios). Cells to be investigated for the α_1 integrin expression were washed and resuspended to 1.8×10^6 cells in 1 ml of 2% paraformaldehyde in PBS^-, digested for 10 min with Triton X-100 (1% in PBS^-), preincubated for 10 min with 1% human serum in PBS^-, then incubated for 1 hr with 5 μl of undiluted polyclonal antibodies against the α_1 integrin (Calbiol-

chem). After a rapid centrifugation, all cells were incubated for 30′ with phycoerythrin-labeled anti-mouse-lgG (Dako, Glostrup, Denmark) (1:10 diluted) or with FITC labeled anti-rabbit lgG (Dako) (1:10 diluted). Controls were established using the secondary antibody alone. Washed cells were resuspended in 250 μl of PBS^{-} and analyzed on a FACScan (Becton Dickinson, San Jose, CA). Debris and dead cells were excluded by forward and side scatter gating. Ten thousand events were collected and evaluated from each cell type using a Lysyl II Software.

RESULTS

Morphology

By light microscopy, normal aponeurotic cells, grown at confluence on glass slides in standard conditions, appeared larger and flatter than dermal fibroblasts and pathologic cells. These features were also confirmed by scanning electron microscopy, whereas transmission electron microscopy did not reveal any peculiar cytoplasmic characteristics of the aponeurotic cells compared with dermal fibroblasts nor revealed differences between normal and pathologic cells (data not shown). In the present study, only normal dermal fibroblasts were taken as reference, since forearm dermal fibroblasts from healthy subjects and from patients affected by Dupuytren's disease were always identical from both the morphological and behavioral points of view (data not shown).

When seeded on different substrates, both normal and pathological aponeurotic cells exhibited a rather peculiar appearance compared with dermal fibroblasts. Figure 1 shows the results after 4 hr from seeding. On bacteriological dishes, palmar aponeurotic cells, either normal (Fig. 1E) or from patients (Fig. 1I,M), spread less efficiently than dermal fibroblasts (Fig. 1A). Vitrogen was mostly effective in inducing an high number of long and thin cytoplasmic protrusions in all aponeurotic cells (Fig. 1F,J,N). All cell types spread efficiently on fibronectin substrate, however aponeurotic cells (Fig. 1G,K,O) were always less efficient than dermal fibroblasts (Fig. 1C). Similar results were obtained when cells were seeded on a mixture of Vitrogen and fibronectin (Fig. 1D,H,L,P).

Cells grown up to 24 hr into a three-dimensional collagen gel, showed a polymorphous shape: normal aponeurotic cells exhibited long cytoplasmic branches, whereas pathologic aponeurotic cells from fibrotic cords were generally less elongated (data not shown). No significant differences were observed by transmission electron microscopy as to the subcellular organization of aponeurotic cells compared with dermal fibroblasts.

Adhesion

In the adhesion experiments, as expected, normal dermal fibroblasts showed high adhesiveness to all substrates tested. By contrast, control aponeurotic cells, compared with dermal fibroblasts, exhibited low adhesiveness to Vitrogen (Fig. 2A, $P < 0.01$ by ANOVA), fibronectin (Fig. 2B, $P < 0.02$ by ANOVA), Vitrogen in combination with fibronectin (Fig. 2C, $P < 0.001$ by ANOVA), collagen type III (Fig. 2D, $P < 0.002$ by ANOVA), and gelatin (Fig. 2E, $P < 0.1$ by ANOVA). Diseased aponeurotic cells showed higher adhesiveness for all the extracellular matrix molecules tested, compared with control aponeurotic cells, approaching that of dermal fibroblasts. Significant differences between control aponeurotic cells and cells from fibrotic cords were observed when cells were seeded on Vitrogen (Fig. 2A, $P < 0.001$ by ANOVA), fibronectin (Fig. 2B, $P < 0.001$ by ANOVA), Vitrogen in combination with fibronectin (Fig. 2C, $P < 0.01$ by ANOVA), collagen type III (Fig. 2D, $P < 0.01$ by ANOVA), and gelatin (Fig. 2E, $P < 0.04$ by ANOVA). Moreover, adhesion to gelatin appeared to be even higher in cells from fibrotic cords than in dermal fibroblasts (Fig. 2E, $P < 0.1$ by ANOVA).

