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Modulation of perisinusoidal cell cytoskeletal features during experimental hepatic fibrosis

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Summary. Hepatic perisinusoidal cells (PSCs) proliferate and are thought to be the principal source of extracellular matrix proteins during the development of liver fibrosis. We have studied the classical model of carbon tetrachloride induced liver fibrosis in order to evaluate the possible modulation of PSCs into a synthetically active and contractile cell: the myofibroblast (MF). At the ultrastructural level, this modulation was characterized by reduction of lipid vacuoles and appearance of a developed rough endoplasmic reticulum as well as of microfilament bundles. On investigating the cytoskeletal equipment of PSCs and MFs using light and electron microscopic immunohistochemistry, we found a heterogeneity of phenotypic features. While typical PSCs in normal and fibrotic livers always contained desmin, MFs expressed α-smooth muscle (SM) actin in areas of tissue injury and active fibrogenesis. Cells co-expressing α-SM actin and desmin were most prominent in the prevenular zone of the lobule (known to be vulnerable to carbon tetrachloride toxicity) and in developing fibrous septa. As demonstrated by immunogold electron microscopy, labelling of microfilament bundles by α-SM actin antibody was noted in PSCs containing lipid droplets in early stages of fibrosis; here MFs gradually accumulated and showed α-SM actin containing microfilament bundles. In scar tissue, α-SM actin expression decreased in both PSCs and myofibroblasts. Our observations support the concept of phenotypic plasticity of PSCs and confirm, at the ultrastructural level, previous suggestions of modulation of these cells into MFs in the course of liver fibrosis.

Key words: Carbon tetrachloride – Liver disease – Myofibroblast – Actin isoforms – Desmin

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Introduction

The evaluation of intermediate filament (IF) and actin isoform expression has revealed that stromal cells may express, both in normal and pathological situations, cytoskeletal and contractile proteins typical of muscle cells (for review, see Sappino et al. 1990). Perisinusoidal cells (PSCs) of rat liver, also called lipocytes, fat-storing – or Ito – cells (for review, see Aterman 1986) are an example of a resident stromal cell containing desmin in vivo (Yokoi et al. 1984) and in vitro (for review, see Ramadori 1991). Moreover, rat PSCs may express α-smooth muscle (SM) actin under culture conditions, while lacking it when freshly isolated (Ramadori et al. 1990; Rockey et al. 1992; Runnger-Brändle and Gabbiani 1983). PSCs of normal adult human liver are devoid of desmin containing IFs and exceptionally stain for α-SM actin (Skalli and Gabbiani 1988). The occurrence of an appreciable number of α-SM actin positive PSCs is a feature of various pathological settings generally associated with fibrogenesis (Schmitt-Gräff et al. 1991). Only a subset of these cells, that is to say, those observed in fibrous bands of connective tissue in cirrhosis and neoplastic or tumor-like lesions may co-express desmin (Schmitt-Gräff et al. 1991). It is well accepted that fibrotic lesions in humans and animals are characterized by the presence of fibroblasts rich in rough endoplasmic reticulum (RER) and bundles of microfilaments, also called myofibroblasts (MFs, for review, see Skalli and Gabbiani 1988). These cells express α-SM actin and desmin more or less permanently.

One of the unresolved problems concerning the biology of MFs is their origin (Gown 1990). Liver fibrosis in man, rat and baboon is known to be characterized by the presence of numerous MFs (French et al. 1988; Leo and Lieber 1983; Mak et al. 1984; Mak and Lieber 1988; Martinez-Hernandez 1985; Nakano and Lieber 1982). Whether or not they are derived from PSCs has been a controversial issue. Takase et al. (1988) demonstrated desmin staining only in cultured PSCs and not in MFs and concluded that the two cell types were dis-

tinct. In contrast, it has been suggested that an appropriate stimulation may induce a progressive modulation of PSCs into MFs (French et al. 1988; Mak et al. 1984; Tsutsumi et al. 1987). This assumption is supported by the finding that cultured PSCs exhibit a reduced number of vitamin A containing vacuoles, produce increased amounts of extracellular matrix proteins and show a modulation to MF-like cells (Friedman et al. 1985, 1989). In experimental models, damage inflicted to liver tissue has been shown to result in an enhanced expression of desmin (Geerts et al. 1991; Ogawa et al. 1986) and total actin (Gabbiani et al. 1975; Ogawa et al. 1986) and in an increased collagen synthesis by mesenchymal cells (Milani et al. 1989, 1990). Recently, the appearance of α-SM actin positive cells within scars forming in zones of necrosis induced by carbon tetrachloride has been documented (Ramadori et al. 1990; Tanaka et al. 1990). However, neither double labelling with anti-desmin and anti-α-SM actin antibodies, nor electron microscopy, has been performed to characterize these cells further. In human cirrhotic liver (Schmitt-Gräff et al. 1991), cells containing lipid droplets and expressing α-SM actin have been seen, but the study was obviously not followed chronologically. In the present study, we have examined various stages of a well accepted liver fibrosis model in the rat systematically in order to answer to the question of the possible modulation of PSCs into typical MFs. Our results support the assumption that, at least in part, MFs are derived from local PSCs.

Materials and methods

Adult Wistar rats were treated with weekly intubation of carbon tetrachloride in corn oil or with corn oil alone (controls). 0.2 ml carbon tetrachloride was administered from the 1st until the 10th week, 0.25 ml from the 11th to the 20th week, and 0.3 ml from the 21st to the 37th week of treatment in corn oil, adjusting the total volume to 0.5 ml. Before sacrifice, rats were fasted for 12 h and anaesthetized with ether. Liver tissue was perfused for 5 min either by sodium chloride 0.9% through the portal vein to wash blood out of hepatic sinusoids for further light microscopic studies, with 2% glutaraldehyde in cacodylate buffer for electron microscopic studies, or with 2.5% freshly prepared paraformaldehyde for immunoelectron microscopic studies.

Liver tissue was fixed in formaldehyde 4%. Paraffin sections were stained with haematoxylin and eosin, Giemsa, Prussian blue, Masson's trichrome for collagen and Gomori's reticulin method. Elastic staining was performed with Verhoeff's iron haematoxylin.

For immunofluorescence, liver tissue samples perfused with phosphate-buffered saline (PBS) were snap frozen in isopentane pre-cooled in liquid nitrogen. Cryostat sections (3 μm thick) were fixed in acetone for 5 min at −20° C. As primary antibodies, monoclonal α-SM actin antibody (hybridoma supernatant; Skalli et al. 1986) diluted 1:10 and affinity-purified rabbit anti-desmin (Kocher et al. 1984) at an IgG concentration of 0.025 mg/ml were employed. The secondary antibodies were rhodamine-conjugated goat anti-mouse IgG (Nordic Immunology, Tilburg, The Netherlands) diluted 1:20 and fluorescein isothiocyanate-conjugated goat anti-rabbit IgG diluted 1:10 (Nordic Immunology). For double immunofluorescence staining, both primary antibodies were applied simultaneously, as were the secondary antibodies. Controls were performed using non-immune IgG in place of the primary antibody.

