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Modulation of Alpha Smooth Muscle Actin and Desmin Expression in Perisinusoidal Cells of Normal and Diseased Human Livers

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It has been suggested that perisinusoidal liver cells (PSC) play a pivotal role in the pathogenesis of fibrocontractive changes. Using light and electron microscopic immunolocalization techniques, a series of 207 normal and pathologic human liver specimens were evaluated for the expression of alpha smooth muscle (SM) actin and desmin in this and other nonparenchymal cell types. In normal adult liver tissue, PSCs were practically devoid of desmin and exceptionally stained for α-SM actin, whereas this actin isoform frequently was encountered in PSCs from the embryonic to the adolescent period. A broad spectrum of pathologic conditions was accompanied by the presence of α-SM actin containing PSCs; these were detected preferentially in periportal or perivenular zones according to the predominant location of the underlying hepatocellular damage. The occurrence of this PSC phenotype generally was associated with fibrogenesis and was in some cases detected earlier than overt collagen accumulation. Fibrous bands subdividing liver tissue in cirrhosis and focal nodular hyperplasia, as well as desmoplastic reaction to malignant tumors, contained α-SM actin-rich cells admixed with variable proportions of cells coexpressing desmin. In end stages, this population was less numerous than in active fibrotic or cirrhotic processes. Using immunogold electron microscopy, α-SM actin was localized in microfilament bundles of typical PSCs. Our results are compatible with the assumption that the appearance of α-SM actin and desmin-expressing myofibroblasts results at least in part from a phenotypic modulation of PSCs. (Am J Pathol 1991, 138:1233–1242)

Perisinusoidal cells (PSC) are multifunctional mesenchymal cells residing beneath the endothelial cells in the space of Disse. They also are called lipocytes, fat-storing, stellate, or Ito cells (for review see Aterman[1]). Although they have already been mentioned by Boll in 1869[2] and described as a specialized cell population by Ito and Nemoto in 1952,[3] their role in retinoid metabolism[4] and collagen production[5–7] remains to be clarified.

Perisinusoidal cells initially were thought to be characterized by the presence of cytoplasmic lipid droplets reacting intensely with gold chloride (for review see Wake[8]). However ultrastructural *in vivo* and *in vitro* studies revealed that proliferation of PSCs may be associated with reduction of lipid droplets,[9] development of rough endoplasmic reticulum,[10] and of microfilament bundles.[11] Conditions associated with liver fibrosis such as treatment of rats with carbon tetrachloride (CCl_4),[12] feeding of baboons with ethanol,[9] or alcoholism in humans[13] have been suggested to promote the transformation of PSCs into transitional cells bearing ultrastructural features intermediate between this cell type and fibroblasts. Furthermore cells with a typical myofibroblastic morphology have been noted in human and experimental hepatic fibrosis[14,15] and cirrhosis.[16,17] Considerable interest recently was directed to the question of whether PSCs and myofibroblasts in liver diseases are distinct populations[18] or represent the same cell type in various stages of differentiation and/or functional activity.[19,20]

It is well accepted that in rats PSCs of the normal[21] and injured[22] liver contain desmin as the major protein of intermediate-sized filaments. However little information is available concerning the cytoskeletal components of PSCs of the human liver.[19] This led us to investigate the expression of desmin and α-SM actin, the actin isoform typical of smooth muscle cell differentiation,[23,24] which

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appears temporarily in myofibroblasts during normal wound healing[25] and more permanently in several fibrocontractive diseases,[26] in nonparenchymal cells of normal human fetal and adult hepatic tissue, as well as during a variety of pathologic settings. We show here that α-SM actin–expressing PSCs are rare in normal adult liver, while the appearance of an appreciable number of this cellular subset might be a hallmark of liver injury and fibrogenesis.

Materials and Methods

Specimens

Samples of liver tissue from 10- to 72-year-old patients were obtained as percutaneous needle (n = 76) or surgical biopsies (n = 24). Furthermore 82 liver specimens were collected from autopsies within 12 hours after death (age range, 1 day to 85 years). Four embryonic and 21 fetal samples were collected from spontaneous miscarriages or legal abortions. Liver tissue from newborn and adult Wistar rats (known to contain desmin-expressing PSCs) were used as controls.

Tissue samples were fixed in 4% phosphate-buffered formaldehyde solution for light microscopy, in 2.5% cacodylate buffered glutaraldehyde for electron microscopy, or in 0.1 mol/l (molar) phosphate buffer containing 2% freshly prepared paraformaldehyde and 0.5% glutaraldehyde for immunogold electron microscopy. Small blocks of tissue were frozen in isopentane precooled in liquid nitrogen and stored at −80°C for immunofluorescence staining.

Light Microscopy

Sections from paraffin-embedded material cut at 3 to 5 μ were stained with hematoxylin and eosin, Gomori's reticulin, Masson's trichrome, elastica van Gieson, and in some cases, Giemsa and periodic acid-Schiff solution. Parallel cryostat sections from samples studied by immunofluorescence microscopy also were processed for conventional light microscopy.