Collagen gel retraction

Cell-matrix interactions were also investigated by growing cells into collagen matrices, either Vitrogen or collagen extracted from rat tails, and by measuring lattice retraction with time up to 3 days. Normal aponeurotic cells were less efficient than dermal fibroblasts in retracting the collagen gel; on the contrary, aponeurotic cells from patients retracted collagen gels to a greater extent compared with normal aponeurotic cells and more similar to dermal fibroblasts (Fig. 3). Moreover, the rate of retraction was different depending on the area of origin of the diseased cells: cells from fibrotic cords were more efficient in gel retraction than cells from clinically normal aponeurotic branches of the same patient.

Gel retraction was further investigated by the use of cell-matrix inhibitors, such as RGD peptides and anti α_2, α_5, and β_1 antibodies (Fig. 4). Among the tested RGD peptides, the GRGDTP peptide produced the most homogeneous and clearest results when added to the medium after gel polymerization. Antibodies to the α_1 subunit of integrins were always the most effective inhibitors of gel retraction, whereas the role of RGD peptides or of the antibodies towards α_2 and α_5 varied according to the cell type. Dermal fibroblasts were mostly sensitive to α_5, and less to α_2 antibodies and to the RGD peptide GRGDTP. Normal aponeurotic cells were mainly sensitive to the peptide, whereas were less influenced by either α_2 or α_5 antibodies. Diseased aponeurotic cells behaved differently from controls: cells derived from the fibrotic cords were unaffected by the RGD peptide and by the α_5 antibodies, at least at the same concentrations which were effective on normal aponeurotic cells; cells derived from the clinically normal aponeurotic branches of patients behaved in between those from control and fibrotic cord-derived cells (Fig. 4).

Integrin analysis

The integrin pattern of dermal fibroblasts and of normal and diseased aponeurotic cells was further investigated by FACS (Fig. 5). Major differences regarded the expression of the α_2 chain which was markedly reduced in all aponeurotic cells, compared with dermal fibroblasts. Moreover, there were differences among aponeurotic cells: the lowest expression was in cells from control aponeuroses, whereas levels were higher in cells derived from clinically unaffected aponeurotic branches, and even higher in cells from fibrotic retracted cords of patients. Differences between cell types were only negligible as far as α_1 integrin expression: compared with dermal fibroblasts, in fact, the α_1 integrin expression was only slightly diminished in cells