Photographs were taken with a Zeiss photomicroscope (Carl Zeiss, Oberkochen, Germany) equipped with epi-illumination using a Kodak TMAX400 black and white or Ektachrome colour films (Kodak Limited, Hemel Hempstead, UK).

Immunoperoxidase staining for α-SM actin and desmin was performed on formalin-fixed and paraffin-embedded tissues. Sections were subsequently exposed to hydrogen peroxide in methanol, and subsequently in 0.1 mol/l periodic acid, 0.005 mol/l sodium borohydride, normal horse serum and then to the primary antibodies for 2 h at room temperature. Anti-α-SM actin was used at a dilution of 1:500 and anti-desmin at IgG concentrations of 0.010 mg/ml. Control sections were incubated with non-immune IgG from mouse or rabbit. After washing in PBS, the avidin-biotin complex peroxidase staining was performed, according to the manufacturer's instructions (Vectastain ABC Kit, Vector, Burlingame, Calif., USA). The peroxidase activity was revealed with 30% 3,3'diaminobenzidine (Serva, Heidelberg, Germany), containing 0.015% hydrogen peroxide. Sections were counterstained with Mayer's haematoxylin, dehydrated and mounted in Eukitt.

For electron microscopy, tissue perfused with cacodylate-buffered glutaraldehyde was minced in 1 mm diameter blocks, fixed for 30 min, post-fixed in osmium tetroxide for 2 h, dehydrated and embedded in Epon 812 (Fluka, Buchs, Switzerland) (Geerts et al. 1991). Thin sections were examined with a Philips 400 electron microscope.

For immunoelectron microscopy, samples of liver tissue were cut into pieces of less than 1 mm^3, fixed for 2 h in 2.5% paraformaldehyde – 0.1% glutaraldehyde in 0.1 M phosphate buffer and rinsed overnight in phosphate buffer. Dehydration, tissue embedding in Lowicryl K_4M resin (Chemische Werke Lowi, Waldkraiburg, Germany) and polymerization in ultraviolet light were done as previously described (Darby et al. 1990). Thin sections were mounted on Formvar-coated nickel grids, exposed to 5% normal goat serum in PBS containing 0.8% bovine serum albumin and 0.1% gelatin, and incubated for 2 h within drops of anti-α-SM actin antibody diluted 1:20 in PBS containing 1% goat serum. Control specimens were incubated with non-immune mouse IgG. After washing three times for 10 min in PBS containing bovine serum albumin and gelatin, sections were exposed to the secondary antibody diluted 1:300 for 2 h (goat anti-mouse IgG labelled with 1 nm gold particles; Janssen Life Science Production, Olen, Belgium), rinsed three times for 5 min in PBS and then in distilled water. The gold staining was amplified by reaction with colloidal silver (Danscher 1981) for 15 min. All staining procedures were performed at room temperature. Grids were counterstained with uranyl acetate and lead citrate and examined with the electron microscope.

Results

Liver from rats treated chronologically with carbon tetrachloride showed the characteristic histological features of the model (Martinez-Hernandez 1985). However, since we used carbon tetrachloride at low doses given to female animals by mouth, tissue damage was established relatively slowly. Thus, we had the opportunity to study the cytoskeletal composition of PSCs and MFs at different phases during the ongoing process of phenotypic modulation, with only slight variations in individual animals. In the first phase (1–8 weeks of treatment), no significant fibrosis was noted. In the second phase (8–12 weeks), perivenular fibrosis developed gradually but constantly. The third period (13–17 weeks) was characterized by the development of fibrous septa expanding from the perivenular area. In a fourth phase (18–32 weeks), active bridging fibrosis was present. In the last phase (32–37 weeks), most animals showed extensive bands of scar tissue without signs of active remodelling.