Antibodies, Immunofluorescence, and Avidin-Biotin-Complex Peroxidase (ABC-P) Staining

We used the following antibodies: 1) anti–αSM-1, a monoclonal IgG2a recognizing α-SM actin[24]; 2) affinity purified polyclonal rabbit IgG against desmin[27] and commercially available monoclonal desmin antibodies (DER-11, D33, Dakopatts, Hamburg, FRG); and 3) antibodies to von Willebrand factor and macrophages (CD68, KPI) purchased from Dako AIS (Gloshun, Denmark). Secondary antibodies for immunofluorescence microscopy were FITC-labeled goat anti-rabbit IgG and TRITC-labeled goat anti-mouse IgG (Nordic Immunological Laboratories, Tilburg, The Netherlands), and for immunogold electron microscopy, goat anti-mouse Ig conjugated to 1-nm gold particles (Janssen Life Science Products, Olen, Belgium).

For avidin-biotin complex peroxidase staining, the Vectastain kits antimouse IgG and anti-rabbit IgG (Vector Laboratories, Burlingame, CA) were used.

For immunofluorescence microscopy, cryostat sections approximately 4 μm thick, were fixed for 5 minutes in cold acetone (−20°C), air dried for 2 hours, and incubated with the primary antibody diluted 1:10 for 45 minutes. After three rinsings in phosphate-buffered saline (PBS), the sections were incubated for 30 minutes with the appropriate second antibody diluted 1:10 or 1:20, respectively. For double-labeled immunofluorescence microscopy, both primary antibodies were added simultaneously, as were secondary antibodies. After three rinsings in PBS, the sections were mounted in buffered polyvinylalcohol.[28] Controls were performed using nonimmune IgG in place of the primary antibody. Immunoperoxidase staining for desmin was performed on cryostat sections. Sections were pretreated with normal goat serum and incubated for 1 hour with rabbit anti-desmin diluted 1:200.

Immunoperoxidase staining for α-SM actin and desmin was done on formalin-fixed and paraffin-embedded tissues. Sections were pretreated with H_2O_2/methanol and subsequently with 0.1 mol/l periodic acid, 0.005 mol/l $NaBH_4$, and normal horse serum. They were incubated overnight at 4°C with anti–αSM-1 or D33 at the dilutions of 1:500 and 1:50, respectively. The first incubation was followed by ABC-P staining according to the manufacturers' instructions. The peroxidase activity was revealed with 30% 3,3′diaminobenzidine (Serva, Heidelberg, FRG) in PBS containing 0.015% H_2O_2. Slides were counterstained weakly with Mayer's hematoxylin, dehydrated, and mounted in Eukitt. Controls were performed using a rabbit or mouse IgG instead of the primary antibody.

Estimation of the number of anti–αSM-1 and anti-desmin immunoreactive PSCs was done independently by two researchers on areas containing mainly longitudinally cut liver cell plates and analyzed separately in the periportal zone, the intermediate zone, and the perivenular zone of liver lobules. Immunoreactivity was categorized arbitrarily as follows: − = nothing; +/− = staining of some PSCs occupying approximately less than 1% of the sinusoidal liver cell surface; + = staining of PSCs occupying approximately 1% to 10% of the sinusoidal

liver cell surface; + + = staining of PSCs occupying approximately 10% to 30% of the sinusoidal liver cell surface; + + + = staining of PSCs occupying more than 30% of the sinusoidal liver cell surface. In conditions associated with the formation of scars, fibrous septa, or desmoplasia, the number of anti–αSM-1 and anti-desmin immunoreactive cells was evaluated using the following scale: − = no positivity; +/− = positivity of less than 10% of mesenchymal cells; + = positivity of 10% to 20% of mesenchymal cells; + + = positivity of 30% to 50% of mesenchymal cells; + + + = positivity of more than 50% of mesenchymal cells.

Photographs were taken with a Zeiss photomicroscope (Carl Zeiss Inc., Oberkochen, FRG) equipped with epi-illumination using plan apo-chromate × 10/1.0 to 63/1.0 objectives and photographed on Kodak TMAX 400 (Kodak Limited, Hemel, Hempstead, UK) or Ilford PANF black and white films (Ilford, Basel, Switzerland).

Electron Microscopy

Tissue samples fixed with glutaraldehyde were postfixed in 1.33% osmium tetroxide, dehydrated, and embedded in Epon. Semithin sections were stained with toluidine blue. Thin sections were stained with uranyl acetate and lead citrate and examined in a Philips 400 electron microscope (Philips SA, Zürich, Switzerland). For immunogold electron microscopy, fixed tissues were rinsed in 0.1 mol/l phosphate buffer, dehydrated, and embedded in Lowicryl K4M resin (Chemische Werke Lowi, Waldkraiburg, FRG). Immunogold staining with anti–αSM-1 was performed on sections collected on Formvar-coated nickel grids as previously described.[25,26] As control, grids were incubated with purified mouse IgG instead of the primary antibody.