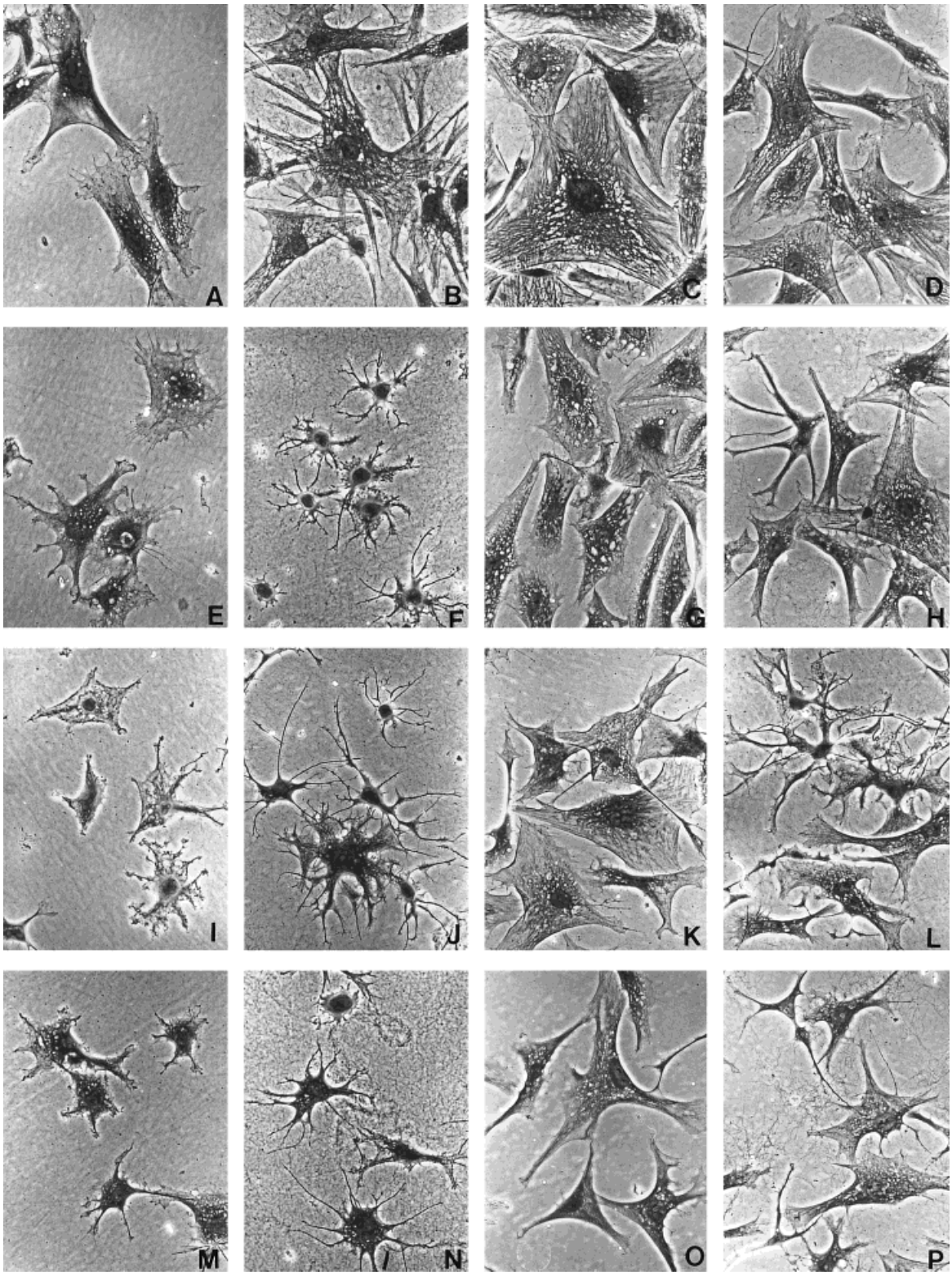

Fig. 1. Light microscopy of normal dermal fibroblasts (**A–D**), control aponeurotic cells (**E–H**), and aponeurotic cells from clinically unaffected areas (**I–L**) or from fibrous cord (**M–P**) of the same Dupuytren's patient, seeded for 4 hr on different substrates. On bacteriological dishes (A,E,I,M), all palmar aponeurotic cells showed an arborized morphology and spread less efficiently than dermal fibroblasts. Vitrogen (B,F,J,N) was mostly effective in inducing an high number of long cytoplasmic protrusions in all aponeurotic cell clones, whereas all cell types spread more efficiently on a fibronectin substrate (C,G,K,O) or on a combination of Vitrogen and fibronectin (D,H,L,P), however, aponeurotic cells were always less efficient than dermal fibroblasts. Cells were observed in a phase contrast mode (×200).