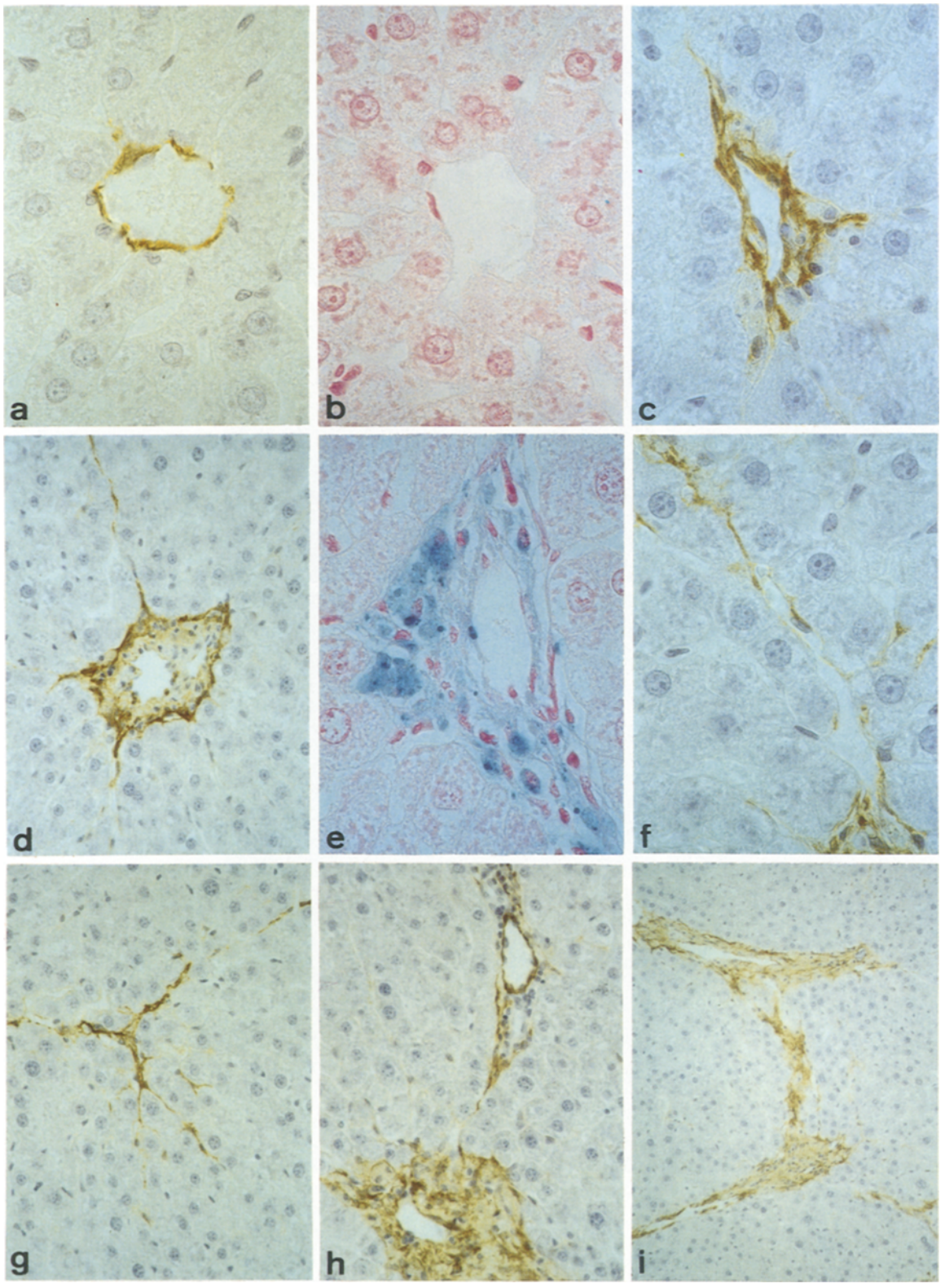

Fig. 1 a–i. Sequential changes induced by carbon tetrachloride. **a, b** Normal rat liver tissue, which shows **a** a thin layer of smooth muscle cells staining for α-SM actin around a terminal hepatic venule and **b** is devoid of Prussian blue stained macrophages. **c–e** After 8–12 weeks of carbon tetrachloride treatment, the prevenular zone contains numerous anti-α-smooth muscle (SM) actin positive cells (**c, d**) and macrophages loaded with haemosiderin (**e**) . **f, g** After 12–14 weeks, scattered foci of typical perisinusoidal cells (PSCs) lining liver cell plates are positive for α-SM actin staining. **h, i** After 17–20 weeks, numerous α-SM actin containing cells are present in septa spreading from the prevenular zone and portal tracts (**h**) and in zones of bridging fibrosis (**i**). **a–c, e, f** ×1000; **d, g, h** ×400; **i** ×200

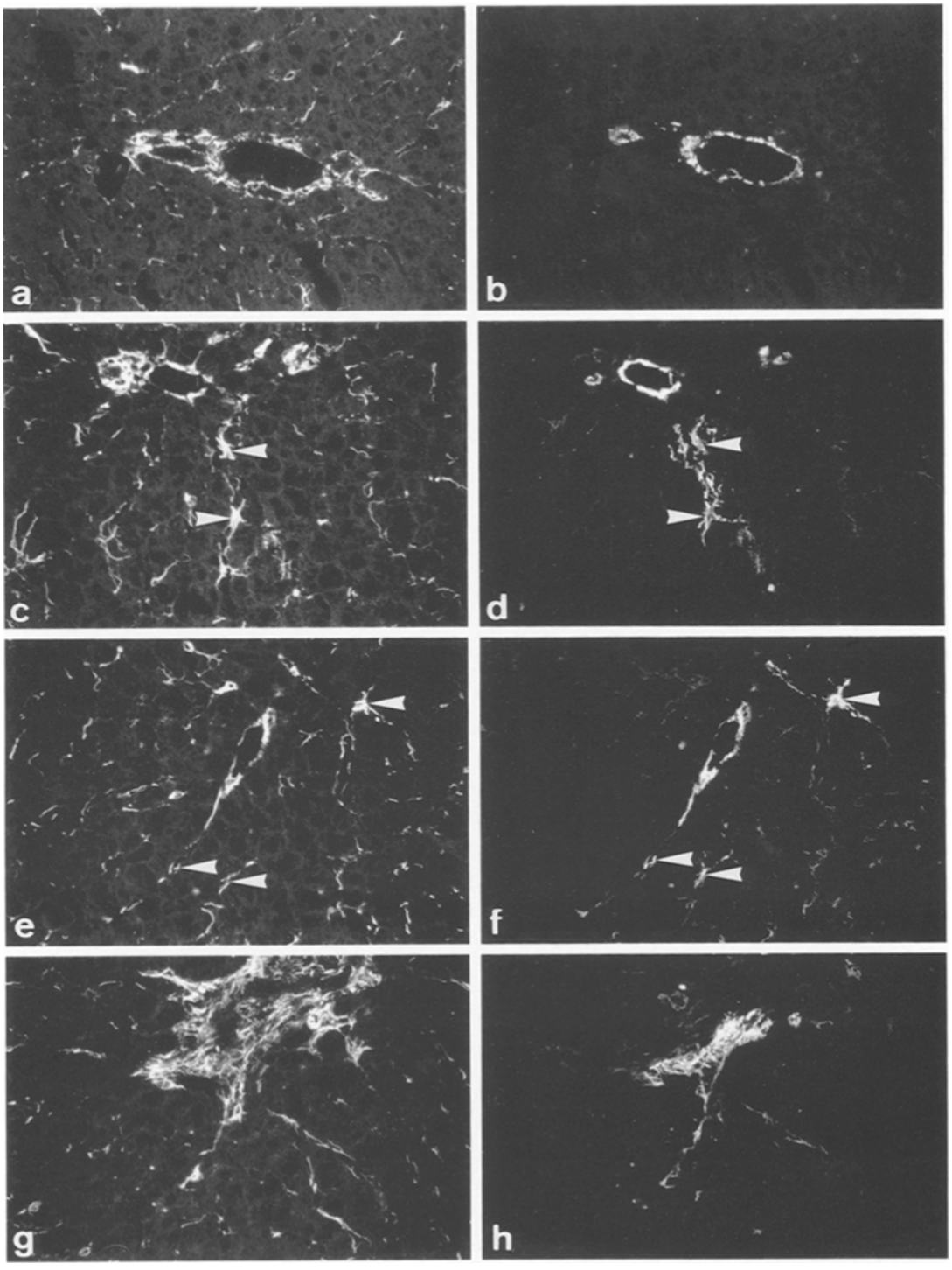

Fig. 2. Double immunofluorescence staining of rat liver tissue with anti-desmin (**a, c, e, g**) and anti-α-SM actin (**b, d, f, h**). In a control animal (**a, b**) the vessel wall is stained with both antibodies, while PSCs within the liver lobule contain desmin only. Chronical treatment with carbon tetrachloride results in an increased number of desmin positive PSCs (**c, e**) and the appearance of cells co-expressing α-SM actin (*arrowheads*) within newly formed slender fibrous bands (**d**) and scattered within the lobules (**f**, *arrowheads*). In advanced stages of fibrosis cells co-expressing desmin and α-SM actin are essentially confined to the centre of inactive scar tissue and rarely present adjacent to hepatocytes (**g, h**). **a–h** × 630

During the first phase, lesions essentially consisted of microvesicular fatty metamorphosis and scanty coagulation necrosis of liver cells. Councilman bodies were confined to the prevenular and midzonal areas of lobules. A recent study has addressed the mechanism of the intralobular heterogeneity of carbon tetrachloride hepatotoxicity (Suematsu et al. 1991). It has been proposed that the highest vulnerability of zone 3 to carbon tetrachloride induced oxidative stress may be due to the low oxygen tension and the high activity of a radical-

generating enzyme, cytochrome P-450, in the prevenular region (Suematsu et al. 1991).