Results

In all cases examined, diagnoses were made on the basis of histologic features correlated with clinical information. The patterns of immunofluorescence and immunoperoxidase staining related to different kinds of liver diseases are given in Tables 1 to 3.

Normal Tissues and Pathologic Liver Specimens with a Retained Lobular Structure

The media of the portal vein, hepatic artery branches, and of intercalated and large hepatic vein branches stained brightly for anti–αSM-1 (Figure 1a). Double immunofluorescence showed that desmin-positive smooth muscle cells (SMC) were less numerous than those positive for anti–αSM-1. Desmin-expressing SMCs usually were located in the external portion of the media (not shown). Rare mesenchymal cells embedded in the connective tissue of portal tracts were positive for anti–αSM-1 and for desmin. Terminal hepatic venules of normal liver generally were surrounded by a discontinuous single layer of SMCs staining for anti–αSM-1 (Figure 1b). Inter-

Table 1. *Expression of α-Smooth Muscle Actin in PSCs of Normal and Diseased Livers with a Retained Lobular Structure*

Condition	No of cases	Periportal zone	Intermediate zone	Perivenular zone
Embryo	4	+	+	+ +
Fetus	21	+	+	+ +
Infant/child	7	+/−	+	+ +
Adolescent	3	+/−	+	+
Adult	6	+/−	+/−	+/−
Steatosis	3	+/−	+/−	+/−
Vitamin A intoxication	2	+	+ +	+ + +
Circulatory shock	6	+/−	+/−	+ +
Septic shock	4	+/−	+	+
Chronic congestion	12	+	+ + +	+ + +
Veno-occlusive disease	1	+	+ +	+ +
Alcoholic hepatitis	15	+	+ +	+ + +
Chronic active hepatitis	4	+ +	+	+/−
Chronic persistent hepatitis	3	+	+/−	+/−
Chronic large bile duct obstruction	7	+ + +	+	+/−
Inactive secondary biliary fibrosis	3	+	+/−	+/−
Primary biliary cirrhosis, stages I and II	2	+ +	+	+
Acute liver rejection	1	+ +	+	+
Acute myeloid leukemia	5	+	+	+
Chronic myeloid leukemia	4	+	+	+
Chronic myelomonocytic leukemia	3	+ +	+ +	+ +

PSC, perisinusoidal cells.

Table 2. *Expression of α-Smooth Muscle Actin and Desmin in Mesenchymal Cells of Cirrhotic Livers*

Condition	No of cases	Perisinusoidal cells		Fibrous septa	
		αsm-1	Desmin	αsm-1	Desmin
Cirrhosis with alcoholic hepatitis	11	+ +	+/−	+ + +	+
Inactive alcoholic cirrhosis	9	+	−	+ +	+/−
Chronic active hepatitis with cirrhosis	9	+	+/−	+ + +	+
Primary biliary cirrhosis, stage IV	2	+ +	ND	+ + +	ND
Chronic graft-*versus*-host disease	2	+ +	ND	+ + +	ND
Secondary biliary fibrosis with developing cirrhosis	9	+	ND	+ + +	ND
Inactive portal fibrosis with partial cirrhosis	3	+	ND	+ +	ND
Cirrhosis in metabolic disorders	4	+	ND	+ +	ND
Inactive cirrhosis of unknown etiology	5	+	ND	+ +	ND

ND, not done.