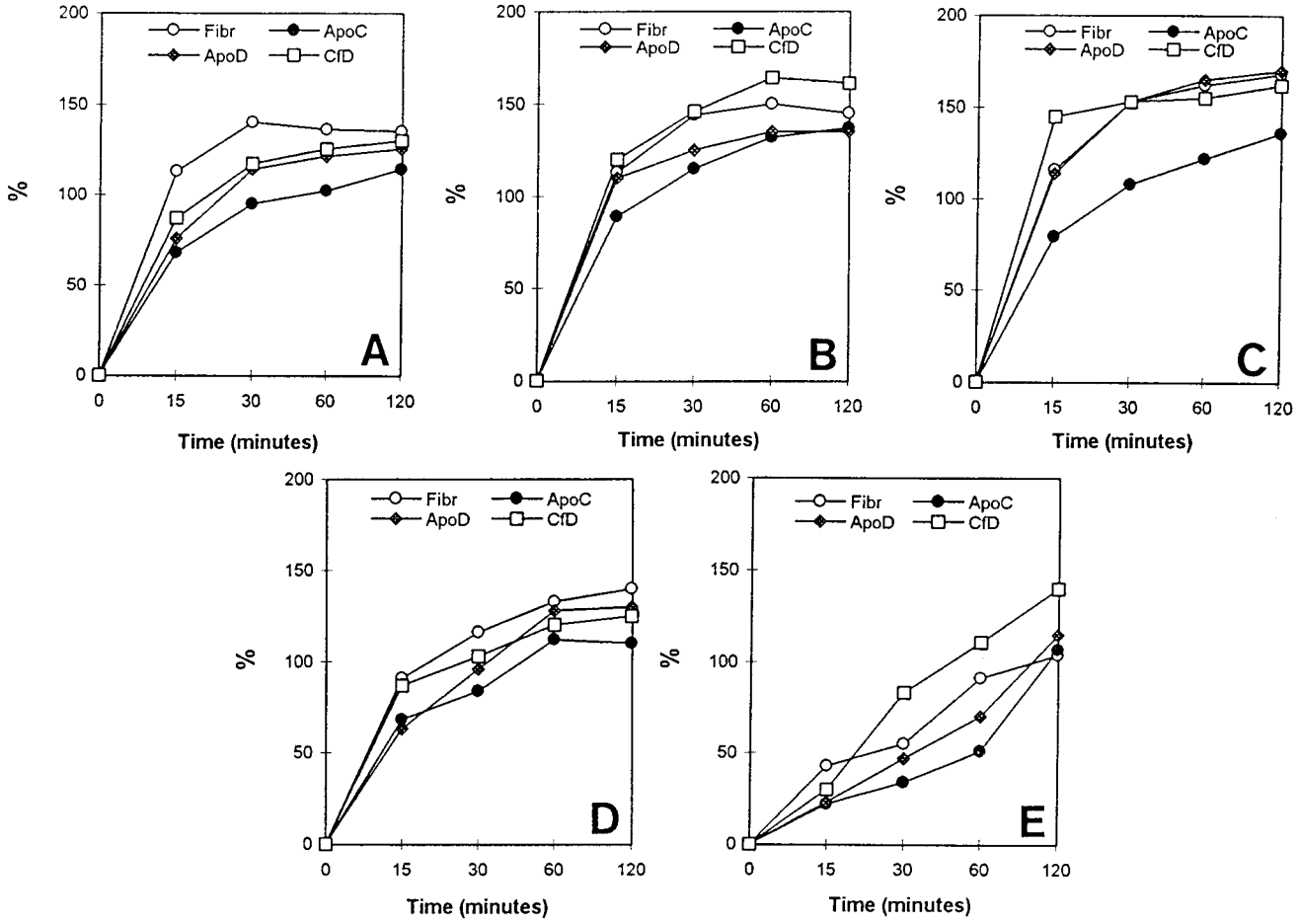

Fig. 2. Adhesion of normal dermal fibroblasts (Fibr), control aponeurotic cells (ApoC), and aponeurotic cells from clinically unaffected areas (ApoD) or from fibrous cord (CfD) of the same Dupuytren's patient, on Vitrogen (**A**), fibronectin (**B**), Vitrogen in combination with fibronectin (**C**), collagen type III (**D**), and gelatin (**E**). Dermal fibroblasts showed high adhesivness to Vitrogen, collagen type III and fibronectin. Control aponeurotic cells exhibited the lowest adhesiveness to all substrates tested, whereas diseased aponeurotic cells, compared with the normal ones, showed increased matrix adhesiveness. Data are from one experiment representative of five.

from normal or clinically unaffected aponeuroses. Furthermore, minor changes were observed in the expression of α_5 integrin chains, by comparing dermal fibroblasts and aponeurotic cells, with the one exception of cells from the fibrotic cords that exhibited the highest expression of this integrin. The expression of HaR appeared increased only in the cells from unaffected aponeuroses of patients (Fig. 5).

DISCUSSION

The present study was designed to characterize, from both structural and behavioral points of view, normal and diseased aponeurotic cells in vitro paying particular attention to cell-matrix interactions.

Cell populations were obtained from control aponeuroses, from still clinically normal aponeurotic branches, and from fibrotic cords of patients, these last two representing the very early and the final stage of the disease, respectively. Cells from nodules were excluded on purpose from this study, because of their heterogeneity (Azzarone et al., 1983; Pasquali Ronchetti et al., 1993). In our experiments, aponeurotic cells exhibited a rather stable phenotype, at least up to the tenth passage in culture. Results were compared with those obtained with dermal fibroblasts of healthy subjects and of Dupuytren's patients. Differences were never observed between dermal fibroblasts from normal subjects and from patients affected by Dupuytren's contracture, confirming that this disease is confined to the aponeurotic tissue and is not a generalized connective tissue disorder.

