Mitogenic Effect of Transforming Growth Factor-β1 on Human Ito Cells in Culture: Evidence for Mediation by Endogenous Platelet-derived Growth Factor

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We assessed the effect of transforming growth factor-β1 on the proliferation of human Ito cells. Ito cells in their myofibroblast-like phenotype were grown from explants of human liver and were characterized with electron microscopy and positive immunostaining for desmin and smooth muscle α-actin. Transforming growth factor-β1 was mitogenic for human Ito cells whatever the culture conditions, although it was, as previously described, inhibitory of growth for rat Ito cells. The mitogenic effect of transforming growth factor-β1 was likely due to induction of autocrine platelet-derived growth factor chain secretion by Ito cells themselves because (a) the mitogenic effect of transforming growth factor-β1 was blocked by specific platelet-derived growth factor antibodies, (b) transforming growth factor-β1 increased platelet-derived growth factor-A chain messenger RNA expression and platelet-derived growth factor-AA secretion by human Ito cells and (c) human Ito cells expressed the α-type platelet-derived growth factor-A receptor messenger RNA. Exogenous platelet-derived growth factor-AA was also mitogenic for human Ito cells, mimicking the effect of transforming growth factor-β1. Our data suggest that results obtained with rat Ito cells must be extrapolated with caution to human ones. The mitogenic effect of transforming growth factor-β1 on human Ito cells probably has pathophysiological relevance because transforming growth factor-β1 has been demonstrated in vivo at sites of active liver fibrogenesis. (HEPATOLOGY 1993;18:137-145.)

The role of hepatic Ito cells in the constitution of liver fibrosis has been well established by a variety of studies. In situ hybridization has demonstrated that Ito cells are the main cell type expressing transcripts for procollagens and laminin in human (1, 2) and experimental liver fibrogenesis (3-5). Studies of purified cell populations from fibrotic rat liver have shown that procollagens and laminin transcripts are much more increased in Ito cells than in hepatocytes (6). Very recently, data from a modified in vivo dual-radiolabel technique have incriminated nonparenchymal cells in more than 90% of collagen formation after injection of CCl4 in rats (7). Finally, many studies have shown that cultured rat Ito cells have the ability to synthesize most liver fibrosis components (8, 9).

During hepatic fibrogenesis, Ito cells proliferate (10-13) and acquire a modified phenotype, which is considered transitional (12, 14), myofibroblast-like (15) or activated (13, 16). The mechanisms responsible for Ito-cell proliferation have been examined mostly with cultures of rat Ito cells. To our knowledge, the regulation of human Ito-cell proliferation has not been studied. The importance of transforming growth factor-β1 (TGF-β1) as a major profibrogenic agent in human liver fibrogenesis has been stressed very recently by two findings. First, TGF-β1 protein (17) or messenger RNA (mRNA) (18, 19) is detected at sites of active liver fibrogenesis in human liver specimens. Second, liver expression of TGF-β1 mRNA has been shown to be increased during chronic hepatitis C, a condition associated with progressive fibrosis; this expression was correlated with the fibrogenic activity of the disease (20). In this study, we demonstrate a mitogenic effect of TGF-β1 on human Ito-cell proliferation and characterize its mechanism using a model of cultured myofibroblastic human Ito cells.