estingly on several occasions, no anti–αSM-1-positive cells were seen in this location. Scattered star-shaped cells within the lobules of embryonic, fetal, childhood, and adolescent livers were decorated with anti–αSM-1 (Figure 1b). These cells were consistently negative for von Willebrand factor and the macrophage marker KPI, and hence they represented PSCs. They were located in the perisinusoidal space adjacent to hepatocytes and were more numerous in perivenular than in intermediate or periportal areas. In fetal tissues, some staining for desmin was noted in these PSCs. In all other specimens, PSCs lacked desmin immunoreactivity, contrary to what we (and previous workers) have observed in rat liver (data not shown). In normal adult liver, some α-SM actin-expressing PSCs were noted, which is contrary to what was observed in rat liver (data not shown). The vast majority of liver lobules of adult controls, however, was devoid of this PSC phenotype (Figure 1a). A pattern similar to controls was observed in nonalcoholic steatosis without inflammation or necrosis (Figure 2a). An increase in α-SM actin-expressing PSCs was encountered in different pathologic situations showing hepatocellular degeneration, inflammation and fibrosis (Table 1). Two patients with chronic hypervitaminosis A showed perivenular fibrosis and prominent anti–αSM-1 immunoreactive PSCs containing cytoplasmic vacuoles indicating fat droplets (Figures 2b, c). This cell type was abundant, especially around the terminal hepatic venule and in the perivenular and intermediate zones of hepatic lobules. Postmortem liver specimens from patients with acute heart failure and shock surviving longer than 48 hours showed anti–αSM-1-positive PSCs around areas of perivenular necrosis and collapse. In patients with prolonged septic shock and hyperbilirubinaemia, canalicular cholestasis and foci of hepatocellular necrosis were associated with a slight increase in anti–αSM-1-positive PSCs. Biopsy and autopsy specimens obtained in chronic central congestion due to right-sided heart failure were characterized by a striking accumulation of anti–αSM-1-positive PSCs surrounding terminal venules and cords of atrophic hepatocytes adjacent to engorged sinusoids (Figure 2d). A similar pattern was observed in veno-occlusive disease complicating allogenic bone marrow transplantation. These findings were most prominent in perivenular and intermediate zones. Alcoholic hepatitis with fatty changes, ballooning of hepatocytes, and Mallory bodies showed increased anti–αSM-1 positivity in the perivenular zone; this change was clear even in cases in which pericellular fibrosis was not detected by means of Gomori's reticulin or Masson's trichrome stain. Alcoholic hepatitis associated with central sclerosis showed consistently perivenular accumulation of anti–αSM-1-positive PSCs (Figure 2e). In the majority of cases with pericellular fibrosis of the 'chicken wire' type, we observed

Table 3. *Expression of α-Smooth Muscle Actin and Desmin in Mesenchymal Cells of Neoplastic and Tumorlike Lesions with Alteration of Lobular Structure*

Condition	No. of cases	Tissue surrounding lesions		Cells within lesions	
		αsm-1	Desmin	αsm-1	Desmin
Focal nodular hyperplasia	6	+ + +	+ +	+ +	−
Adenoma	5	+ +	ND	+	ND
Nodular transformation	4	+	ND	+/−	ND
Hepatocellular carcinoma	4	+ + +	ND	+ +	ND
Metastatic carcinoma	12	+ + +	+ +	+ +	+
Lymphoma	7	+ +	ND	+	ND

ND, not done.