Aponeurotic cells, grown in monolayer in vitro, were morphologically different from dermal fibroblasts, and these differences were even more pronounced when cells were seeded on, or within, extracellular matrix substrates, suggesting that aponeurotic cells have a peculiar phenotype which may reflect in vivo-specific functional behavior. Diseased aponeurotic cells, derived either from still clinically unaffected aponeurotic branches or from fibrotic retracted cords, were, morphologically, only slightly different from the control ones. This may suggest that Dupuytren's disease might be

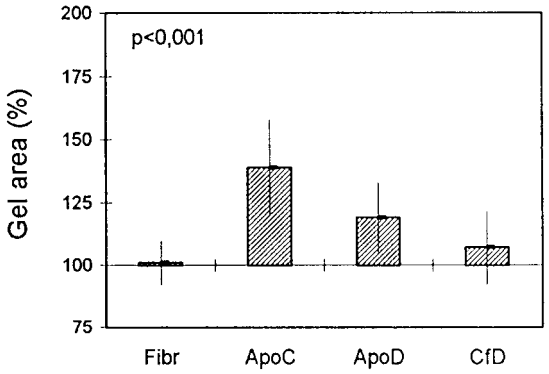

Fig. 3. Rat tail collagen gel retraction, after 24 hr from cell seeding, by normal dermal fibroblasts (Fibr), control aponeurotic cells (ApoC), and aponeurotic cells from clinically unaffected areas (ApoD) or from fibrous cord (CfD) of the same Dupuytren's patient. Differences between cell types were significant ($P \leq 0.001$ by analysis of variance) indicating that i) normal aponeurotic cells were less efficient than dermal fibroblasts in retracting the collagen gel ($P \leq 0.0001$ by the Student's t-test), and ii) diseased aponeurotic cells retracted the gels to a greater extent than normal cells (ApoD vs. ApoC: $P \leq 0.06$; CfD vs. ApoC: $P \leq 0.001$ by the Student's t-test). Data represent the mean value ± standard deviation of eight experiments and are expressed as percentage of the gel area, considering 100 the area after retraction by dermal fibroblasts.

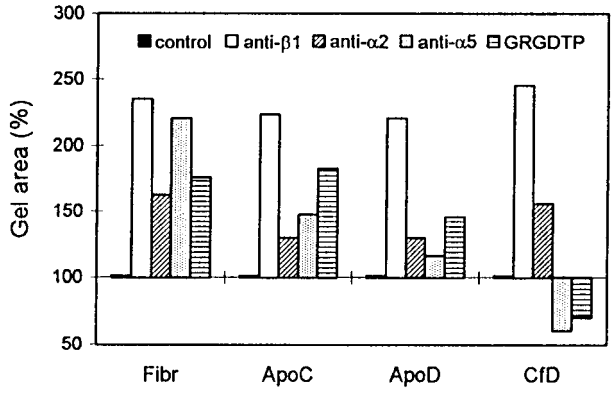

Fig. 4. Role of cell-matrix interaction inhibitors (anti-β1, anti-α_2, anti-α_5, GRGDTP) on rat tail collagen gel retraction. Lattice retraction was always inhibited by β_1 antibodies ($P \leq 0.0001$). Dermal fibroblasts (Fibr) were mostly sensitive to α_5 ($P \leq 0.001$) and less to α_2 antibodies and GRGDTP ($P \leq 0.05$). Normal aponeurotic cells (ApoC) were mainly sensitive to the peptide ($P \leq 0.001$), whereas were less influenced by α_2 or α_5 antibodies ($P \leq 0.06$). Cells derived from the fibrotic cords (CfD) were unaffected by the RGD peptide and by the α_5 antibodies ($P \leq 0.05$), whereas cells derived from clinically unaffected aponeurotic branches (ApoD) behaved in between those from control and fibrotic cord-derived cells. Data are expressed as percentage variation of the gel area resulting after inhibitor supplementation, compared with that normally occurring, within each cell type, in the absence of inhibitors (control) and statistically evaluated with the Student's t-test. One experiment representative of five is shown.

related also to altered cell-matrix interactions and to test this hypothesis, dermal fibroblasts, control aponeurotic cells, and cells from different areas of diseased aponeuroses were grown on various matrix substrates (Majewski et al., 1992; Raghow, 1994; Schmidt et al., 1994). During the first hour, as expected, dermal fibroblasts showed a rapid and prominent spreading on

