Virchows Arch (1997) 430:195–207

REVIEW ARTICLE

Marc L. Hautekeete · Albert Geerts

The hepatic stellate (Ito) cell: its role in human liver disease

Received: 19 December 1995 / Accepted: 27 September 1996

Abstract The hepatic stellate (Ito) cell lies within the space of Disse and has a variety of functions. Stellate cells store vitamin A in characteristic lipid droplets. In the normal human liver, the cells can be identified by the presence of these lipid droplets; in addition, many stellate cells in the normal liver express α-smooth muscle actin. In acute liver injury, there is an expansion of the stellate cell population with increased α-smooth muscle actin expression; stellate cells appear to play a role in extracellular matrix remodelling after recovery from injury. In chronic liver injury, the stellate cell differentiates into a myofibroblast-like cell with marked expression of α-smooth muscle actin and occasional expression of desmin. Myofibroblast-like cells have a high fibrogenic capacity in the chronically diseased liver and are also involved in matrix degradation. In vitamin A intoxication, hypertrophy and proliferation of the stellate and myofibroblast-like cells may lead to non-cirrhotic portal hypertension, fibrosis and cirrhosis. In liver tumours, myofibroblast-like cells are involved in the capsule formation around the tumour and in the production of extracellular matrix within it. The transition of stellate cells into myofibroblast-like cells is regulated by an intricate network of intercellular communication between stellate cells and activated Kupffer cells, damaged hepatocytes, platelets, endothelial and inflammatory cells, involving cytokines and nonpeptide mediators such as reactive oxygen species, eicosanoids and acetaldehyde. The findings suggest that the stellate cell plays an active role in a number of human liver diseases, with a particular reactivity pattern in fibrotic liver disorders.

Key words Hepatic stellate cell · Ito cell · Fat-storing cell · Lipocyte · Fibrosis

M.L. Hautekeete (✉) · A. Geerts
Laboratory for Cell Biology and Histology,
Free University of Brussels (VUB),
Laarbeeklaan 103, B-1090 Brussels, Belgium
Tel.: (32) 2-477 6300, Fax: (32) 2-477 6325

Introduction

The space of Disse of the liver harbours an intriguing cell which is known under a variety of names: Ito cell, fat-storing cell, lipocyte, perisinusoidal cell, parasinusoidal cell, or, as was recently proposed, hepatic stellate cell. The cell was originally described as "Sternzelle" (stellate cell) by von Kupffer in 1876 who identified the cells with a gold impregnation technique [118]. However, von Kupffer later confused these cells with liver macrophages which now bear his name. Ito and Nemoto [62] showed that the stellate cells are distinct from macrophages and sinusoidal endothelial cells, and Wake demonstrated their vitamin A storing capacity [119] (for a historical review, see Aterman [4]).

Stellate cells are located in the space of Disse, under the endothelial cell layer. The cells have long cytoplasmic processes which underly the endothelium and embrace the sinusoid on the one hand, and which make contact with a great number of hepatocytes, on the other (Fig. 1). They also make contact with neighbouring stellate cells and nerve endings. An intriguing aspect of stellate cell function is the capacity to express a dual phenotype. In healthy liver, the cells show the "quiescent phenotype", that is to say, they contain vitamin A-rich lipid droplets, under developed organelles and show a low proliferation rate. In the chronically diseased liver, the cells acquire the "activated phenotype" – they differentiate into myofibroblast-like cells with high proliferative capacity, thereby losing their vitamin A-containing fat-droplets, and developing a prominent rough endoplasmic reticulum, extensive microtubules and bundles of actin filaments with local condensations. A variety of functions have been ascribed to the stellate cells and the myofibroblast-like cells derived from them: they take up, store, and release retinoids; they, particularly in their myofibroblast-like form, synthesize and secrete extracellular matrix proteins (collagens I, III, IV, V and VI, fibronectin, laminin, tenascin, undulin, hyaluronan and proteoglycans); stellate cells and derived myofibroblast-like cells in particular synthesize and secrete extracellu-