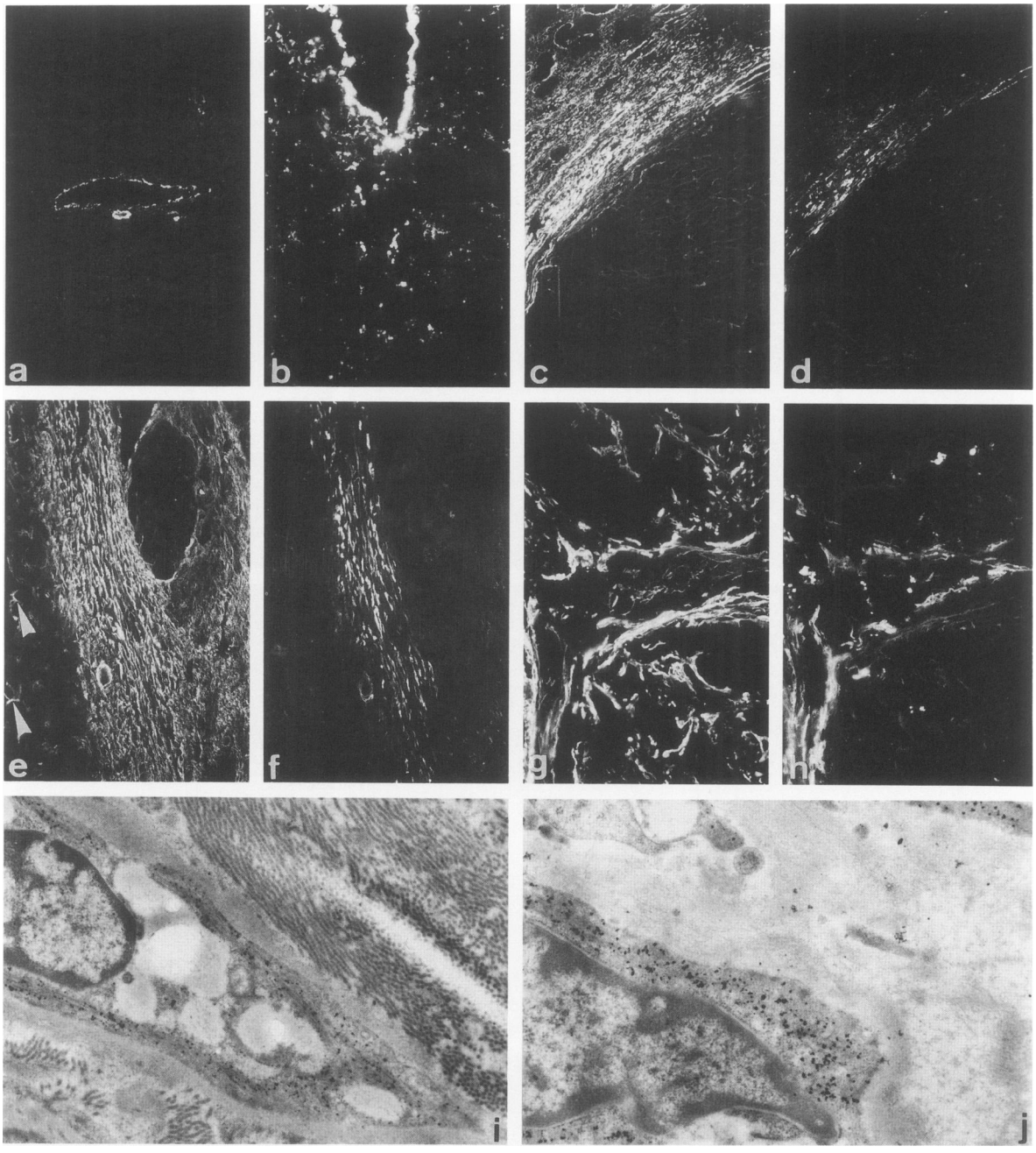
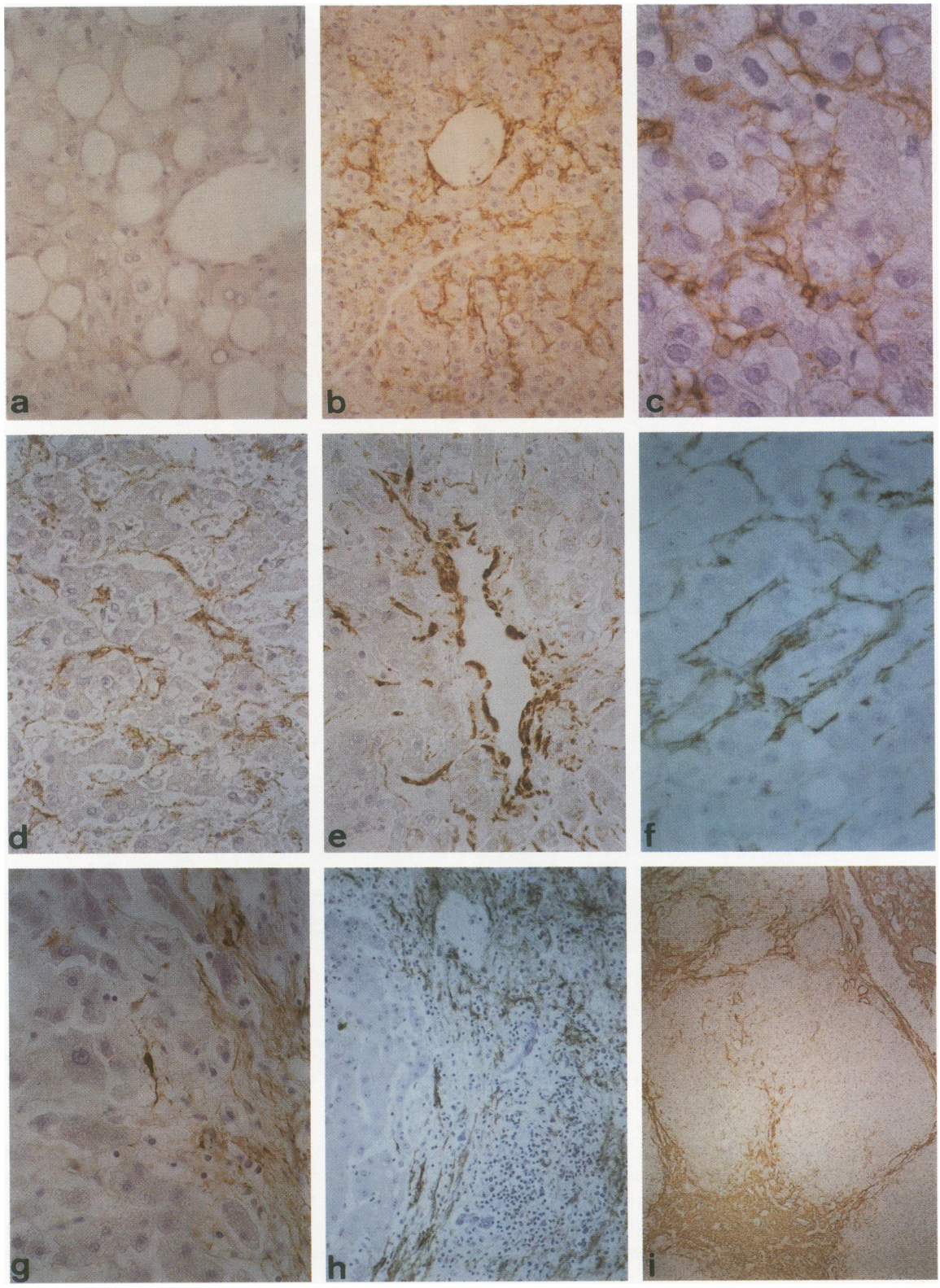