MATERIALS AND METHODS

Materials. All reagents for culture media were from Gibco (Paisley, Scotland). All tissue-culture plasticware was from Falcon Plastics (Becton Dickinson, Oxnard, CA) except for 96-well plates, which were from Costar (Cambridge, MA). LabTek culture slides were obtained from Miles (Puteaux, France). TGF-β1 (purified from human platelets) recombinant human platelet-derived growth factor (PDGF)-AA homodimer and rabbit PDGF-AA antibody were from Genzyme (Cam-
bridge, MA). Human recombinant PDGF-BB homodimer and epidermal growth factor (EGF) were from Upstate Biotechnology (Lake Placid, NY). Human recombinant basic fibroblast growth factor (bFGF) was a gift from G. Bouche, Ph.D. (CNRS, Toulouse, France). Heparin-Sepharose was from Pharmacia (St. Quentin-en-Yvelines, France). Monoclonal desmin antibody was from Biosys (Compiegne, France), and purified purified chicken desmin was from Boehringer-Mannheim (Meylan, France). Monoclonal antibody to smooth muscle α-actin, MOPC21 mouse monoclonal IgG, normal goat and rabbit IgG, dianobenzidine, hydrogen peroxide, nitro-blue tetrazolium and 5-bromo-4-chloro-3-indolyl phosphate were from Sigma Chemical Co. (St. Louis, MO). Affinity-purified biotinylated antibodies to mouse or rabbit IgG, avidin and biotinylated alkaline phosphatase or peroxidase were from Vector Laboratories (Burlingame, CA). Polyclonal goat antihuman PDGF neutralizing the three different isoforms of PDGF (AB, AA and BB) was from British Biotechnology (Oxford, UK). [Methyl-\(^3\)H](thymidine (925 GBq/mmol), Hybond C “Extra” nitrocellulose sheets and Hybond N were obtained from Amersham (Les Ulis, France). Normal human serum, blood group AB, was obtained from the local blood transfusion department. All other reagents were of the highest grade available.

Isolation of Human Ito Cells. Human Ito cells in their activated phenotype were isolated from explants of discarded nontumoral liver tissue obtained during hepatectomy from patients with cirrhosis with associated HCC (three patients), primary hemochromatosis without fibrosis but with coincident focal nodular hyperplasia (one patient) or histologically normal livers (one normal liver with HCC and one patient with primary oaxalos who underwent liver transplantation). Handling of human tissue was performed in accordance with French ethical regulations. The procedure was essentially that described by Chen et al. (21), with a few modifications. The explants were placed in Dulbecco’s MEM (DMEM) containing antibiotics, 2 mmol/L glutamine, 5% FCS and 5% pooled human AB serum (DMEM 5/5) [with 5% FCS and 5% human serum]. Growing Ito cells were detected at day 10. They were recovered by trypsinization after about 3 wk after the start of the explant culture and seeded at 5 × 10^6/dish in 10-cm diameter dishes. They were used for growth experiments between the third and eighth passages without noticeable differences in results.

Isolation of Rat Ito Cells. Rat Ito cells in their activated phenotype were isolated by overgrowth according to the technique described by Chen et al. for mouse Ito cells (22), with some modifications. Hepatocytes were isolated from male Sprague-Dawley rats as described previously (23). These preparations are always contaminated by a small percentage of Ito cells demonstrable on desmin staining (24). After 2 wk in DMEM with 20% FCS, only a few hepatocytes remained in the flasks. Most cells were Ito cells, which were recovered on brief trypsinization. With this protocol, no further purification was necessary to obtain pure cultures of rat Ito cells (100% desmin and α-actin positive). These cells were used between the second and fourth passages.

Characterization of Cultured Ito Cells with Immunological Methods. For immunocytochemical study, human or rat cells were seeded on LabTek slides. After washing cells with buffer, we fixed the monolayers with acetone at −20 °C for 60 min. The cells were incubated with desmin (1:10) or smooth muscle α-actin (1:100) antibody; this was followed by biotinylated anti-mouse IgG, avidin-biotin-peroxidase complex and color development with diaminobenzidine in H\(_2\)O\(_2\). The slides were lightly counterstained and mounted. Construction of controls included (a) omission of the primary antibody; (b) replacement of primary antibody with an irrelevant, isotype-matched monoclonal mouse IgG, MOPC 21; and (c) abolition of staining by incubation of the antibody in the presence of an excess (100 μg/ml) of soluble desmin. The presence of smooth muscle α-actin was also demonstrated on Western blotting: Ito cells were lysed in Laemmli’s reducing buffer (25). Lysates were subjected to SDS-PAGE on 12% gels and transferred to nitrocellulose with a semidy system (Semi-Phor; Hoefer Scientific Instruments, San Francisco, CA). The membrane was incubated successively with monoclonal antibody to smooth muscle α-actin, biotinylated antimouse IgG and avidin-biotin-alkaline phosphatase complex before color development with a mixture of 5-bromo-4-chloro-3-indolyl phosphate and nitro-blue tetrazolium. As a negative control, identical protein amounts from cells of nonmuscle origin were run in parallel with the Ito cell lysates.

