Histol Histopathol (1999) 14: 719-727

http://www.ehu.es/histol-histopathol

Histology and Histopathology

Cells with morphological and immunohistochemical features of hepatic stellate cells (Ito cells) form an extralittoral (extrasinusoidal) compartment in the cirrhotic rat liver

A. Zimmermann, H. Zimmermann, M. Fellay and J. Reichen

Departments of Pathology and Clinical Pharmacology, University of Berne, Berne, Switzerland

Summary. Systematic studies on hepatic stellate cells and myofibroblasts have so far mainly focused on cells located in the perisinusoidal space of Disse, the so-called littoral compartment. Here, these cells play a key role for intralobular fibrogenesis and sinusoidal capillarization. However, advanced hepatic fibrosis and cirrhosis are characterized by portal tract fibrosis and septal fibrosis, thus involving cells outside the perisinusoidal space. To study the question as to whether hepatic stellate cells occur and are expanded in an extralittoral (extrasinusoidal) compartment in cirrhogenesis, we systematically analyzed the distribution and density of desmin-reactive stellate cells in a rat model of hepatic fibrosis. Fibrosis and remodeling of the liver were induced by bile duct ligation, and stellate cells were identified by single and double immunohistochemistry. We can show that desmin-reactive cells are reproducibly detectable in extralittoral compartments of the normal and fibrotic rat liver. Periductular extralittoral stellate cells are significantly more frequent in cirrhosis, indicating that extralittoral stellate cells expand in concert with proliferating ductules. The findings suggest that ductular proliferation thought to represent a pacemaker of hepatic remodeling is accompanied by a population of cells exhibiting the same phenotype as perisinusoidal stellate cells.

Key words: Hepatic fibrogenesis, Cirrhosis, Hepatic stellate cells, Ito cells

Introduction

Hepatic perisinusoidal stellate cells (HSCs; Ito cells, fat-storing cells, lipocytes) play a pivotal role among cells of the non-hepatocyte parenchyma (Mathew et al., 1996). They are characterized both by a distinct morphology reminiscent of pericytes and several specific functions. Morphologically, HSCs located in the perisinusoidal space of Disse (the so-called littoral compartment) are small cells with numerous thin and branching processes, the latter covering long distances sometimes extending over more than one hepatocyte plate and their endings being in close contact with both the sinusoidal endothelium and the hepatocyte surface (Wake, 1980). Apart from their storage of lipids containing vitamin A, HSCs, which are not easily identifiable in routine preparations, are reproducibly detectable owing to their expression of several markers (Yokoi et al., 1984; Burt et al., 1986a; Buniatian et al., 1996; Nakatani et al., 1996; Neubauer et al., 1996). Functionally, HSCs are regarded as the principal producers of the hepatic extracellular matrix (ECM; Nakano and Lieber, 1982; Friedman et al., 1985; Ogawa et al., 1986; Arenson et al., 1988; Milani et al., 1990; Weiner et al., 1990; Mathew et al., 1996). Intiation and progression of ECM protein and proteoglycan synthesis critically depend on the transformation of HSCs into myofibroblast-like cells (Gabbiani, 1996; Gressner, 1996) expressing alpha-smooth muscle actin (SMA).

Most of the systematic studies on HSCs and MFBs have focused on desmin- and alpha-SMA-reactive cells located in the so-called littoral compartment (LC) of the perisinusoidal space of Disse. In fact, important pathways of hepatic fibrogenesis and sinusoidal capillarization are mediated by ECM-producing cells located in the LC. However, advanced phases of hepatic fibrosis and liver cirrhosis are morphologically characterized by portal tract fibrosis and septal fibrosis, fibrous septa frequently taking their origin in portal tracts and sometimes at interface lesions (piece-meal necroses; Portmann et al., 1985; Nakanuma, 1991; Lory et al., 1994). In most situations, the production of septa is associated with a numerical increase of small bile ducts and ductules (ductular proliferations), the latter being embedded in newly formed fibrous connective

Offprint requests to: Arthur Zimmermann, M.D., Professor of Pathology, Institute of Pathology of the University, Murtenstrasse 30, CH-3010 Berne, Switzerland. Fax: 0041-31-632 99 38

tissue (Nakanuma and Ohta, 1986; Thung, 1990; Slott et al., 1990; Burt and MacSween, 1993). The question therefore arises as to which types of cells are involved in formation of extralobular hepatic fibrosis, as the perisinusoidal space and its LC ends at the peripheral lobular border or at the limiting plate itself. Theoretically, fibrogenic cells of the types defined for the LC might also occur outside the LC, i.e. in an extralittoral (extrasinusoidal) compartment (ELC) being defined as a space outside the perisinusoidal space of Disse. In fact, MFBs and cells reactive for either desmin or alpha-SMA have previously been detected in portal tracts, portal tract-like structures and fibrous septa (Callea et al., 1982; Callea and Desmet, 1985; Burt and MacSween, 1986; Takahara et al., 1988; Yokoi et al., 1988; Milani et al., 1990; Nakatsukasa et al., 1990; Tanaka et al., 1990; Burt, 1993; Lory et al., 1994). In the present study, we systematically assessed the spatial distribution of desmin-reactive cells showing the morphology of HSCs in a rat model of liver fibrosis, emphasis being placed on cells located in an extralittoral compartment.

Materials and methods

Materials

Male Sprague-Dawley rats (Deutsche Versuchstier-farm Hartmutt-Voss, Tuttlingen, Germany) were kept on a standard rat diet with free access to tap water in temperature- and humidity-controlled animal quarters with a 12-h light/dark cycle. The body weight of the animals was 202±7 grams at the time of first surgery.

Experimental design

Details with respect to the experimental design and surgical techniques are given in our previous report on HSC morphometry in rat biliary cirrhosis (Zimmermann et al., 1997). Material from the same animals was analyzed for the present investigation, employing two experimental groups: A) bile duct ligation for 3 weeks (BDL, n=7) (Zimmermann et al., 1992); B) sham operation, control group (SHAM, n=6); laparotomy at the beginning and at 3 weeks. Surgical procedures are specified in our previous study (Zimmermann et al., 1997). The investigation was approved by the local animal ethics committee.