In the second phase, deposition of increasing amounts of collagen and elastic fibres emerging from the prevenular zone developed concomitantly with hepatic cell injury. Accumulation of cells expressing α-SM actin was conspicuous around terminal hepatic venules (Fig. 1c, d) while in controls only smooth muscle cells of vessel wall stained for this actin isoform (Fig. 1a). Prussian blue staining failed to show macrophages loaded with haemosiderin in controls (Fig. 1b); carbon tetrachloride treatment produced an inflammatory infiltration of the prevenular area adjacent to liver cell necrosis. In addition to polymorphonuclear leucocytes and mast cells, numerous macrophages containing haemosiderin were noted (Fig. 1e). Foci of PSCs lining liver cell plates in the central and midzonal areas stained for α-SM actin (Fig. 1f, g). In the third phase, α-SM actin staining was prominent in fibrous septa expanding from the prevenular zone and to a lesser degree also from the portal fields (Fig. 1h). In the fourth phase, trichrome stain for collagen revealed fibrous bands joining central veins and portal fields resulting in the formation of focal nodules of small size (data not shown). These irregular fibrous septa contained numerous cells positive for α-SM actin (Fig. 1h). Until 30–32 weeks, there was a progression of bridging fibrosis giving a nodular appearance to liver tissue. After about 33 weeks, progression of the fibrogenic process was no longer obvious and complete cirrhotic remodelling failed to develop, suggesting that animals became less susceptible to carbon tetrachloride hepatotoxicity. In foci of apparently inactive scar tissue lacking inflammatory cells and necrosis, staining for α-SM actin was less pronounced than in active fibrous septa.

Double immunofluorescence staining of normal rat liver tissue with anti-desmin and anti-α-SM actin confirmed the previous observation (Yokoi et al. 1984) that PSCs express desmin (Fig. 2a) but not α-SM actin (Fig. 2b). Chronic carbon tetrachloride treatment resulted in an increase in PSCs delineated by a strong positivity for desmin (Fig. 2c, e, g). Moreover, cells co-expressing desmin and α-SM actin were noted in slender, newly formed septa (Fig. 2d, f) and scattered throughout the liver lobule in a characteristic perisinusoidal localization (Fig. 2d, f). However, PSCs expressing only desmin were encountered more frequently than PSCs staining for both proteins during all phases of the experiment. In advanced stages, cells co-expressing desmin and α-SM actin were essentially confined to the scar tissue (Fig. 2g, h).

Electron microscopically, control PSCs were characterized by a stellate configuration of the cytoplasm forming numerous irregular processes and by the presence of abundant lipid droplets. Examination of liver tissue obtained from treated animals at about 13–17 weeks showed PSCs in the Disse space containing still lipid droplets but exibiting a well-developed RER and microfilaments at the periphery of the cytoplasm and particularly in cell processes (Fig. 3). This cell type was most frequently encountered in the prevenular area and was associated with a deposition of collagen fibres. Interspersed between extracellular matrix (ECM) containing

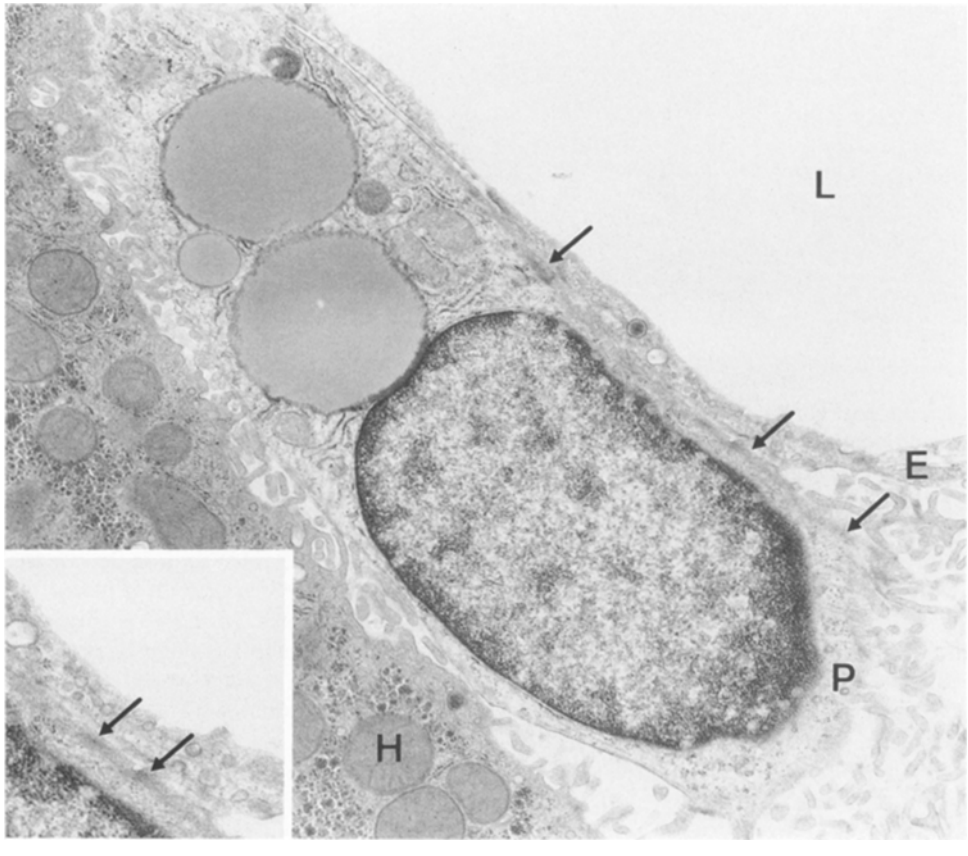

Fig. 3. Ultrastructural appearance of a perisinusoidal cell (*P*) situated between a hepatocyte (*H*) and a sinusoidal endothelial cell (*E*) after 10 weeks of carbon tetrachloride treatment. Typical lipid droplets are still present, and in addition microfilament bundles (*arrows*) appear at the cell periphery; the *inset* shows a detail of cytoplasmic microfilaments. *L*, sinusoidal lumen (×16000; *inset* ×30000)