Transmission Electron Microscopy. For transmission electron microscopy, cells were grown in 35-mm culture dishes. They were fixed with 1.25% glutaraldehyde in cacodylate buffer and processed with standard techniques, as described previously (26).

Preparation of Depleted Human Serum. Twenty milliliters of human serum was subjected to chromatography on a 1 × 4-cm heparin-Sepharose column preequilibrated with Tris HCl 0.02 mol/L (pH 7.4) and 0.15 mol/L NaCl. The column was washed extensively with the same buffer, and the effluent was collected. Bound material was eluted with 2 mol/L NaCl in the initial buffer and discarded. The column was reequilibrated with the original buffer, and the effluent from the first pass was subjected to chromatography under the same conditions. The final effluent was dialyzed against distilled water (cut-off = 10,000 Da), lyophilized, reconstituted in 20 ml 0.15 mol/L NaCl, filtered through a 0.22-μm filter and stored in aliquots at −20 °C. This preparation is henceforth referred to as depleted human serum (DHS).

Assay of Cell Proliferation. Two cell-proliferation assays were used. (a) In \[^{3}H\]thymidine incorporation, cells were seeded at a density of 5 × 10^4/well in 96-well plates in DMEM 5/5 medium. After overnight attachment, the medium was aspirated and replaced with Waymouth’s medium/0.5% DHS to which growth factors, antibodies or both were added. In some experiments, a 5% human serum concentration was used. Waymouth’s medium, with varying concentrations of FCS, was used for rat cells. The cells were incubated for 3 days with \[^{3}H\]thymidine (0.5 μCi/well) present in the preceding 16 to 18 hr. At the end of the incubation, trichloroacetic acid (10% vol/vol) was added and the cells were kept on ice for 10 min. After another wash with trichloroacetic acid and one with methanol, the cells were solubilized in 0.2N NaOH and the radioactivity of an aliquot was measured in a scintillation counter. Sextuplicate determinations were used for every experimental point. Unless specified, the results are expressed as the ratio over those obtained with basal test medium, × 100. (b) In direct cell counting, cells were seeded at a density of 10^5/35-mm dish in DMEM 5/5 medium. After overnight attachment, one set of replicates was counted in a hemocytometer after trypsinization to assess plating efficiency. The other cells were washed with serum-free medium and exposed to test medium as described above. Medium and additives were renewed after 3 days, and cells were counted after 6 days. Duplicate or triplicate determinations were performed for each experimental point.

RNA Preparation and Northern-blot Analysis. Cells were seeded at a density of 7 × 10^5/10-cm dish and were made quiescent by a 2- to 3-day incubation in DMEM containing 0.25% DHS. They were then incubated for 6 hr in serum-free
Waymouth's medium in the presence or absence of 0.1 ng/ml TGF-β1. Total RNA was extracted according to the method of Chomczynski and Sacchi (27) and quantified by measure of its absorbance at 260 nm. After denaturation, RNA (30 to 50 µg/lane) was fractionated on a 1.2% agarose/formaldehyde gel and transferred to a nylon membrane as described (28). RNAs were crosslinked by UV irradiation, and the membrane was baked for 2 hr at 80°C. Prehybridization and hybridization were carried out at 42°C in 50% deionized formamide, 1% SDS, 5 × standard saline citrate (1 × standard saline citrate = 300 mmol/L NaCl and 30 mmol/L sodium citrate [pH 7]), 1 × Denhardt’s solution, 50 mmol/L NaH₂PO₄ (pH 6.5) and 0.25 mg/ml salmon-sperm DNA. The following complementary DNAs (cDNAs) were used: a 1.3-kb human PDGF-A chain cDNA (29), a 1.2-kb human PDGF-B chain cDNA (30), a 0.65-kb human PDGF α-type receptor cDNA (31) and a 0.65-kb human PDGF β-type receptor cDNA (32). Probes were labeled with [α-³²P]deoxy ATP with a multiprime kit (Megaprime; Amersham) according to the manufacturer’s instructions. After hybridization, blots were washed at a final stringency of 0.1 × SSPE/0.5% SDS (1 × SSPE = 150 mmol/L NaCl, 10 mmol/L NaH₂PO₄ and 1 mmol/L EDTA [pH 7.4]) at 68°C and exposed for autoradiography (Hyperfilm; Amersham). To control for loading variations, blots were rehybridized with a 1.3-kb rat glyceraldehyde-3-phosphate dehydrogenase (G3PDH) cDNA (33).