Figure 1. *Anti–αsm-1-positive cells are seen only in the blood vessel wall of normal adult liver (***a**, *×50), while a specimen obtained from a 9-year-old child shows staining of both vascular SMCs and PSCs (***b**, *×200). Double-immunofluorescence staining shows abundant α-SM actin-expressing cells in fibrous tissue of posthepatitic cirrhosis (***c**, *×100), focal nodal hyperplasia (***e**, *×100), and of desmoplasia associated with metastatic breast cancer (***g**, *×200). Note coexpression of desmin by a small (***d**, *×100) to moderate (***f**, *×100; **g**, ×200) number of stromal cells. In focal nodal hyperplasia (***e***), anti–αsm-1-immunoreactive cells are seen also within hepatocyte nodules (arrowheads). By means of immunoelectron microscopy, α-SM actin appears localized in stromal cells of focal nodal hyperplasia, which contain lipid droplets (arrowheads;* **i**, *×10,000;* **j**, *×13,000). Intense labeling of microfilament bundles and of attenuated cytoplasmic processes is noted. The extracellular matrix is rich in collagen fibers.*

anti–αSM-1-positive PSCs surrounding hepatocytes (Figure 2f). Active alcoholic hepatitis contained many immunoreactive cells in fibrous scars linking perivenular areas to portal tracts. Findings in acute viral hepatitis were inconsistent and therefore not included in Table 1. In chronic active hepatitis with piecemeal necrosis, anti–αSM-1-positive PSCs were observed adjacent to the inflammatory infiltrate trapping clusters of hepatocytes (Figure 2g). In chronic persistent hepatitis, an increase in anti–αSM-1-positive PSCs was not evident. Chronic large bile duct obstruction yielded many anti–αSM-1-positive PSCs adjacent to portal areas presenting cholestasis,

bile ductular proliferation, inflammation, and fibrosis. Anti–αSM-1 immunoreactive cells also were present in fibrous septa of chronic biliary obstruction. Secondary biliary fibrosis, without signs of activity, ie, inflammation or cholestasis, showed only a small number of anti–αSM-1-positive PSCs. In primary biliary cirrhosis with florid duct



lesions, ductular proliferation and expanding portal tracts (stages I and II), anti–αSM-1-positive PSCs were encountered predominantly in the periportal zone. Furthermore scattered immunoreactive cells were present in intermediate and perivenular zones. In acute liver rejection with mixed portal infiltrate, bile duct damage and central vein endothelialitis, scattered anti–αSM-1-positive cells were seen, predominantly in the perivenular zone. Portal tract and sinusoidal infiltration by acute and chronic leukemias was associated with a slight increase in anti–αSM-1-positive SMCs. A more pronounced proliferation of these cells was observed in chronic myelomonocytic leukemia.

Cirrhosis

Bands of fibrous tissue surrounding parenchymal nodules in all types of cirrhosis examined contained mesenchymal cells immunoreactive for anti–αSM-1 (Table 2, Figure 1c). Double immunofluorescence showed that a portion of them coexpressed desmin (Figure 1d). No differences were noted between micro- and macronodular patterns. The frequency of anti–αSM-1-positive cells encountered in fibrous septa and scars was related to the activity of the cirrhotic process. In active cirrhosis with a blurred interface between fibrous tissue and hepatocytes, inflammatory infiltrates and cell damage, many α-SM actin-expressing cells were present in expanding septa as well as in the perisinusoidal space of residual hepatic parenchyma (Figure 2i). However, in areas demonstrating regenerative activity, the number of anti–αSM-1-positive PSCs was restricted to the periphery of the regenerative cell plates. End stages with sharply outlined nodules showed a diminished number of immunoreactive cells in fibrous septa and absence of anti–αSM-1-positive cells in a perisinusoidal localization. Chronic graft-*versus*-host disease with piecemeal necrosis and developing cirrhosis was characterized by the presence of α-SM actin-expressing cells in the periportal zone and within fibrous septa (Figure 2h).