Histopathological and immunohistochemical methods

From harvested rat livers, five 1 ml samples were randomly selected (Gross et al., 1987), precooled in liquid nitrogen and stored at -70 centigrade for later processing. For specificity reasons, frozen sections were chosen for the experiment. Frozen liver sections were produced by use of a cryostat, fixed in acetone for one minute, afterwards incubated with normal rabbit serum (DAKO, Glostrup, Denmark, X0.902, 1:5) for 20 minutes, and then incubated with a monoclonal mouse desmin antibody (DAKO, clone D33, code no. M0760; final dilution: 1:50) for 45 minutes. Afterwards, preparations were incubated with rabbit anti-mouse Ig (rat Ig-absorbed, DAKO 20456, dilution 1:50) for 30 minutes and immunostained employing the APAAP procedure (DAKO D0651, dilution 1:30, for 30 minutes). As a substrate, new fuchsin-naphthol AS-BI (30 minutes) was used. Sections processed without specific antibody served as negative controls. Parallel sections were stained with hematoxylin and eosin and were used for the assessment of overall hepatic changes.

For immunohistochemical double staining (desmin and cytokeratin 7), 5-μm cryostat sections were fixed in acetone for 10 min, air-dried, rehydrated in tris-buffered saline, pH 7.5 (TBS), and immersed in methanol containing 0.3% H_2O_2 for 20 min to block endogenous peroxidase. Sections were then (and following all subsequent steps) washed in TBS. Endogenous rat immunoglobulins that might interfere with cross-reacting anti-mouse Ig secondary antibodies used in later steps were blocked by incubating the slides for 30 min with a rabbit-anti-rat immunoglobulin antibody (DAKO, Glostrup, Denmark; cat. no. Z0455) diluted 1:150 in TBS containing 5% rabbit serum (Life Technologies Inc., Basel, Switzerland), 0.5% casein sodium salt (Sigma, St. Louis, MO, USA), and 0.1% sodium azide. This step was followed by the primary antibody, either mouse-anti-desmin monoclonal antibody, clone D33 (DAKO, M 0760; concentration 4.5 μg Ig/ml, same dilution buffer as above) or mouse-anti-cytokeratin 7, clone OV-TL12/30 (DAKO, M7018; concentration 4.4 μg Ig/ml, same dilution buffer as above) for 45 min at room temperature. Thereafter a horseradish peroxidase-labeled goat-anti-mouse Ig antibody was applied (DAKO, P0447; 1:100 in TBS, 30 min) and the slides were developed in 0.02% 3,3'-diaminobenzidine with 0.005% H_2O_2. Sections were stripped of the antibodies used for the first staining by immersion in 50 mM glycine buffer, pH 2.2 for 30 min (Nakane, 1968; Zhao et al., 1993) and then incubated with the second layer of primary antibodies, i.e. anti-cytokeratin 7 for slides first stained for desmin and vice versa (same antibodies and conditions as above). A rabbit-anti-mouse IgG antibody absorbed with rat serum (DAKO, Z0456; 1:50 in same dilution buffer as above, 30 min) served as the link antibody to the alkaline phosphatase-anti-alkaline phosphatase (APAAP) mouse monoclonal antibody (DAKO, D0651; 1:50 in TBS, 30 min) that was applied as the 3rd layer. Finally, slides were developed in new fuchsin-naphtol AS-BI, counterstained with haematoxylin, and mounted. Sections processed without specific antibodies were used as negative controls.

For the assessment of desmin reactivity, the following cell systems were considered in a preliminary analysis: 1) Slender cells with numerous thin and partly branched cytoplasmic processes, strong immunoreactivity for desmin being detectable both in the cell body and the processes (Fig. 1a). This type of cell was,

in descriptive terms and irrespective of its localization, classified as a stellate cell (STC). 2) Spindle-shaped cells with elongated and thin nuclei showing marked desmin reactivity, but lacking cytoplasmic processes. This type of cell which occurs in blood vessel walls and in the walls of bile ducts, was classified as a smooth muscle cell and was mainly assessed as a control for a good quality of staining (SMC; Fig. 1b). 3) Desmin-reactive cells exhibiting an irregular shape, without cytoplasmic processes, and not forming components of vessel and bile duct walls, descriptively classified as desmin-reactive cells, not otherwise specified (NOSC). After a first analysis of randomly selected sections it was realized that STC were easily distinguishable from both, SMC and NOSC. Therefore, the final analysis was restricted to the assessment of STC exclusively.

For the spatial allocation of STC, the following tissue compartments were analyzed: 1) Littoral compartment (LC), morphologically defined as the parenchymal (intralobular or intranodular) tissue space situated between hepatocyte cords, not extending beyond the peripheral lobular hepatocyte limiting plate or the peripheral border of nodules, and including the peri-sinusoidal space of Disse. 2) Extralittoral compartment (ELC), morphologically defined as the portal tracts and the tissue of fibrous septa (in the case of biliary cirrhosis). Within the LC, desmin-reactive STC were assessed in a qualitative manner only; the ease of detectability of these cells was employed as an internal quality control of immunostaining. Within the ELC, the presence and distribution of STC were studied qualitatively and quantitatively in both the whole