**Western-blot Analysis of PDGF-AA.** Human Ito cells were seeded at a density of 10⁶/10-cm dish and made quiescent by a 2-day incubation in serum-free Waymouth’s medium. They were incubated for 2 more days in the same medium with or without 1 ng/ml TGF-β1. Media from eight replicate dishes were pooled, dialyzed against distilled water, lyophilized and reconstituted directly in electrophoresis buffer without reducing agents. The samples were subjected to SDS-PAGE on a 12% gel and transferred to nitrocellulose. Each blot was probed with PDGF-AA antibody (1 µg/ml). Detection was achieved as described for α-actin.

**Statistical Analysis.** Results were analyzed with Student's t test for paired data.

**RESULTS**

**Isolation, Characterization and Propagation of Human Ito Cells.** Cultures of liver explants consistently yielded a homogeneous cell population growing actively after 10 to 15 days of culture. After trypsinization and seeding in new dishes, the cells reached confluency within 1 wk. On immunocytochemical study, all the cells were positive for desmin and smooth muscle α-actin (Fig. 1). No staining was seen when the primary antibody was omitted or replaced with an irrelevant IgG, and staining for desmin was abolished in the presence of an excess of purified desmin. The presence of smooth muscle α-actin was independently confirmed by the demonstration of a 42-kD actin band on a Western blot of Ito cell extract. No such band was detected in control cell lysates from rat hepatocytes, hepatoma tissue culture hepatoma cells or fibroblastic L 929 cells (Fig. 2). On transmission electron microscopy, features typical of myofibroblast-like cells, such as abundant rough endoplasmic reticulum, large bundles of microfilaments and numerous pinocytotic vesicles were observed; typical lipid droplets were not detectable (Fig. 3). The cells were propagated by weekly trypsinization at a split ratio of 1:4 to 1:6 for at least 10 passages without detectable phenotypic alterations.

**Effect of TGF-β1 on Proliferation of Human Ito Cells.** When tested in a low-mitogenicity medium (0.5% DHS), TGF-β1 induced a marked increase in [³²P]thymidine incorporation by human Ito cells (Fig. 4). The mitogenic
FIG. 2. Demonstration of smooth muscle α-actin on Western blotting. Equivalent amount of protein (1 μg) from cell extracts of (A) L929 fibroblasts, (B) hepatoma tissue culture hepatoma cells, (C) rat hepatocytes and (D) human Ito cells were subjected to SDS-PAGE, transferred to nitrocellulose and probed with α-actin antibody (1:1,000). Numbers on the left denote molecular-weight markers (in thousands). Arrow denotes the 42-kD actin band.

Effect of TGF-β1 was observed with concentrations ranging from 0.01 to 5 ng/ml, with half-maximal stimulation between 0.05 and 0.1 ng/ml and a plateau at 0.5 ng/ml. The increase in [³H]thymidine incorporation was completely blunted in the presence of 10 mmol/L hydroxyurea, showing that the increase in [³H]thymidine incorporation represented true DNA synthesis. Furthermore, direct cell counting confirmed results obtained with [³H]thymidine incorporation; cell numbers were increased with the same dose response (Fig. 5). TGF-β1 was also mitogenic in the presence of 5% human serum, but the dose-response curve differed, with a plateau at 0.1 ng/ml and a decrease toward control values at higher concentrations (Fig. 6). Identical results were obtained when TGF-β1 was added to medium containing FCS instead of human serum (data not shown). No consistent variations were seen between the different cell isolates whether they originated from normal or fibrotic liver. Table 1 shows, by comparison with TGF-β1, the mitogenic response of human Ito cells to other polypeptide growth factors.

Effect of TGF-β1 on [³H]thymidine Incorporation by Rat Ito Cells. As shown in Fig. 7, in contrast with results obtained with human cells, TGF-β1 had a dose-dependent inhibitory effect on [³H]thymidine incorporation by rat Ito cells, regardless of FCS concentration. Similar results were obtained by direct cell counting (25% and 62% inhibition at 1 and 5 ng/ml, respectively). A similar inhibitory effect was also seen when cells were grown in the presence of 0.5% DHS or various concentrations of human serum instead of FCS (data not shown). No cytotoxic effect of TGF-β1 on rat Ito cells was detectable, either on microscopic examination or on measurement of lactate dehydrogenase activity in the culture medium (not shown).