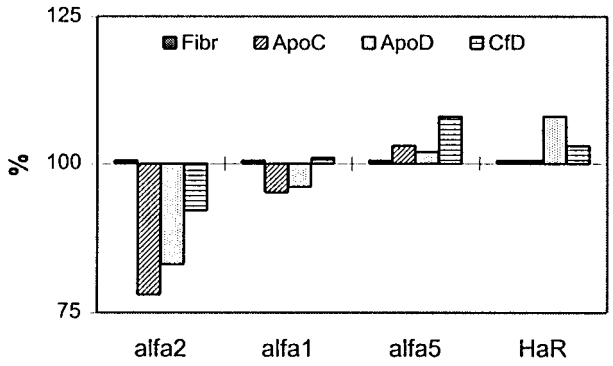

Fig. 5. Expression of integrins by FACS analysis. Compared with dermal fibroblasts (Fibr), all aponeurotic cells had lower expression of α_2 integrin chains: the lowest expression was in cells derived from control aponeuroses (ApoC) (ApoC vs. Fibr: $P \leq 0.05$ by the Student's t-test), whereas levels were slightly higher in cells derived from clinically unaffected aponeurotic branches (ApoD) and even higher in cells from fibrotic retracted cords of the same patient (CfD) (CfD vs. ApoC: $P \leq 0.05$ by the Student's t-test). Expression of the α_1 integrin chain was only moderately diminished in control and clinically normal cells, whereas levels in cells from fibrotic branches approached that of dermal fibroblasts. Expression of the α_5 integrin chains was rather similar between cells from normal or unaffected aponeuroses and dermal fibroblasts, whereas it was increased in cells from fibrotic cords (CfD vs. ApoC: $P \leq 0.07$ by the Student's t-test) A moderate increased expression of HaR was observed in diseased aponeurotic cells, especially from those derived from clinically unaffected areas (ApoD vs. ApoC: $P \leq 0.06$ by the Student's t-test). Data are from one experiment representative of seven and are expressed as percentage variation of values obtained in dermal fibroblasts that were always considered as 100.

plastic, as well as on collagen type I, fibronectin, or a mixture of these two substrates. On the contrary, both normal and diseased aponeurotic cells remained star-shaped and exhibited only few, long and thin cytoplasmic branches protruding from the roundish cell body, clearly demonstrating that aponeurotic cells spread less efficiently than dermal fibroblasts. In the adhesion tests, as already known, dermal fibroblasts showed high adhesiveness to collagens and fibronectin (Grinnell and Lamke, 1984), whereas, in the same experimental conditions, control aponeurotic cells revealed reduced adhesion to these matrix substrates. On the contrary, Dupuytren's aponeurotic cells, compared with the control ones, showed higher adhesiveness to all matrix molecules tested, including gelatin which is a randomly hydrolyzed collagen comprised of a mixture of short peptides (Isemura et al., 1982; Santoro et al., 1994).

These data have been reinforced by experiments in which cells were grown into three-dimensional matrices of Vitrogen or of collagen extracted from rat tail tendons (Guidry and Grinnell, 1987; Heath and Paechey, 1989; Nakagawa et al., 1989; Doane and Burk, 1991; Eastwood et al., 1996). Rather interestingly, although in vivo normal aponeurotic cells have a myofibroblast-like phenotype (Baccarani Contri et al., 1994), when cultured in vitro, they exhibited a significantly lower retraction capability compared with dermal fibroblasts; on the contrary, diseased aponeurotic cells induced a relevant lattice retraction, more similar to that of dermal fibroblasts.

It has been recently shown that cells derived from

various tissues have different interactions with matrix molecules and that, for instance, tenocytes have delayed and reduced contractile forces compared with dermal fibroblasts (Eastwood et al., 1996). Our data clearly indicate that normal aponeurotic cells behave rather similar to tenocytes in spite of their myofibroblast-like appearance in vivo; furthermore, adhesion and spreading results show that diseased aponeurotic cells exhibit higher adhesiveness to matrix molecules than the control ones, and this could be relevant for the comprehension of the process of aponeurotic contracture in Dupuytren's disorder.

More information concerning the receptors and the integrins involved in the phenotype of aponeurotic cells was obtained in experiments in which the lattice retraction was inhibited by adding to the culture medium RGD peptides (Pytela et al., 1987; Ruoslahti and Pierschbecher, 1987; Williams, 1992) or anti-integrin antibodies and by FACS analyses of isolated cells for the expression of some integrins (α_1 α_2 α_5) and of the HaR (human heterotypic adhesion receptor): the former being some of the major cell binding sites of collagen type I and fibronectin to cells (Fogerty et al., 1990; Ruoslahti, 1991; Bosman, 1993), and the latter representing an unspecific receptor for matrix molecules (Gallatin et al., 1989).