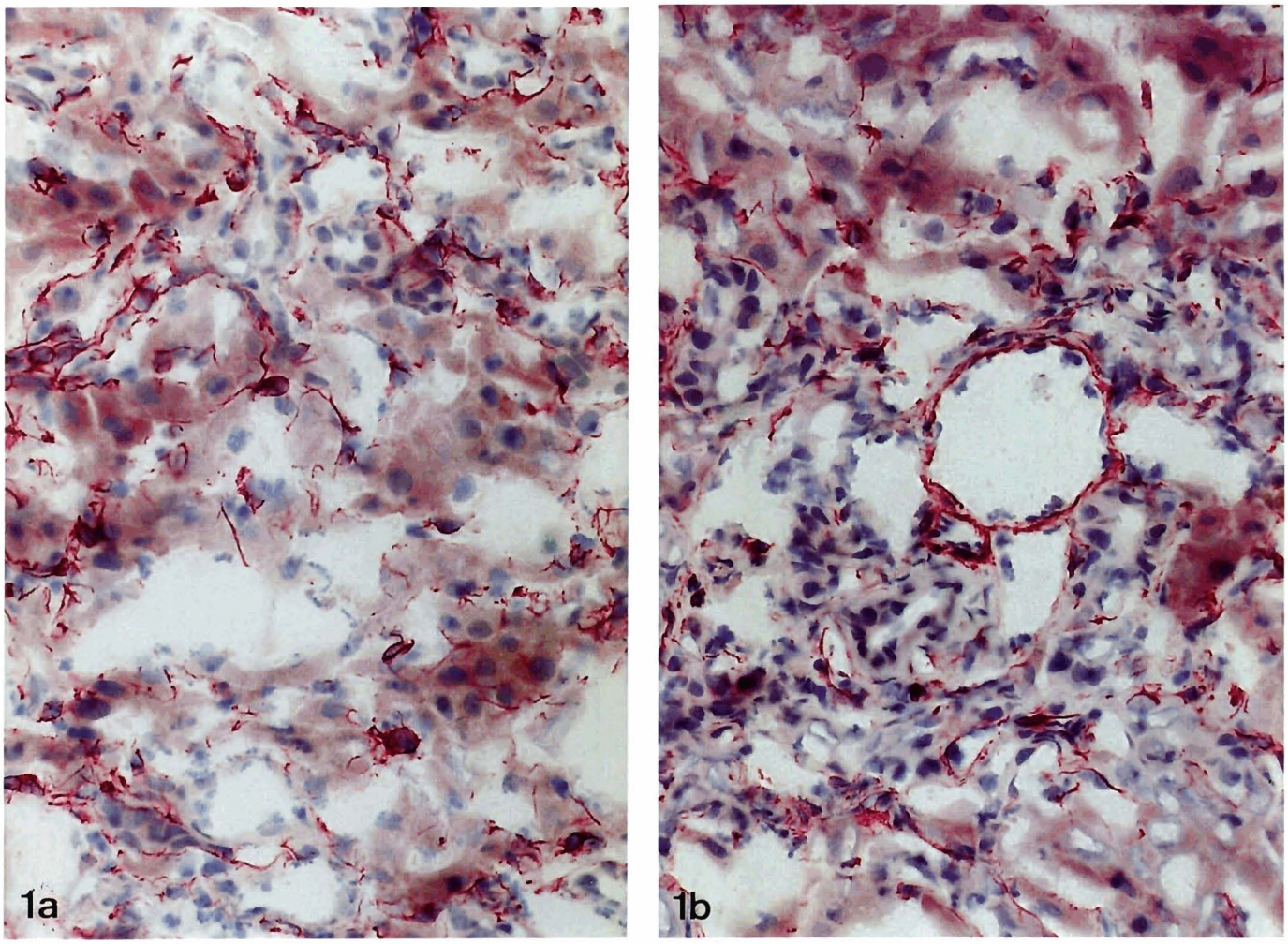


Fig. 1. a. Frozen section of rat liver, parenchyma and areas with ductular reaction. Desmin-reactive cells shown in this figure are characterized by numerous thin and in part branched cytoplasmic processes and are descriptively classified as stellate cells. In this preparation, desmin-reactive stellate cells are located both, within the parenchyma and portal tracts. Tissue gaps represent artifacts due to frozen section technique. Anti-desmin antibody, APAAP immunostain. x 180. **b.** An enlarged portal tract is visualized in the central part of the figure. Apart from stellate cells as specified in a, desmin-reactive spindle cells without processes and representing smooth muscle cells are seen in the walls of a portal vein and a hepatic artery branch. Clear distinction between vessel wall cells without processes and arborizing stellate cells was important for the detection of an extralittoral compartment of stellate cells. Anti-desmin antibody, APPAP immunostain. x 180

compartment and its subcompartments (periductal, periductular and perivascular tissue spaces). For this purpose, sections stained for desmin alone and double-immunostained preparations were used. The latter served to reliably identify bile duct and ductular cells owing to their reactivity for cytokeratin 7 (CK 7). A spatial relationship between desmin-positive STC and CK-7-positive bile duct cells could thus easily be assessed in one and the same section. As an additional control, double immunohistochemistry was performed cross-wise with respect to the staining procedure (immuno-peroxidase and APAAP for desmin and CK-7, respectively, and vice versa).

For the BDL and SHAM groups, 50 randomly chosen areas per tissue section were selected and analyzed using a x40 objective. The number of STC spatially associated with periductal, periductular and perivascular spaces in ELC was assessed in each area, and positivity for STC was expressed as percent of all structures thus involved.

Statistical Evaluation

The results are expressed as mean±SD. The Wilcoxon test was used to compare groups. $P<0.05$ was accepted as the significance level.

Results

In the control group (SHAM), qualitative analysis of desmin-reactive cells disclosed cells with the typical morphology and reactivity of STCs and representing typical HSCs mainly within the parenchyma, and here

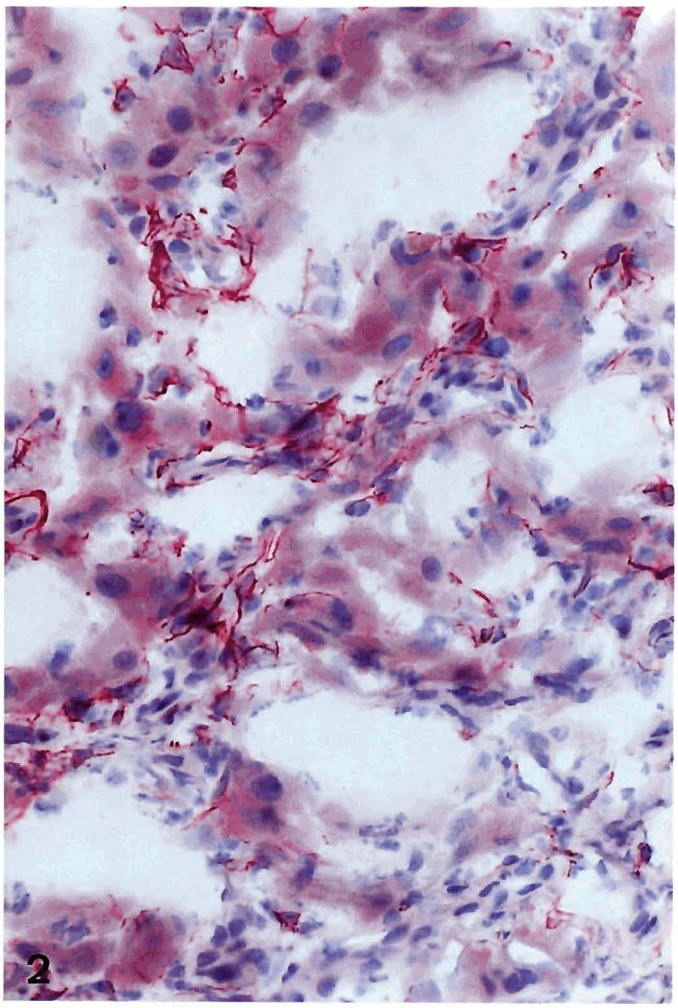


Fig. 2. Frozen section of liver tissue of a BDL rat. Note that desmin-reactive stellate cells appear to be more densely distributed in areas occupied by portal tracts exhibiting ductular proliferations. Anti-desmin antibody, APAAP immunostain. x 180