Effect of PDGF Antibody on TGF-β1–induced Human Ito Cell Proliferation. In several models, the mitogenic effect of TGF-β1 has been shown to be mediated by endogenous PDGF (34). We thus questioned whether this was the case with human Ito cells. In these experiments, TGF-β1 was coincubated with a polyclonal PDGF antibody or normal goat IgG at an equivalent concentration. As shown in Table 2, PDGF antibody at 40 μg/ml reduced by 70% the mitogenic effect of TGF-β1, whereas normal goat IgG had no such inhibitory effect. Furthermore, purified PDGF-AA and PDGF-BB were mitogenic for human Ito cells (Table 1), and their effect was blocked by the antibody with the same magnitude of
FIG. 4. Effect of TGF-β1 on [3H]thymidine incorporation by human Ito cells grown in a low-mitogenicity medium. The cells were seeded at an initial density of 5,000/well in 96-well culture plates. After overnight incubation of cells, the medium was replaced with Waymouth's medium with 0.5% DHS and the indicated concentrations of TGF-β1. Incubation lasted 3 days; [3H]thymidine was present for the last 16 to 18 hr. Results are expressed as stimulation of cells with TGF-β1 over baseline values and are the mean ± 1 S.E.M. of three to 12 determinations performed in sextuplicate. *p < 0.01. Control values ranged between 240 and 2,670 cpm.

**TABLE 1. Effects of several polypeptide growth factors on [3H]thymidine incorporation by human Ito cells**

<table>
<thead>
<tr>
<th>Growth factor (ng/ml)</th>
<th>Stimulation*</th>
<th>p Valueb</th>
</tr>
</thead>
<tbody>
<tr>
<td>TGF-β1 (0.5)</td>
<td>2.60 ± 0.72</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>PDGF-AA (20)</td>
<td>5.60 ± 1.95</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>PDGF-BB (10)</td>
<td>1.95 ± 0.27</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>EGF (5)</td>
<td>4.10 ± 1.30</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>bFGF (0.5)</td>
<td>1.27 ± 0.03</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

The experimental protocol was the same as that described in Figure 4.

*Results are shown for the growth factor concentrations yielding the maximal effect and are the mean ± 1 S.E.M. of four to nine experiments conducted in sextuplicate.

*bCompared with cells exposed to medium without growth factors.

inhibition (Table 2). Similar results were found on counting of cells after a 6-day exposure to TGF-β1 in the presence of PDGF antibody or control IgG (the mitogenic effect of 1 ng/ml TGF-β1 was inhibited by 70% with 40 μg/ml PDGF antibody in two separate experiments).

**Northern-blot Analysis of PDGF and PDGF Receptor mRNA Expression.** In the experiment depicted in Figure 8, two PDGF-A chain mRNA species, one of 2.4 and one of 2.9 kb were detectable in unstimulated human Ito cells. After 6-hr exposure to TGF-β1, steady-state levels of these mRNAs were increased 2.5 times as measured by scanning laser densitometry. Hybridization with the G3PDH probe confirmed that an identical RNA amount was loaded in each lane. Similar results were obtained in two other independent experiments. No PDGF-B chain mRNA could be detected in stimulated or unstimulated cells under our hybridization conditions. As shown in Figure 9, human Ito cells expressed transcripts for α- and β-type PDGF receptors.

**Demonstration of PDGF-AA in Conditioned Medium from TGF-β1-treated Human Ito Cells.** Western-blot analysis of concentrated conditioned medium from human Ito cells treated for 48 hr with 1 ng/ml TGF-β1 showed a band comigrating with human recombinant PDGF-AA. This band was absent in concentrated medium from control cells (Fig. 10).
Fig. 7. Effect of TGF-β1 on proliferation of rat Ito cells as measured by [3H]thymidine incorporation. The experimental design was the same one detailed in Figure 4 except that test medium contained varying concentrations of FCS, as indicated. Results are expressed as the percentage of [3H] thymidine incorporation in TGF-β1-treated cells compared with that in untreated cells and are the mean ± 1 S.E.M. of three to five experiments conducted in sextuplicate. *p < 0.05; □p < 0.001.