Neoplastic or Tumorlike Lesions with Alteration of Lobular Structure

Focal nodular hyperplasia was characterized by the presence of abundant anti–αSM-1-positive cells, some of which coexpressed desmin (Table 3, Figures 1e, f). This phenotype was encountered mainly in the central stellate scar and the fibrous septa with chronic inflammation and proliferating bile ductules. Venules revealed a thickened layer of anti–αSM-1- and anti–desmin-positive SMCs consistent with fibromuscular hyperplasia. The nodular parenchyma bore many α-SM actin-expressing cells adjacent to hepatocytes. This pattern was quite different from that seen in inactive cirrhosis, despite the histologic resemblance that sometimes occurred between the two lesions. The hepatocellular adenoma contained anti–αSM-1-positive cells mainly in the fibrous capsule and foci of hemorrhage. In nodular transformation, anti–αSM-1-positive PSCs were present in zones of atrophic internodular hepatocytes; within the nodules, a proliferation of PSCs expressing α-SM actin was exceptional. All specimens obtained from hepatocellular carcinomas, metastatic carcinoma, and lymphomas of high-grade malignancy producing bulky masses resulted in a striking proliferation of anti–αSM-1-positive cells in bands of fibrous tissue surrounding tumor masses as well as within the lesions (Figure 1g). In these desmoplastic reactions, a small number of cells coexpressing α-SM actin and desmin were present (Figure 1h). No differences in the staining pattern for anti–αSM-1 were noted in hepatocellular carcinomas compared to metastatic tumors. Generally the non-neoplastic liver tissue adjacent to a benign or malignant lesion contained an increased number of anti–αSM-1-positive cells associated with congestion and inflammation.

Immunogold Electron Microscopy

Immunogold electron microscopy was done on surgical specimens from focal nodular hyperplasia and primary biliary cirrhosis; it showed anti–αSM-1 labeling of microfilament bundles present in stellate-shaped nonparenchymal cells embedded in a collagenous matrix (Figures 1i, j). The cells displayed indented nuclei, a well-developed endoplasmic reticulum, and microfilament bundles with dense bodies scattered within, predominantly in the peripheral cytoplasm. Furthermore conspicuous lipid droplets, as seen in typical fat-storing PSCs, were noted in several of them. These morphologic features, which correlated well with those observed in conventional transmission electron microscopy, were com-

Figure 2. *ABC-P staining of liver tissue for α-SM actin. In a fatty liver of a diabetic patient that does not show inflammatory infiltration, immunoreactive PSCs are absent* (**a**, ×*200*). *α-SM actin containing PSCs are visible in a case of vitamin A intoxication characterized by a conspicuous proliferation of PSCs containing lipid vacuoles* (**b**, ×*100*; **c**, ×*550*); *in chronic congestion* (**d**, ×*200*); *in alcoholic liver disease with central sclerosis associated with many PSCs around terminal hepatic venule* (**e**, ×*200*); *in alcoholic hepatitis with prominent pericellular fibrosis* (**f**, ×*450*); *in chronic active hepatitis with stellate-shaped PSCs at the margin of piecemeal necrosis* (**g**, ×*400*); *in chronic graft-*versus*-host disease with piecemeal necrosis containing PSCs in fibrous tissue near the portal tract* (**h**, ×*100*); *in active cirrhosis with alcoholic hepatitis a conspicuous immunoreactivity for anti–αsm-1 is visible in stromal cells of the scars, fibrous septa expanding in the parenchyma, and in PSCs scattered within the nodules* (**i**, ×*60*).

patible with the assumption that α-SM actin develops in hepatic PSCs.

Discussion

It appears more and more clear that stromal fibroblastic cells are heterogeneous in several respects, including the expression of cytoskeletal markers.[29] Thus they may express markers of myogenic differentiation such as α-SM actin and/or desmin in several locations such as the lymph nodes and spleen,[30,31] tonsil and thymus,[32] bone marrow,[33] and uterine mucosa.[34] In addition, stromal cells can acquire, temporarily[25] or more permanently,[26] these markers during pathologic situations such as wound healing or fibrocontractive diseases. We consider that a high proportion of the α-SM actin-expressing cells that we have identified in liver parenchyma belong to the PSC population because of their characteristic location, their stellate morphology with the presence of long cytoplasmic processes, and the electron microscopic immunolocalization showing lipid droplets and microfilaments labeled by gold particles in the same cell. Furthermore lipid droplets were clearly visible even by light microscopy in cells expressing α-SM actin in vitamin A intoxication. However we cannot exclude that some α-SM actin-expressing cells, especially those of fibrous septa, originate from pre-existing fibroblastic cells, as well as from SMCs or pericytes. It was documented previously that myofibroblasts of cirrhotic rat liver produced by CCl_4 possess a contractile potential.[17] Connective tissue septa of cirrhosis and focal nodular hyperplasia or desmoplastic stroma of tumor metastasis contain not only α-SM actin-positive cells but also a smaller proportion of cells also expressing desmin. Desmin antibodies did not stain PSCs of normal adults livers, while rat PSCs were highly labeled. It was mentioned previously that human PSCs may lack desmin intermediate filaments.[19] In human fetal specimens of our series, however, some PSCs were indeed stained by desmin antibodies. Furthermore α-SM actin-expressing PSCs were numerous in the livers from the embryonic period to the age of adolescence, whereas this phenotype was rare in normal adult liver tissue. These observations suggest that the cytoskeletal composition of PSCs may be modulated during development and in response to various stimuli in the adult, as it was shown previously for stromal cells of other organs such as bone marrow[33] or intestine.[35]