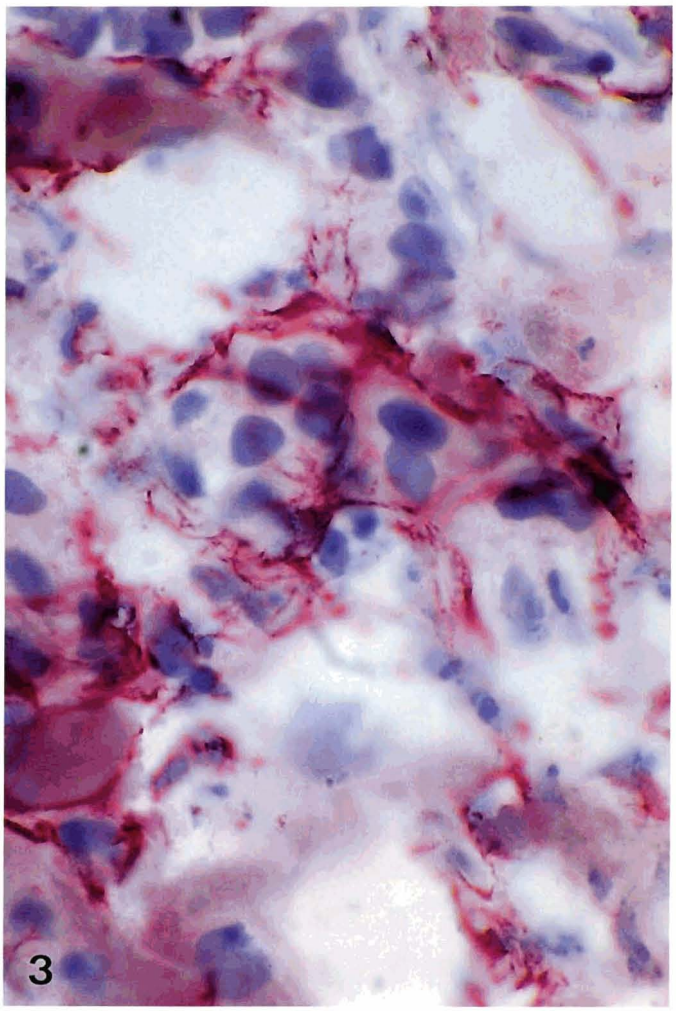
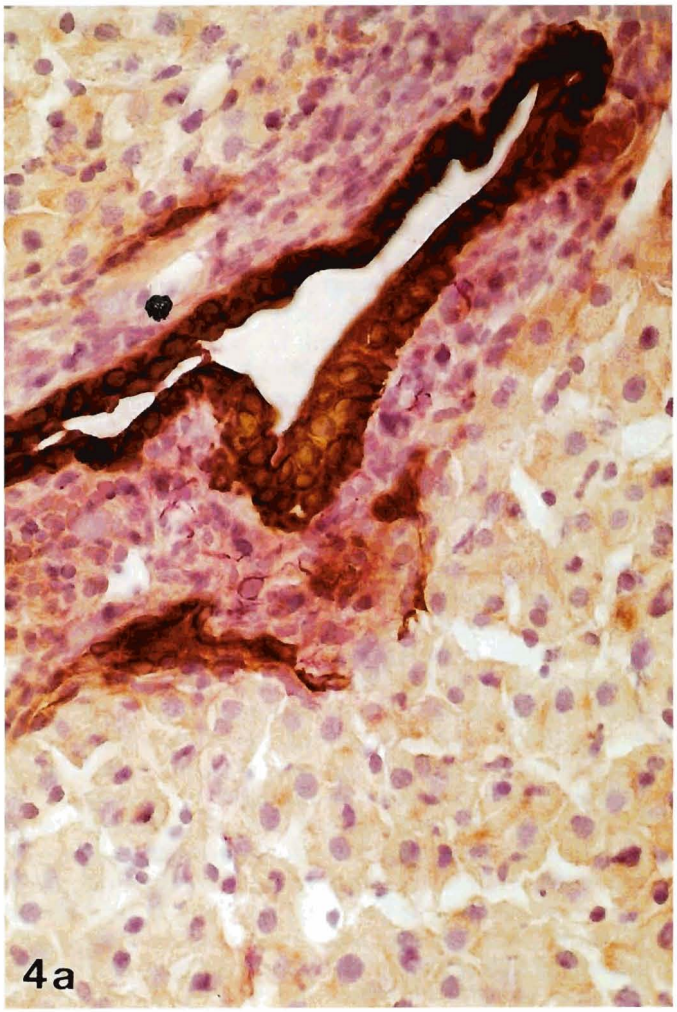


Fig. 3. Frozen section of liver tissue of a BDL rat at high magnification. In the center of the figure, profiles of small bile ducts (ductules) are seen. These ductules are in close spatial contact with a dense desmin-reactive meshwork of cytoplasmic processes. As periductal or periductular smooth muscle cells do not exhibit this type of processes, the cells involved are classified as extralittoral stellate cells. Anti-desmin antibody, APAAP immunostain. x 240

preferentially along the sinusoids (LC). As expected, the overall density of these cells was low in control sections but, in some lobules, appeared to be somewhat higher in the pericentral zone and/or around portal tracts. Conversely, a qualitatively higher overall density of STCs was detected in the BDL group (Fig. 2). It was seen that STCs were on the one hand present within the parenchyma similar to the situation in SHAM, but on the other hand they appeared to be clustered in areas occupied by portal tracts, proliferated ductules and septa. A regularly observed finding consisted of a higher density of STCs in the LC adjacent to ductules that had sprouted into the periportal parenchyma; STCs in these areas sometimes formed an arc-like arrangement around sectors of parenchyma occupied by ductules. No stained cells were detectable in sections processed without specific antibody.