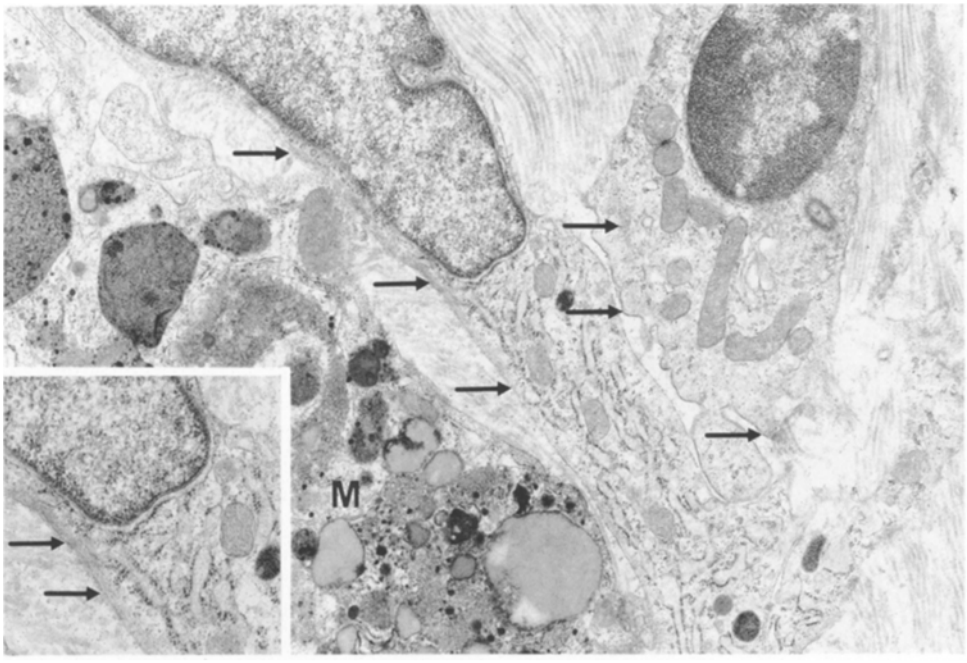

Fig. 4. After 12 weeks of carbon tetrachloride treatment, a perivenular area of fibrosis contains macrophages (*M*) and myofibroblast-like cells showing a well-developed rough endoplasmic reticulum and thin bundles of microfilaments; the *inset* shows a detail of cytoplasmic microfilaments (*arrows*) (×16000; inset ×30000)

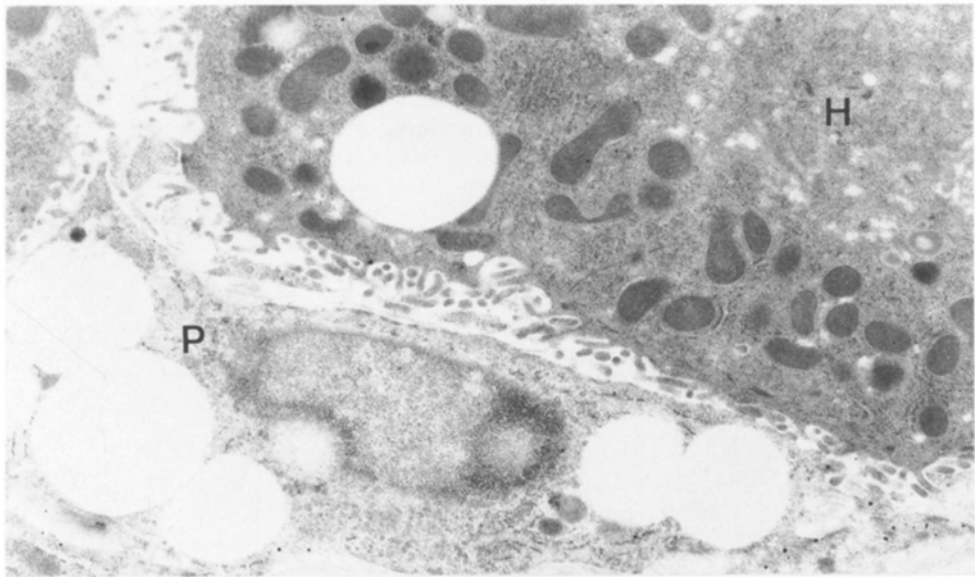

Fig. 5. Immunoelectron microscopic localization of α-SM actin within a typical perisinusoidal cell (*P*) of a rat treated for 10 weeks with carbon tetrachloride. Silver particles delineate thin microfilament aggregates present beneath the plasmalemma and in cell processes. *H*, Hepatocyte; ×17500

bundles of collagen fibres were few macrophages, mast cells, and several slender mesenchymal cells devoid of lipid droplets. They showed oval or indented nuclei and cytoplasmic microfilaments, marking them as MF-like cells (Fig. 4). However, when compared with characteristic MFs described in granulation tissue previously (Darby et al. 1990; Geerts et al. 1991), microfilament bundles appeared less numerous, were often confined to attenuated cell processes and only occasionally showed the presence of dense bodies scattered within. In more advanced stages of fibrosis, these MF-like cells and more typical MF were encountered in fibrous septa expanding in the liver lobule and adjacent to hepatocytes in the space of Disse. Here, deposition of ECM resulted in a capillarization of hepatic sinusoids.

Immunogold electron microscopy performed on controls failed to show staining of PSCs for α-SM actin (not shown) and was positive only in smooth muscle cells of vessel walls. Starting around 10 weeks, scattered PSCs were stained with anti-α-SM actin. Labelling with colloidal silver was confined to faint aggregates of microfilaments (Fig. 5), whereas PSCs devoid of microfilaments were negative. At 20 weeks, several MF-like cells surrounded by collagen bundles in the zone of prevenular fibrosis and in expanding septa showed moderate to marked staining of microfilaments. Moreover, typical PSCs still containing few lipid droplets were clearly laden with microfilament bundles decorated by anti-α-SM actin (Fig. 6a, b). In advanced stages, zones of scar tissue contained MFs labelled with colloidal silver over micro-