Nonparenchymal liver cells are now generally considered to be the primary source of collagen in normal and fibrotic liver,[5–7,10,12,36] although hepatocytes also have been suggested to contribute to collagen production.[37–39] However, in a rat model of CCl_4-induced liver fibrosis[6] and in experimental biliary fibrosis,[7] increasing amounts of α_2 (I), α_1 (III), and α_1 (IV) procollagen transcripts were demonstrated in cells of expanding fibrous septa and in PSCs, but not in hepatocytes. As shown by Milani et al,[7] procollagen-expressing PSCs were colocalized with desmin-immunoreactive cells suggesting that PSCs and modified PSCs (transitional cells) were implicated in collagen deposition. This correlates with an *in vitro* study indicating that collagen derives from PSCs in primary hepatocyte cultures.[20]

Our findings indicate that a large variety of hepatic pathologic conditions is accompanied by an increase in PSCs expressing α-SM actin. Most of these pathologic settings are characterized by fibrosis. Appearance of anti–αSM-1-positive PSCs was more prominent in active stages of fibrosis and cirrhosis accompanied by cell death than in advanced or inactive stages. However it is worth noting that the occurrence of α-SM actin-expressing PSCs is already clear at a stage of disease earlier than overt hepatic fibrosis. Examples include early stages of alcoholic hepatitis and acute liver allograft rejection. Furthermore a high proportion of anti–αSM-1-positive PSCs in the absence of fibrosis was detected in shock necrosis and leukemic infiltration. In the context of our study, it is of special interest that the response of rat liver to acute CCl_4 injury has been found to result in an increase in desmin-positive PSCs in areas of necrosis as early as 48 hours after intoxication,[40] while in other experiments increased procollagen expression has been demonstrated after 2 weeks of CCl_4 treatment.[6] In humans the anti–αSM-1-positive population may correspond to PSCs with morphologic traits of transitional cells or myofibroblasts described in experimental rat liver injury. This phenotypic change of PSCs has become known as activation of PSCs.[9,41,42] Feeding of rats with alcohol and a high-fat low-protein diet resulted in perisinusoidal cell activation characterized by a significantly increased area of rough endoplasmic reticulum both in PSCs located near sinusoids and within scars.[42] Matsuoka et al[43] have reported that PSCs isolated from rat liver with alcoholic fibrogenesis are activated to produce more collagen and are stimulated more efficiently by Kupffer cell-derived factors compared to PSCs from normal livers. It has been suggested that cytokines such as transforming growth factor β (TGF-β1), interleukin-1 (IL-1), and tumor necrosis factor (TNF) may be released from monocytes/macrophages in conditions such as CCl_4 or alcohol intoxication and schistosomiasis.[44] Recent evidence indicates an important role of TGF-β_1 in hepatic fibrogenesis.[44] In a study of the effect of cytokines on cell proliferation and extracellular matrix formation by cultured PSCs, TGF-β1 inhibited fat-storing cell proliferation while enhancing collagen accumulation in the medium.[45] Interleukin-1α and TNFα had a mitogenic effect but induced an inhibition of collagen formation. It is tempting to

speculate that cytokines may modulate the expression of α-SM actin by PSCs in human liver. Indeed α-SM–positive cells frequently are visible around focal hepatocellular necrosis during septic shock. This condition is characterized by high levels of TNF.[46] Increased serum levels of TNF also have been described in patients with chronic congestive heart failure.[47] In our study, chronic venous congestion was associated with a striking accumulation of PSCs expressing α-SM actin. Thus this pattern may not merely be a consequence of the mechanical hydrostatic pressure and hypoxic cell damage, but also may be related to the effects of TNF. Tumor necrosis factor α has been incriminated in the pathogenesis of pulmonary fibrosis.[48] However we do not know whether cytokines influence PSCs directly or indirectly, eg, *via* Kupffer cells. Furthermore infiltration of sinusoids by monocytes in chronic and acute myelomonocytic leukemia is accompanied by the presence of many α-SM actin-positive cells. This finding is in agreement with the previous observation that bone marrow stromal cells are modulated to express α-SM actin in these types of leukemia.[33]

Little is presently known on the features influencing the appearance of α-SM actin and desmin in stromal fibroblastic cells. It has been reported that γ-interferon decreases the expression of α-SM actin in SMCs and fibroblasts,[49] whereas local application of GM-CSF increases the expression of this marker in fibroblasts surrounding the application site.[50] Our findings correlate with the assumption that the appearance of α-SM actin and desmin-positive fibroblasts coincides with the development of fibrotic changes in several organs and tissues[26] (for review Sappino et al[29]). Further information on the factors regulating the expression of these proteins in stromal cells will help the understanding of the pathogenetic mechanism leading to the development of fibrocontractive diseases, including those of the liver.

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