In oversized and fibrotic portal tracts present in the BLD group having developed biliary cirrhosis, increased numbers of small bile ducts were observed (ductular reaction with proliferation of ductules). At high magnification, desmin-reactive STCs were detected in close spatial relationship with ductules (Fig. 3). Similar to the situation in the LC lining the hepatocyte plates within the parenchyma, where processes of PSCs are known to be in direct contact with the hepatocyte surface



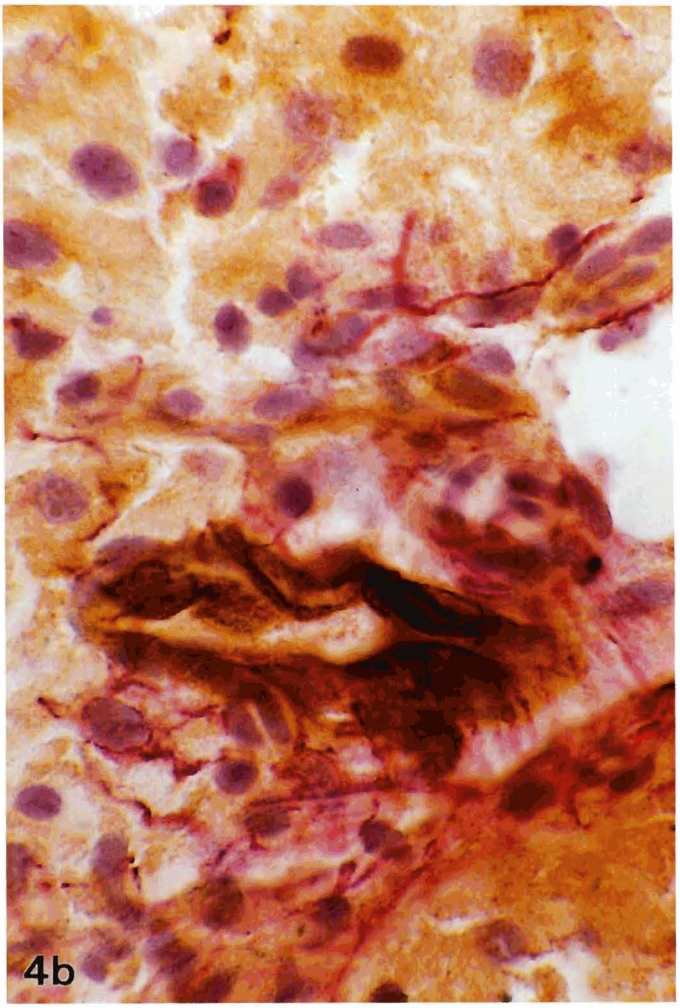
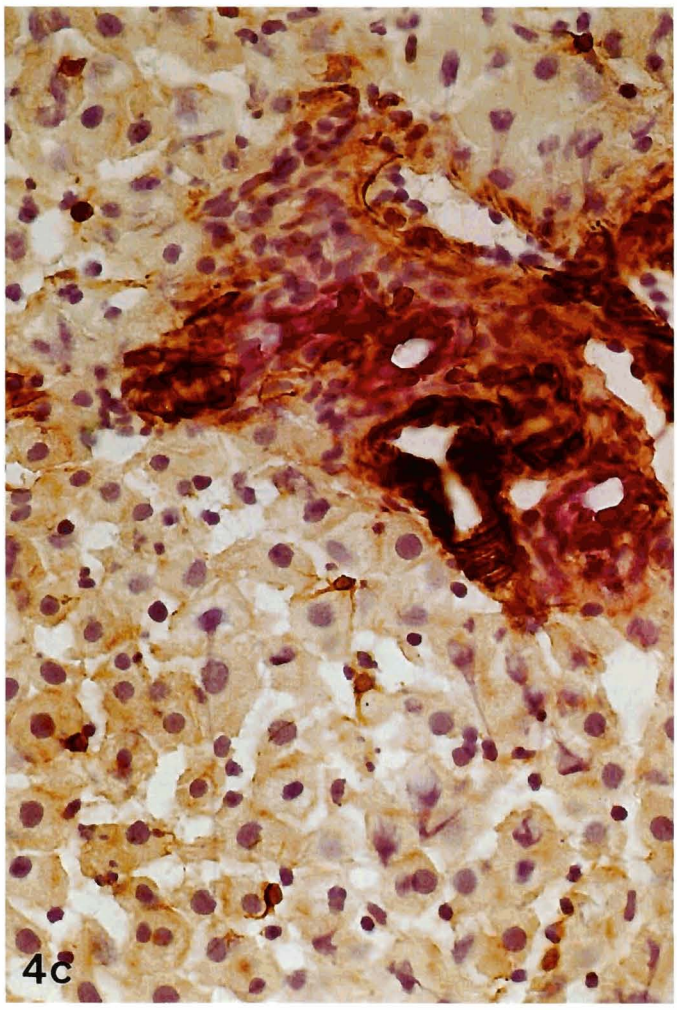


Fig. 4. a. Double-immunostained liver tissue, BDL group. Cholangiocytes are visualized owing to their expression of cytokeratin 7 (dark brown reaction product), whereas desmin-reactive stellate cells exhibit red cytoplasm and processes. Note that desmin-positive stellate cells are present within the portal tract space (extralittoral compartment), sometimes in close spatial relationship with the bile duct and ductules. CK 7 and desmin double immunohistochemistry. x 180. **b.** Peripheral part of a portal tract, BDL group, double immunostain for cytokeratin 7 (dark brown reaction product). and for desmin (red reaction product). At this high magnification, a desmin-reactive cell with the typical features of a stellate cell is seen to be in close contact with a CK 7-reactive ductule. CK 7 and desmin double immunohistochemistry. x 240. **c.** Double-immunostained liver tissue, BDL group. In this preparation, cholangiocytes are depicted in red and desmin-expressing cells in dark brown, i.e. vice versa as to Figures 4a and 4b. Desmin-reactive brown stellate cells are observed in the hepatic parenchyma (bottom half of figure; littoral compartment). In addition, few desmin-positive stellate cells are located within the portal tract (extralittoral compartment), in part being close to a small CK 7-reactive bile duct. The larger brown structures represent the muscle layers of arteries and veins. CK 7 and desmin double immunohistochemistry. x 180

and to sometimes extend over several hepatocytes or even hepatocyte plates, STCs situated along or around ductular profiles were seen to form a delicate network of fine processes surrounding single ductular cells or small groups thereof (Fig. 3). STCs clearly located within parenchyma were sometimes found to be in apparent continuation with STCs forming a population around ductules. Sometimes, desmin-reactive cells being in close contact with a ductule disclosed a larger cytoplasmic body, even though they also showed long processes. Cells of this type may represent transition forms between HSCs and MFBs.