Table 2. Effects of PDGF antibody and normal IgG on mitogenic effects of TGF-β1, PDGF-AA and PDGF-BB on human Ito cells

<table>
<thead>
<tr>
<th>Growth factors/antibodies</th>
<th>No. of experiments</th>
<th>% of Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>TGF-β1 + PDGF antibody</td>
<td>3</td>
<td>31 ± 20*</td>
</tr>
<tr>
<td>TGF-β1 + normal IgG</td>
<td>3</td>
<td>163 ± 77*</td>
</tr>
<tr>
<td>PDGF-AA + PDGF antibody</td>
<td>2</td>
<td>31</td>
</tr>
<tr>
<td>PDGF-AA + normal IgG</td>
<td>2</td>
<td>135</td>
</tr>
<tr>
<td>PDGF-BB + PDGF antibody</td>
<td>2</td>
<td>20</td>
</tr>
<tr>
<td>PDGF-BB + normal IgG</td>
<td>2</td>
<td>138</td>
</tr>
</tbody>
</table>

The experimental design was the same described in Figure 4. TGF-β1 was used at a concentration of 0.1 ng/ml; PDGF-AA and -BB were used at 20 ng/ml and PDGF antibody and normal IgG were used at 40 μg/ml. In the absence of antibody, TGF-β1 and PDGF-AA and -BB stimulated [3H]thymidine incorporation by factors of 1.8, 6.0 and 2.7, respectively.

Results are expressed as the percentage of [3H]thymidine incorporation in growth factor and antibody (PDGF antibody or normal IgG)-treated cells vs. cells receiving only growth factor.

DISCUSSION

In this study, culture of human liver explants led to isolation of homogeneous populations of cells with features indicating that they were activated Ito cells. Their morphological and immunohistological characteristics are similar to those of the activated Ito cells that differentiate during culture of freshly isolated Ito cells from human (35) or rat liver (36, 37). They also resemble the transitional, activated, or myofibroblastlike Ito cells found in vivo in the fibrous septa during experimental (13, 14, 38) or human liver fibrogenesis (12, 39). Remarkably similar features include loss of fat droplets, development of abundant rough endoplasmic reticulum and neoeexpression of smooth muscle α-actin coexpressed with desmin (40). Expression of α-actin is widely regarded as a marker of the activation of Ito cells in rat (39), or human liver (40, 42). Whereas desmin is an unambiguous marker of rat Ito cells (43, 44), controversial results have been obtained in human subjects (21, 40, 42, 45). However, we have found desmin immunoreactivity in lobular Ito cells from normal human liver and in many cells in fibrotic septa that are likely to be activated Ito cells from normal human liver and in many cells in fibrotic septa that are likely to be activated Ito cells (Charlotte F et al., Unpublished observations, 1991). The discrepancies between the different studies probably result from use of different antibodies, different tissue-processing methods or both. We can therefore conclude that the cells isolated from liver explants represent a good culture model for activated Ito cells of the fibrotic liver. We did not detect consistent variations in experimental results.
whether the cells originated from a normal or a fibrotic liver. This is probably due to the fact that quiescent Ito cells from normal liver undergo spontaneous activation during culture (35, 36).