The use of cell-matrix interaction inhibitors revealed that the lattice retraction could be efficiently reduced in dermal fibroblasts and, at progressively reduced rate, in normal and diseased aponeurotic cells, respectively. As already known for RGD peptides (Williams, 1992), GRGDTP peptide was rather efficient in inhibiting collagen lattice retraction by dermal fibroblasts probably because of its broad inhibition spectrum (i.e., collagen, fibronectin, and vitronectin) (Pierschbacher and Ruoslahti, 1984). Gel retraction by aponeurotic cells was less and less inhibited by GRGDTP passing from control aponeurosis towards clinically unaffected aponeurotic branches to the fibrotic cord of patients, where, on the contrary, the RGD peptide seemed to favor gel retraction. This paradoxical phenomenon has been already described for other cell culture systems and interpreted as an RGD peptide-induced relocation of membrane integrin/s with a net increase of cell-matrix interactions (Ylanne, 1990; Koivunen et al., 1994).

Collagen gel retraction by dermal fibroblasts was inhibited by antibodies towards β_1, α_2, and α_5 integrin subunits, in agreement with the finding that dermal fibroblasts mainly interact with collagen and fibronectin (Gillery et al., 1986). Antibodies towards β_1 integrin subunits induced a dramatic reduction of lattice retraction in all aponeurotic cells tested, suggesting that aponeurotic cell-matrix interactions are mainly mediated by β_1-containing integrins (Gullberg et al., 1990). By contrast, antibodies against α_2 chains, and especially those against α_5 integrin subunits, had little effect in inhibiting gel retraction in all strains of aponeurotic cells, compared with dermal fibroblasts. These results seem to indicate that aponeurotic cells have scarce interaction with collagen and fibronectin through $\alpha_2\beta_1$ and $\alpha_5\beta_1$, respectively. Therefore, it must be admitted that integrins other than $\alpha_2\beta_1$ and $\alpha_5\beta_2$ are involved in cell-matrix interactions, especially in pathologic aponeurotic cells where collagen gel retraction data show that diseased cells are more efficient than normal cells in retracting collagen lattice.

Data from collagen gel retraction experiments have been confirmed by FACS analysis which showed that α_2 integrin subunit expression was very low in control aponeurotic cells, and was higher in diseased cells without reaching, however, the level of dermal fibroblasts. Moreover, FACS revealed that the expression of the α_5 integrin chain was similar in dermal fibroblasts, and in control and clinically unaffected aponeurotic cells, whereas was higher on cells from fibrotic branches. These data are consistent with those from both adhesion and collagen lattice retraction experiments. In fact, although fibronectin seems to be implicated in the process of collagen gel retraction (Gillery et al., 1986; Asaga et al., 1991), it has been demonstrated that antibodies against the α_5 integrin chain can efficiently inhibit attachment and migration of cells on fibronectin, but have poor effects on collagen gel contraction, suggesting that other integrins may be involved in this process (Schiro et al., 1991; Tomasek and Akiyama, 1992; Carver et al., 1995). For instance, the α_1 containing integrins, when compared with dermal fibroblasts, appeared slightly reduced only in normal and unaffected aponeurotic cells. By contrast, the HaR receptor, which represents a less specific binding site for matrix molecules, such as collagen (Gallatin et al., 1989), was increased in cells derived from still clinically unaffected areas of Dupuytren's aponeuroses, suggesting that this event could represent an early sign of the general reorganization of cell-matrix interactions occurring in Dupuytren's disease.

The main results from this study can be summarized as: 1) normal human palmar aponeurotic cells are characterized, in vitro by low interaction with the matrix, and this indicates that cells confer low constraint to the normal aponeurotic tissue: 2) cell-matrix interactions, approaching that of dermal fibroblasts, appeared to be higher in Dupuytren's than in normal aponeurotic cells and may be related, at least in part, with the matrix retraction occurring in Dupuytren's disease: and 3) cells from the still clinically unaffected aponeurotic branches of patients are already altered and committed to disease as previously suggested (Pasquali Ronchetti et al., 1993).

In conclusion, this study provides a further characterization of human palmar aponeurotic cells in comparison with dermal fibroblasts, and with cells derived from palmar aponeuroses of Dupuytren's patients, also in the attempt to understand the role of cell-matrix interactions in Dupuytren's contracture.

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LITERATURE CITED

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