Double immunostaining for desmin and CK-7 confirmed that desmin-reactive STC can occur in close contact to CK-7-reactive small bile ducts or ductules (Fig. 4a-c).

Quantitatively, STCs located in the ELC of the control group (SHAM) were detected in periductal/periductular and perivascular subcompartments in 8.5±4.6% and 2.2±1.7%, respectively. In the BDL group, periductal/periductular and perivascular STCs were observed in 85±22.6% and 5.0±2.5%, respectively (control vs. BDL: periductal/periductular compartment $p<0.01$; perivascular compartment: NS).



Discussion

The present study shows that stellate desmin-reactive cells (STC) with a morphology similar to or identical with perisinusoidal hepatic stellate cells (HSC) are reproducibly detectable in extralittoral compartments of the normal and cirrhotic rat liver. Extralittoral cells with this morphological and immunohistochemical phenotype mostly occur in close spatial relationship to small bile ducts and ductules and, albeit less frequently, close to small hepatic artery branches accompanying ducts and ductules. Periductal and periductular extralittoral STC are encountered significantly more frequently in cirrhosis induced by BDL, suggesting that the extralittoral population of STC is expanding in concert with ductular proliferation developing in hepatic fibrosis and nodular change. Similar to perisinusoidal stellate cells, extralittoral rat STC are characterized, apart from their desmin reactivity, by numerous branching cytoplasmic processes forming a delicate array of structures apparently being in direct contact with other cells; this is particularly evident for bile duct and ductule cells which thereby are engulfed by a network of processes. Littoral, i.e. perisinusoidal stellate cells were apparently in direct continuation with extralittoral STC. These findings suggest that in normal and cirrhotic rat liver a significant extralittoral compartment of desmin-positive stellate, HSC-like cells exists; we propose that these cells make part of the same or at least a similar cell lineage as HSC. Myofibroblasts and spindle cells being either reactive for desmin or smooth-muscle actin (SMA) have previously been detected in hepatic portal tracts and in fibrous septa, but not by use of systematic investigations (Callea et al., 1982; Callea and Desmet, 1985; Burt et al., 1986a,b; Takahara et al., 1988; Yokoi et al., 1988; Milani et al., 1990; Nakatsukasa et al., 1990; Tanaka et al., 1990; Lory et al., 1994). The reasons and the implications of the phenomenon that cells currently thought to be chiefly localized to the perisinusoidal space of Disse (the so-called littoral compartment) are also located to an extralittoral compartment are not known. Moreover, it is not clear whether desmin-reactive cells observed in the two compartments involved are members of the same or a similar cell lineage, irrespective of their similarity regarding morphology, immunophenotype and contacts to adjacent cells. However, the presence of significant numbers of STC with a function purportedly similar or identical to that of typical perisinusoidal hepatic stellate cells (HSC) in extralittoral compartments of portal tracts and, in case of cirrhosis, in fibrous septa has an impact on our understanding of cirrhogenesis. Perisinusoidal stellate cells, which express desmin in the rat (Yokoi et al., 1984) but not in humans (Schmitt-Gräff et al., 1991) have a key role in fibrosing liver disease, where they perform a transition from a quiescent to an activated

phenotype chiefly characterized by expansion of the cell population by proliferation and subsequent differentiation first into transitional cells and then into ECM-producing myofibroblast-like cells (Burt, 1993; Friedman, 1993; Gressner, 1994, 1995; Desmoulière et al., 1995; Hautekeete and Geerts, 1997; Kawada, 1997).
In acute liver damage, HSC are known to be capable of expanding beyond the space of Disse; e.g., they can spread into large areas of necrosis or focal necroses, in case of massive hepatic necrosis and in acute viral hepatitis (Inuzuka et al., 1990; Kobayashi and Fujiyama, 1981). In addition, SMA-positive derivatives of HSC have been demonstrated to localize around areas of perivenular necrosis in acute heart failure and shock (Schmitt-Gräff et al., 1991). Conversely, expansion and spread of HSC and myofibroblasts in chronic liver disease are less well known. The number and density of SMA-reactive cells have been reported to be increased in areas of piecemeal necrosis in chronic active viral hepatitis and these cells have been found to be adjacent to damaged hepatocytes and infiltrating inflammatory cells (Yamaoka et al., 1993; Enzan et al., 1994). In obstructive cholestatic disorders, myofibroblast-like cells are accumulated predominantly in periportal areas (Schmitt-Gräff et al., 1991), and we recently detected a numerical increase of this cell type within biliary piecemeal necrosis, together with vascular sprouts, in hepatic atrophy caused by biliary obstruction (Lory et al., 1994). Taken together, these previous findings demonstrated the phenomenon that HSC and myofibroblast-like cells may accumulate at certain sites of damage within liver lobules, and particularly at their periphery, but this does not sufficiently explain the pathogenesis of portal tract fibrosis and septal fibrosis occurring in cirrhosis of the liver. Therefore, other cells have been proposed, mainly fibroblasts in portal tracts, the hepatic capsule and the second-layer cells being characterized by fibroblasts situated around distal segments of the central veins (Bhunchet and Wake, 1992). In fact, portal tract fibroblasts are activated and extend into parenchyma in cholestatic liver injury after bile duct ligation (Desmoulière et al., 1997), and it has been shown that portal fibroblasts participate in the development of connective tissue deposits surrounding proliferating ductules in experimental fibrosis induced by bile duct ligation (Tuchweber et al., 1996). The matrix-producing cell lineage involved and the differentiation pathways resulting in a periductular cell population with the morphological features of fibroblasts are only starting to be better understood. In a recent investigation it has been clearly shown that during the initial stages of cholestatic fibrosis in the rat, stromal cells adjacent to proliferating ductules start to be reactive for alpha-SMA at 48 hours after bile duct ligation, and that the number of alpha-SMA-positive cells increased until the seventh day (Desmoulière et al., 1997). In this rat model of hepatic fibrosis it was suggested that myofibroblastic differentiation represents an adaptive response to a modification of the cellular environment which is modified by ECM deposition very early after injury (Desmoulière et al., 1997), whereas, in early stages after bile duct ligation, expression of alpha-SMA in hepatic stellate cells was not in evidence (Tuchweber et al., 1996). The findings of the present investigation may, however, suggest an alternate or additional pathogenic mechanism involved in the accumulation of matrix-producing myofibroblast-like cells in portal tracts and septa. We propose that in this rat model of biliary cirrhosis, an extralittoral compartment of desmin-reactive stellate cells being present under normal conditions and expanding during fibrogenesis represents at least a fraction of precursor cells later undergoing differentiation into periductal, periductular and periarterial myofibroblast-like cells, utilizing a differentiation pathway similar to that known for perisinusoidal HSC (Enzan et al., 1994). Provided this hypothesis is significant, the question arises as to what relationships may exist between stellate cells situated in littoral vs. extralittoral compartments, and as to how the two compartments may be linked. Littoral stellate cells are located in Disse's space which, per definition, represents an interstitial space where most of the hepatic lymph is formed (Barrowman, 1991; Vollmar et al., 1997). The connection of this space with interstitial spaces of portal tracts, and particularly the periportal space of Mall surrounding bile ducts and ductules has only partially been clarified (Hardonk and Armosoerodjo-Briggs, 1993). However, it has been shown that fluid formed in Disse's space leaves the littoral compartment through gaps between hepatocytes of the limiting plate to reach the space of Mall and, subsequently, the periductular and periarterial interstitial space. The intercellular gaps at the limiting plate contain slender processes of portal tract fibroblast-like cells extending into the parenchyma from the periportal space of Mall (Al-Jomard et al., 1985). We therefore suggest that these ultrastructural findings represent the morphological substrate for a spatial continuum of littoral and extralittoral stellate cell compartments, but further investigations are needed to clarify this point.