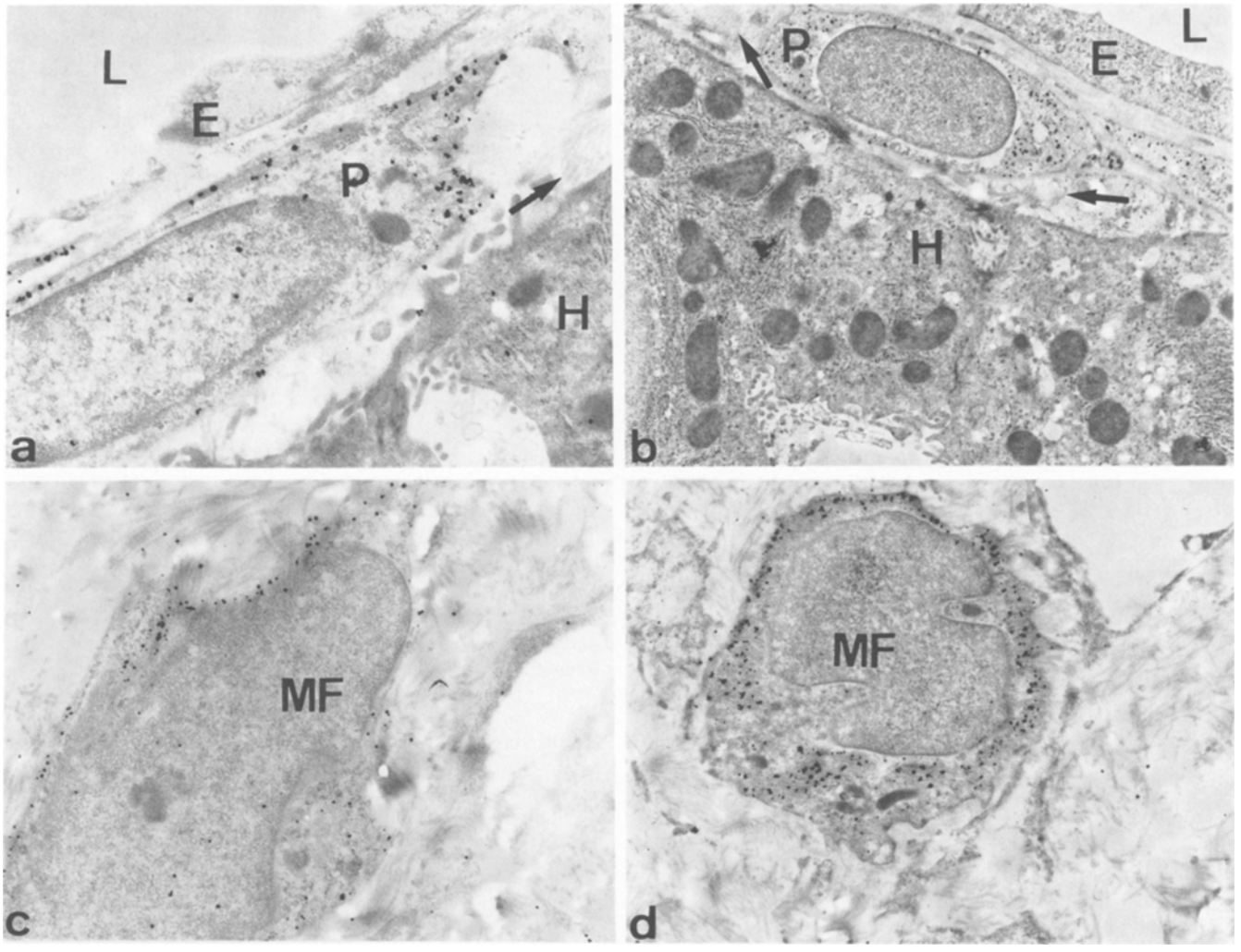

Fig. 6a–d. Immunoelectron microscopic localization of α-SM actin in non-parenchymal hepatic cells of liver during different stages of carbon tetrachloride induced damage. **a, b** After 20 weeks of treatment, α-SM actin is localized within microfilament bundles arranged parallel to the long axis of perisinusoidal cells (*P*) and in slender subendothelial cell processes. Note the presence of collagen fibrils (*arrows*) located between PSCs and hepatocytes (*H*). **c, d** In later stages, dense collagen deposits in a perivenular scar and expanding septa contain typical myofibroblasts with irregular nuclear outlines (**d**). Bundles of microfilaments are decorated by anti-α-SM actin. *E*, Endothelial cell; *L*, sinusoidal lumen; **a** ×17300; **b** ×7500; **c** ×12400; **d** ×10000

filaments (Fig. 6c, d). However, a proportion of MFs showed little or no labelling.

Discussion

Although it is generally agreed that MFs may bear temporary or permanent muscle-like features, the question of their lineage and relationship to other cell types has been a subject of debate since their initial description (Gabbiani et al. 1971; Gown 1990). Recent experimental data (Darby et al. 1990) confirm the postulate that granulation tissue MFs develop from local fibroblasts (Gabbiani et al. 1971). Moreover, it has become apparent that MFs may be derived from histogenetically diverse tissues such as serosal tissue (Bolen et al. 1986), ovarian theca (Self et al. 1988), bone marrow stroma (Schmitt-Gräff et al. 1989), lens (Schmitt-Gräff et al. 1990) or mammary gland stroma (Ronnov-Jessen et al. 1990). Developmental studies have shown that the PSCs of the liver originate from the mesenchymal cells of the septum transversum (for review, see Blomhoff and Wake 1991). Our results suggest that PSCs can modulate into MF-like cells during experimental liver fibrosis. By means of light microscopy and ultrastructural immunohistochemistry, we provide evidence that microfilamentous bands decorated with α-SM actin antibody develop in several PSCs of treated animals, containing lipid droplets and abundant RER. The filaments were observed in cell processes branching along the space of Disse. Moreover, fibrous septa expanding from the prevenular zone contained mesenchymal cells generally devoid of lipid droplets but strongly labelled by α-SM actin. This cell type occurred in close topographic relationship to the predominant localization of hepatocyte necrosis, macrophage infiltration and collagen deposition, and may correspond to an advanced stage of transition from a perisinusoidal to a MF phenotype. However, we cannot exclude the possibility that a subset of MF-like cells arises from

other cell types such as vascular smooth muscle cells, fibroblasts or pericytes. Indeed, PSCs have been designated as liver-specific pericytes (for review, see Blomhoff and Wake 1991). The absence of α-SM actin in normal human (Schmitt-Gräff et al. 1991) and rat PSCs does not contradict this assumption, since this isoform of actin is not common to all microvascular pericytes (Nehls and Drenckhahn 1991). Consistent with the suggestion that the expression of α-SM actin by pericytes may be significantly modified by increased hydrostatic pressure (Nehls and Drenckhahn 1991) is our observation that chronic venous congestion is associated with the appearance of α-SM actin positive PSCs in human liver tissue (Schmitt-Gräff et al. 1991). There have been previous indications that MFs in carbon tetrachloride induced liver cirrhosis are contractile (Irle et al. 1980). Using this model, contraction of fibrous septa in complete cirrhotic transformation was demonstrated; however, our study is focused on the slowly progressing remodelling of liver tissue. In this condition, MF-like cells are, at least in part, still located perisinusoidally. PSCs, which acquire microfilaments in ramified cytoplasmic extensions, show some resemblance to pericytes and may influence sinusoidal blood flow by contractile mechanisms. The coexpression of α-SM actin and desmin by rat PSCs may be considered to be a hallmark of a contractile function; in addition, MFs have been shown to be synthetically active (for review, see Rungger-Brändle and Gabbiani 1983). In foci of fibrosis that were quiescent, judged by the absence of hepatocyte necrosis or of inflammatory cells, most PSCs failed to be decorated by α-SM actin and only expressed desmin. This is in accordance with the observations that the expression of α-SM actin is more pronounced in active fibrotic processes in human lung (Kuhn and McDonald 1991) or liver (Schmitt-Gräff et al. 1991) tissue than in inactive stages. The appearance of α-SM actin in PSCs was accompanied by a deposition of increased amounts of ECM in the space of Disse resulting in a capillarization of sinusoids.