The factors triggering Ito-cell proliferation are still poorly understood. Furthermore, to our knowledge, no study has addressed this issue using human cells. The role of TGF-β1 was recently stressed in animal (46) and human liver fibrogenesis (17, 20). We thus measured the mitogenic response of cultured human Ito cells to TGF-β1. We found that TGF-β1 was a strong mitogenic factor for human Ito cells whether assessed by [3H]thymidine incorporation or direct cell counting. This effect was dose dependent; it was detectable with concentrations as low as 0.01 ng/ml and reached a plateau at 0.5 ng/ml. In other cell models it has been shown that TGF-β1 can stimulate or inhibit cell growth depending on culture conditions (47). With human Ito cells, the mitogenic effect of TGF-β1 could not be ascribed to a specific set of culture conditions. It was found as well in a low-mitogenicity medium (0.5% DHS), under submaximal stimulation (5% human serum) and in the presence of FCS or of other mitogenic growth factors such as EGF or bFGF (data not shown). Our results further show that the mitogenic effect of TGF-β1 on human Ito cells is indirect and is mediated at least in part through autocrine secretion of PDGF by Ito cells themselves on exposure to TGF-β1. This is evidenced by the effect of PDGF antibody, which markedly inhibited TGF-β1-mediated proliferation of human Ito cells; control IgG had no such effect. The intervention of PDGF as a mediator of TGF-β1 mitogenic effect is not unique to human Ito cells. It has already been demonstrated for other cultured mesenchymal cells such as vascular smooth muscle cells (34, 48), fibroblasts (34, 49, 50) and chondrocytes (34). Depending on cell type, the A chain (34, 48, 49) or the B chain of PDGF (50) is induced. In our study, we demonstrated induction of PDGF-A chain mRNA by TGF-β1; PDGF-B chain mRNA was undetectable. In agreement with the hypothesis of a PDGF-A autocrine loop, we found that human Ito cells had a marked mitogenic response to exogenous PDGF-AA. Furthermore, they secreted PDGF-AA in response to TGF-β1 and expressed transcripts for the α-type PDGF receptor necessary for PDGF-AA binding to the cells.

All these results contrast deeply with those obtained in several studies that have examined the effect of TGF-β1 on the proliferation of isolated rat Ito cells. In no case did TGF-β1 alone stimulate rat Ito cell proliferation; it had no effect (51) or decreased this proliferation (15, 52) by as much as 100% (53). The inhibitory effect of TGF-β1 was observed as well in low-mitogenicity medium, such as that containing 0.5% FCS (15), as in the presence of 10% FCS (53). Our own results confirm this inhibitory effect of TGF-β1 on rat Ito-cell proliferation, whatever the FCS concentration. The reasons for this major difference between rat and human Ito cells with regard to TGF-β1 mitogenic effect are not clear. It is neither due to a cytotoxic effect of TGF-β1 on rat Ito cells (which is unlikely to have been overlooked) nor to the use of a heterologous human (15, 51) or bovine factor (52) because TGF-β1 produced by cultured rat Kupffer cells was also inhibitory for rat Ito cells (16). The response to TGF-β1 has been shown to depend on the extracellular matrix on which the cells are
grown (52). In the case of the normal rat kidney cell line, it was shown that the growth-inhibitory effect of TGF-β1 was directly linked to its ability to induce collagen secretion by the NRK cells themselves (54). One could therefore speculate that TGF-β1 induces different quantitative or qualitative extracellular matrixes in rat and human cells and that this could explain the proliferation results. Finally, we believe that the difference might arise from the existence of the PDGF-A autocrine loop in human Ito cells, which cannot exist in rat Ito cells. Rat Ito cells almost completely lack the α-type PDGF receptor and thus do not respond to exogenous PDGF-AA (55-57). In the absence of an indirect mitogenic effect by PDGF-AA, rat Ito cells might be growth inhibited by a direct effect of TGF-β1. A situation in which TGF-β1, while inducing PDGF and human mammary epithelial cells, which lack PDGF receptors (58). Whatever the explanation, the difference existing between the human and rat Ito-cell responses to TGF-β1 necessitates caution on extrapolation of results obtained with rat Ito cells to the human situation.

TGF-β1 is probably not the sole mitogenic factor for human Ito cells in vivo. It is likely acting together with other growth factors such as bFGF, EGF/transforming growth factor-α and PDGF, all of which are expressed in human liver and mitogenic for cultured human Ito cells (59). Nevertheless, the mitogenic effect of TGF-β1 on human Ito cells may have a special pathophysiological relevance because increased liver expression of TGF-β1 mRNA has been reported during human liver fibrogenesis (17) and TGF-β1 has been found at sites of active fibrogenesis (17-19) (i.e., where activated Ito cells are present).

Acknowledgments: We thank C.H. Heldin for PDGF-A-chain and PDGF-α- and β-type receptor cDNAs, S.A. Aaronson for PDGF-B chain cDNA and M. Piechaczyk for G3PDH cDNA.

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