Acknowledgements. Supported by a grant from the Swiss National Foundation for Scientific Research to JR (32.45349.85) and HZ (No 32-29970.90). The secretarial help of E. Müller and the technical assistance of A. Kappeler are gratefully acknowledged.

References

Al-Jomard A., Reid O. and Scothorne R.J. (1985). An EM Study of the route of drainage of interstitial fluid from the space of Disse into portal tract lymphatics. Proc. XIIth Int. Anatom. Congress. p 9 (Abstract).

Arenson D.M., Friedman S.L. and Bissell D.M. (1988). Formation of extracellular matrix in normal rat liver: Lipocytes as a major source of proteoglycan. Gastroenterology 95, 441-447.

Barrowman J.A. (1991). Hepatic lymph and lymphatics. In: Oxford textbook of clinical hepatology. Vol. 1. McIntyre N., Benhamou J.P.,

Bircher J., Rizzetto M. and Rodes J. (eds). Oxford University Press. Oxford. pp 37-40.

Bhunchet E. and Wake K. (1992). Role of mesenchymal cell populations in porcine serum-induced rat liver fibrosis. Hepatology 16, 1452-1473.

Buniatian G., Gebhardt R., Schrenk D. and Hamprecht B. (1996). Colocalization of three types of intermediate filament proteins in perisinusoidal stellate cells: glial fibrillary acidic protein as a new cellular marker. Eur. J. Cell Biol. 70, 23-32.

Burt A.D. (1993). Cellular and molecular aspects of hepatic fibrosis. J. Pathol. 170,105-114.

Burt A.D. and MacSween R.N.M. (1993). Bile duct proliferation - its true significance. Histopathology 23, 599-602.

Burt A.D. and MacSween R.N.M. (1986a). Immunolocalisation of desmin in fixed rat and human liver. In: Cells of the hepatic sinusoid. Rjiswijk: The Kupffer cell foundation. Kirn A., Knook D.L. and Wisse E. (eds). Vol.1: Proceedings of the 3rd Int Kupffer cell symposium held in Strasbourg. pp 253-254.

Burt A.D., Robertson J.L., Heir J. and MacSween R.N.M. (1986b). Desmin-containing stellate cells in rat liver; distribution in normal animals and response to experimental acute liver injury. J. Pathol. 150, 29-35.

Callea F. and Desmet V.J. (1985). Transformation of fat storing cells (Ito cells) to myofibroblasts. J. Hepatol. (Suppl.) 2, 206 (Abstract).

Callea F., Mebis J. and Desmet V.J. (1982). Myofibroblasts in focal nodular hyperplasia of the liver. Virchows Arch. (A) 369, 155-166.

Desmoulière A., Tuchweber B. and Gabbiani G. (1995). Role of the myofibroblast differentiation during liver fibrosis. J. Hepatol. (Suppl 2) 22, 61-64.

Desmoulière A., Darby I., Costa A.M.A., Raccurt M., Tuchweber B., Sommer P. and Gabbiani G. (1997). Extracellular matrix deposition, lysyl oxidase expression, and myofibroblastic differentiation during the initial stages of cholestatic fibrosis in the rat. Lab. Invest. 76, 765-778.

Enzan H., Himeno H., Iwamura S., Saibara T., Onishi S., Yamamoto Y. and Hara H. (1994). Immunohistochemical identification of Ito cells and their myofibroblastic transformation in adult human liver. Virchows Arch. 424, 249-256.

Friedman S.L. (1993). The cellular basis of hepatic fibrosis: mechanisms and treatment strategies. N. Engl. J. Med. 328, 1828-1835.

Friedman S.L., Roll F.J., Boyles J., Bissell D.M. (1985). Hepatic lipocytes. The principal collagen-producing cells of normal rat liver. Proc. Natl. Acad. Sci. USA 82, 8681-8685.

Gabbiani G. (1996) The cellular derivation and the life span of the myofibroblast. Pathol. Res. Pract. 192, 708-711.

Gressner A.M. (1994). Perisinusoidal lipocytes and fibrogenesis. Gut 35, 1331-1333.

Gressner A.M. (1995). Cytokines and cellular crosstalk involved in the activation of fat-storing cells. J. Hepatol. (Suppl 2) 22, 28-36.

Gressner A.M. (1996). Transdifferentiation of hepatic stellate cells (Ito cells) to myofibroblasts: a key event in hepatic fibrogenesis. Kidney Int. Suppl. 54, S39-45.

Gross J.B., Reichen J., Zeltner T. and Zimmermann A. (1987). The evolution of changes in quantitative liver function tests in a rat model of cirrhosis. Hepatology 7, 457-463.

Hardonk M.J. and Armosoerodjo-Briggs J. (1993). Evidence for the anatomical connection between the space of Disse and the portal tract in human and rat liver. Cells of the Hepatic Sinusoid. Vol 4. Knook D.L. and Wisse E. (eds). Kupffer Cell Foundation. Leiden. pp 182-184.

Hautekeete M.L. and Geerts A. (1997). The hepatic stellate (Ito) cell: its role in human liver disease. Virchows Arch. 430,195-207.