Although much new information has emerged regarding PSCs as the predominant source of collagen in the fibrotic liver (Milani et al. 1989, 1990; for review, see Friedman and Bissell 1990), relatively little is known about the stimuli responsible for activation and phenotypic modulation of PSCs under in vivo conditions. Activation of PSCs may be mediated through various pathways of signalling including ECM constituents, cell-to-cell contacts and soluble factors.

Proteoglycans are probably implicated in the modulation of cytoskeletal proteins in cultured rat PSCs (Bachem et al. 1992; Gressner 1991). The expression of proto-oncogenes, such as c-*fos* and c-*jun* by carbon tetrachloride damaged rat hepatocytes and PSCs has suggested subtle relationships between parenchymal and non-parenchymal cell populations in toxic liver injury (Herbst et al. 1991). Cytokines may have multiple impacts of ECM synthesis, cytoskeletal protein expression and production of tissue retraction by PSCs. Based on in vitro and in vivo observations, transforming growth factor-β (TGF-β), tumour necrosis factor-α, (TNF-α), platelet derived growth factor (PDGF) and Kupffer cell derived factors may be important candidates to exert various effects on PSCs (Czaja et al. 1989; Friedman and Arthur 1989; Matsuoka et al. 1989, 1990; Pinzani et al. 1989; Weiner et al. 1990). Miao et al. (1990) have shown that elevated TGF-β levels are associated with an increased synthesis of type I collagen in carbon tetrachloride induced fibrosis. Studies performed on fibrotic reactions of rat subcutaneous tissue failed to show an induction of α-SM actin expression by TNF-α or PDGF, while granulocyte-monocyte colony stimulating factor upregulated this isoform in skin fibroblasts (Rubbia-Brandt et al. 1991). In view of the possible heterogeneous nature of stromal cells, we cannot exclude the possibility that a coordinate release of cytokines in the microenvironment of the liver has different effects when compared with a local application to the subcutaneous tissues. It is conceivable that soluble factors produced by Kupffer cells and other inflammatory cells including mast cells and macrophages infiltrating areas of tissue damage may not only regulate PSC proliferation and ECM deposition, but also induce MF differentiation, characterized by the appearance of α-SM actin.

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References

Aterman K (1986) The parasinusoidal cells of the liver: a historical account. Histochem J 18:279–305

Bachem MG, Meyer D, Melchior R, Sell KM, Gressner AM (1992) Activation of rat liver perisinusoidal lipocytes by transforming growth factors derived from myofibroblastlike cells. A potential mechanism of self perpetuation in liver fibrogenesis. J Clin Invest 89:19–27

Blomhoff R, Wake K (1991) Perisinusoidal stellate cells of the liver: important roles in retinol metabolism and fibrosis. FASEB J 5:271–277

Bolen JW, Hammar SP, McNutt MA (1986) Reactive and neoplastic serosal tissue. A light microscopic, ultrastructural, and immunocytochemical study. Am J Surg Pathol 10:34–47

Czaja MJ, Weiner FR, Flanders KC, Giambrone MA, Wind R, Biempica L, Zern MA (1989) In vitro and in vivo association of transforming growth factor-β1 with hepatic fibrosis. J Cell Biol 108:2477–2482

Danscher G (1981) Localization of gold in biological tissue. A photochemical method for both light and electron microscopy. Histochemistry 71:81–89

Darby I, Skalli O, Gabbiani G (1990) α-Smooth muscle actin is transiently expressed by myofibroblasts during experimental wound healing. Lab Invest 63:21–29

French SW, Miyamoto K, Wong K, Jui L, Briere L (1988) Role of the Ito cell in liver parenchymal fibrosis in rats fed alcohol and a high fat-low protein diet. Am J Pathol 132:73–85

Friedman SL, Arthur MJP (1989) Activation of cultured rat hepatic lipocytes by Kupffer cell conditioned medium. Direct enhancement of matrix synthesis and stimulation of cell proliferation via induction of platelet-derived growth factor receptors. J Clin Invest 84:1750–1755

Friedman SL, Bissell DM (1990) Hepatic fibrosis: new insights into pathogenesis. Hosp Pract 25:43–50

Friedman SL, Roll FJ, Boyles J, Bissell DM (1985) Hepatic lipocytes: the principal collagen-producing cells of normal rat liver. Proc Natl Acad Sci USA 82:8681–8685

Friedman SL, Roll JF, Boyles J, Arenson DM, Bissell DM (1989) Maintenance of differentiated phenotype of cultured rat hepatic