Inuzuka S., Ueno T., Torimura T., Sata M., Abe H. and Tanikawa K. (1990). Immunohistochemistry of the hepatic extracellular matrix in acute viral hepatitis. Hepatology 12, 249-256.

Kawada N. (1997). The hepatic perisinusoidal stellate cell. Histol. Histopathol. 12, 1069-1080.

Kobayashi Y. and Fujiyama S. (1981). Pathological study on gold impregnation of fat-storing cell in human liver. Acta Pathol. Jpn. 31, 65-74.

Lory J., Schweizer W., Blumgart L.H. and Zimmermann A. (1994). The pathology of the atrophy/hypertrophy complex (AHC). of the liver. A light microscopic and immunohistochemical study. Histol. Histopathol. 9, 541-554.

Mathew J., Geerts A. and Burt A.D. (1996). Pathobiology of hepatic stellate cells. Hepatogastroenterology 43, 72-91.

Milani S., Herbst H., Schuppan D., Kim K.Y., Riecken E.O. and Stein H. (1990). Procollagen expression by nonparenchymal rat liver cells in experimental biliary fibrosis. Gastroenterology 98, 175-184.

Nakane P.K. (1968). Simultaneous localization of multiple tissue antigens using the peroxidase-labeled antibody method: a study on pituitary glands of the rat. J. Histochem. Cytochem. 16, 557-560.

Nakano M. and Lieber C.S. (1982). Ultrastructure of initial stages of perivenular fibrosis in alcohol-fed baboons. Am. J. Pathol. 106, 145-155.

Nakanuma Y. (1991). Pathology of septum formation in primary biliary cirrhosis: A histological study in the non-cirrhotic stage. Virchows Arch. (A) 419, 381-387.

Nakanuma Y. and Ohta G. (1986). Immunohistochemical study on bile ductular proliferation in various hepatobiliary diseases. Liver 6, 205-211.

Nakatani K., Seki S., Kawada N., Kobayashi K. and Kaneda K. (1996). Expression of neural cell adhesion molecule (N-CAM). in perisinusoidal stellate cells of the human liver. Cell Tissue Res. 283, 159-165.

Nakatsukasa H., Nagy P., Evarts R.P., Hsia C-C., Marsden E. and Thorgeirsson S.S. (1990). Cellular distribution of transforming growth factor-beta1 and procollagen types I, III, and IV transcripts in carbon tetrachloride-induced rat liver fibrosis. J. Clin. Invest. 85, 1833-1843.

Neubauer K., Knittel T., Aurisch S., Fellmer P. and Ramadori G. (1996). Glial fibrillary acidic protein - a cell type specific marker for Ito cells in vivo and in vitro. J. Hepatol. 24, 719-730.

Ogawa K., Suzuki J., Mukai H. and Mori M. (1986). Sequential change 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.

Portmann B., Popper H., Neuberger J. and Williams R. (1985). Sequential and diagnostic features in primary biliary cirrhosis, based on serial histologic study in 209 patients. Gastroenterology 88, 1777-1790.

Schmitt-Gräff A., Krüger S., Bochard F., Gabbiani G. and 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.

Slott P.A., Liu M.H. and Tavaloni N. (1990). Origin, pattern, and mechanism of bile duct proliferation following biliary obstructin in the

rat. Gastroenterology 99, 466-477.

Takahara T., Kojima T., Miyabayashi C., Inoue K., Sasaki H., Muragaki Y. and Ooshima A. (1988). Collagen production in fat-storing cells after carbon tetrachloride intoxication in the rat. Immunoelectron microscopic observation of type I, type III collagens, and prolyl hydroxylase. Lab. Invest. 59, 509-521.

Tanaka Y., Mak K.M. and Lieber C.S. (1990). Immunohistochemical detection of proliferating lipocytes in regenerating rat liver. J. Pathol. 160, 129-134.

Thung S.N. (1990). The development of proliferating ductular structures in liver disease. An immunohistochemical study. Arch. Pathol. Lab. Med. 114, 407-411.

Tuchweber B., Desmouliere A., Bochaton-Piallat M.L., Rubbia-Brandt L. and Gabbiani G. (1996). Proliferation and phenotypic modulation of portal fibroblasts in the early stages of cholestatic fibrosis in the rat. Lab. Invest. 74, 265-278.

Vollmar B., Wolf B., Siegmund S., Katsen A.D. and Menger M.D. (1997). Lymph vessel expansion and function in the development of hepatic fibrosis and cirrhosis. Am. J. Pathol. 151, 169-175.

Wake K. (1980). Perisinusoidal stellate cells (fat-storing cells, interstitial cells, lipocytes), their related structure in and around the liver sinusoids, and vitamin A-storing cells in extrahepatic organs. Int. Rev. Cytol. 66, 303-353.

Weiner F.R., Giambrone M-A., Czaja M.J., Shah A., Annoni G., Takahashi S., Eghbali M. and Zern M.A. (1990). Ito-cell expression and collagen regulation. Hepatology 11, 111-117.

Yamaoka K., Nouchi T., Marumo F. and Sato C. (1993). α-Smooth-muscle actin expression in normal and fibrotic human livers. Dig. Dis. Sci. 38, 1473-1479.

Yokoi Y., Namihisa T., Kuroda H. and Komatsu I. (1984). Immunocytochemical detection of desmin in fat-storing cells (Ito-Cells). Hepatology 4, 709-714.

Yokoi Y., Namihisa T., Matsuzaki K., Miyazaki A. and Yamaguchi Y. (1988). Distribution of Ito cells in experimental hepatic fibrosis. Liver 8, 48-52

Zhao M., Laissue J.A. and Zimmermann A. (1993). "Neuroendocrine" differentiation in hepatocellular carcinomas (HCCs): Immunohistochemical reactivity is related to distinct tumor cell types, but not to tumor grade. Histol. Histopathol. 8, 617-626.

Zimmermann H., Reichen J., Zimmermann A., Sägesser H., Thenisch B. and Höflin F. (1992). Reversibility of secondary biliary fibrosis by biliodigestive anastomosis in the rat. Effect of relief of biliary obstruction on microsomal lipids, portal pressure and hepatic structure. Gastroenterology 103, 579-589.

Zimmermann H., Fellay M. and Zimmermann A. (1997). Hepatic stellate cells (Ito cells) but not collagen IV may partly be responsible for lower portal pressure after reversing secondary biliary cirrhosis in the rat. J. Hepatol. 26, 158-166.

Accepted November 20, 1998