lipocytes by basement membrane matrix. J Biol Chem 264:10756–10762
Gabbiani G, Ryan GB, Majno G (1971) Presence of modified fibroblasts in granulation tissue and their possible role in wound contracture. Experientia 27:549–550
Gabbiani G, Montesano R, Tuchweber B, Salas M, Orci L (1975) Phalloidin-induced hyperplasia of actin filaments in rat hepatocytes. Lab Invest 33:562–569
Geerts A, Lazou JM, De Bleser P, Wisse E (1991) Tissue distribution, quantitation and proliferation kinetics of fat-storing cells in carbon tetrachloride-injured rat liver. Hepatology 13:1193–1202
Gown AM (1990) The mysteries of the myofibroblast (partially) unmasked. Lab Invest 63:1–3
Gressner AM (1991) β-D-xyloside induced modulations of glycosaminoglycans, proliferation, and cytoskeletal organization of rat liver myofibroblast-like cells (transformed fat storing cells). Cell Mol Biol 37:549–564
Herbst H, Milani S, Schuppan D, Stein H (1991) Temporal and spatial patterns of proto-oncogene expression at early stages of toxic liver injury in the rat. Lab Invest 65:324–334
Irle C, Kocher O, Gabbiani G (1980) Contractility of myofibroblasts during experimental liver cirrhosis. J Submicrosc Cytol 12:209–217
Kocher O, Skalli O, Bloom WS, Gabbiani G (1984) Cytoskeleton of rat aortic smooth muscle cells. Normal conditions and experimental intimal thickening. Lab Invest 50:645–652
Kuhn C, McDonald JA (1991) The roles of the myofibroblast in idiopathic pulmonary fibrosis. Ultrastructural and immunohistochemical features of sites of active extracellular matrix synthesis. Am J Pathol 138:1257–1265
Leo MA, Lieber CS (1983) Hepatic fibrosis after long-term administration of ethanol and moderate vitamin A supplementation in the rat. Hepatology 3:1–11
Mak KM, Lieber CS (1988) Lipocytes and transitional cells in alcoholic liver disease: a morphometric study. Hepatolgy 8:1027–1033
Mak KM, Leo MA, Lieber CS (1984) Alcoholic liver injury in baboons: transformation of lipocytes to transitional cells. Gastroenterology 87:188–200
Martinez-Hernandez A (1985) The hepatic extracellular matrix. II. Electron immunohistochemical studies in rats with CCl_4-induced cirrhosis. Lab Invest 53:166–186
Matsuoka M, Pham NT, Tsukamoto H (1989) Differential effects of interleukin-1α, tumor necrosis factor α, and transforming growth factor β1 on cell proliferation and collagen formation by cultured fat-storing cells. Liver 9:71–78
Matsuoka M, Zhang MY, Tsukamoto H (1990) Sensitization of hepatic lipocytes by high-fat diet to stimulatory effects of Kupffer cell-derived factors: implication in alcoholic liver fibrogenesis. Hepatology 11:173–182
Miao S, Bao-En W, Annoni G, Degli Esposti S, Biempica I, Zern MA (1990) Two rat models of hepatic fibrosis. A morphologic and molecular comparison. Lab Invest 63:467–475
Milani S, Herbst H, Schuppan D, Hahn EG, Stein H (1989) In situ hybridization for procollagen types I, III and IV mRNA in normal and fibrotic rat liver: evidence for predominant expression in nonparenchymal liver cells. Hepatology 10:84–92
Milani S, Herbst H, Schuppan D, Kim KY, Riecken EO, Stein H (1990) Procollagen expression by nonparenchymal rat liver cells in experimental biliary fibrosis. Gastroenterology 98:175–184
Nakano M, Lieber CS (1982) Ultrastructure of initial stages of perivenular fibrosis in alcohol-fed baboons. Am J Pathol 106:145–155
Nehls V, Drenckhahn D (1991) Heterogeneity of microvascular pericytes for smooth muscle type alpha-actin. J Cell Biol 113:147–154
Ogawa K, Suzuki JI, Mukai H, Mori M (1986) Sequential changes of extracellular matrix and proliferation of Ito cells with enhanced expression of desmin and actin in focal hepatic injury. Am J Pathol 125:611–619
Pinzani M, Gesualdo L, Sabbah GM, Abboud HE (1989) Effects of platelet-derived growth factor and other polypeptide mitogens on DNA synthesis and growth of cultured rat liver fat-storing cells. J Clin Invest 84:1786–1793
Ramadori G (1991) The stellate cell (Ito-cell, fat-storing cell, lipocyte, perisinusoidal cell) of the liver. New insights into pathophysiology of an intriguing cell. Virchows Arch [B] 61:147–158
Ramadori G, Veit T, Schwögler S, Kienes HP, Knittel T, Rieder H, Meyer zum Büschenfelde KH (1990) Expression of the gene of the α-smooth muscle-actin isoform in rat liver and in rat fat-storing (Ito) cells. Virchows Arch [B] 59:349–357
Rockey DC, Boyles JK, Gabbiani G, Friedman SL (1992) Rat hepatic lipocytes express smooth muscle actin upon activation in vivo and in culture. J Submicrosc Cytol Pathol 24:193–203
Ronnov-Jessen L, Van Deurs B, Celis JE, Petersen OW (1990) Smooth muscle differentiation in cultured human breast gland stromal cells. Lab Invest 63:532–543
Rubbia-Brandt L, Sappino AP, Gabbiani G (1991) Locally applied GM-CSF induces the accumulation of α-smooth muscle actin containing myofibroblasts. Virchows Arch [B] 60:73–82
Rungger-Brändle E, Gabbiani G (1983) The role of cytoskeletal and cytocontractile elements in pathological processes. Am J Pathol 110:359–392
Sappino AP, Schürch W, Gabbiani G (1990) Differentiation repertoire of fibroblastic cells: expression of cytoskeletal proteins as marker of phenotypic modulations. Lab Invest 63:144–161
Schmitt-Gräff A, Skalli O, Gabbiani G (1989) α-Smooth muscle actin is expressed in a subset of bone marrow stromal cells in normal and pathological conditions. Virchows Arch [B] 57:291–302
Schmitt-Gräff A, Pau H, Spahr R, Piper HM, Skalli O, Gabbiani G (1990) Appearance of alpha-smooth muscle actin in human eye lens cells of anterior capsular cataract and in cultured bovine lens-forming cells. Differentiation 43:115–122
Schmitt-Gräff A, Krüger S, Bochard F, Gabbiani G, Denk H (1991) Modulation of alpha smooth muscle actin and desmin expression in perisinusoidal cells of normal and diseased human livers. Am J Pathol 138:1233–1242
Self DA, Schroeder PC, Gown AM (1988) Hamster thecal cells express muscle characteristics. Biol Reprod 39:119–130
Skalli O, Gabbiani G (1988) The biology of the myofibroblast: relationship to wound contraction and fibrocontractive disease. In: Clark RAF, Henson PM (eds) The molecular and cellular biology of wound repair. Plenum, New York, pp 373–402
Skalli O, Ropraz P, Trzeciak A, Benzonana G, Gillessen D, Gabbiani G (1986) A monoclonal antibody against α-smooth muscle actin: a new probe for smooth muscle differentiation. J Cell Biol 103:2787–2796
Suematsu M, Kato S, Ishii H, Asako H, Yanagisawa T, Suzuki H, Oshio C, Tsuchiya M (1991) Intralobular heterogeneity of carbon tetrachloride-induced oxidative stress in perfused rat liver visualized by digital imaging fluorescence microscopy. Lab Invest 64:167–173
Takase S, Leo MA, Nouchi T, Lieber CS (1988) Desmin distinguishes cultured fat-storing cells from myofibroblasts, smooth muscle cells and fibroblasts in the rat. J Hepatol 6:267–276
Tanaka Y, Nouchi T, Yamane M, Yamaoka K, Irie T, Sato C, Marumo F (1990) Expression of alpha-smooth muscle actin in lipocytes in hepatic fibrosis: an evidence for modulation in lipocytes (abstract). Hepatology 12:953
Tsutsumi M, Takada A, Takase S (1987) Characterization of desmin-positive rat liver sinusoidal cells. Hepatology 7:277–284
Weiner FR, Giambrone MA, Czaja MJ, Shah A, Annoni G, Takahashi S, Eghbali M, Zern MA (1990) Ito-cell gene expression and collagen regulation. Hepatology 11:111–117
Yokoi Y, Namihisa T, Kuroda H, Komatsu I, Miyazaki A, Watanabe S, Usui K (1984) Immunocytochemical detection of desmin in fat-storing cells (Ito cells). Hepatology 4:709–714