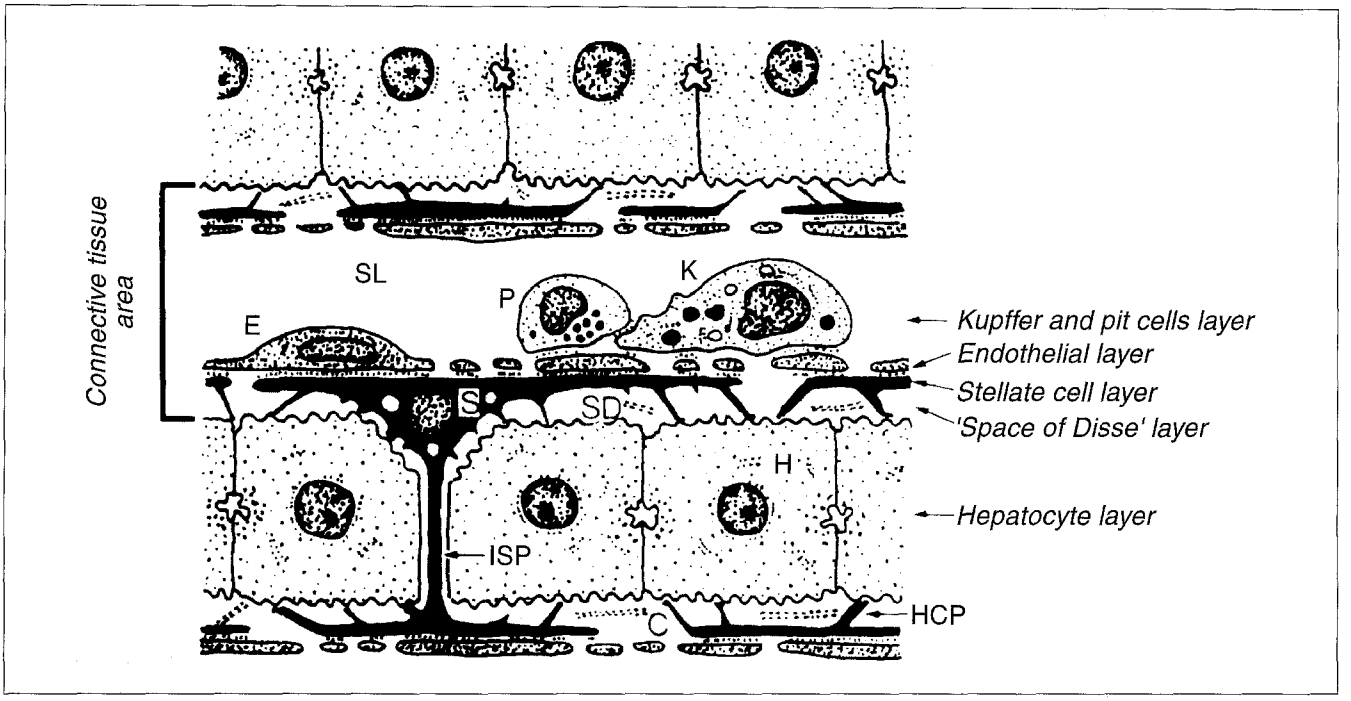


Fig. 1 Schematic representation of the localization of the stellate cell in the sinusoidal wall. *S* stellate cell, *E* endothelial cell, *H* hepatocyte, *K* Kupffer cell, *P* pit cell, *ISP* intersinusoidal processes, *HCP* hepatocyte-contacting processes, *SD* space of Disse, *C* collagen fibrils, *SL* sinusoidal lumen. (From [120] with permission of the author, courtesy of Professor K. Wake, Tokyo University)

lar matrix degrading metalloproteinases and tissue inhibitors of metalloproteinase-1; in both forms they synthesize and secrete cytokines and, especially, as myofibroblast-like cells they are able to contract in response to vasoactive substances and are thus potentially involved in the regulation of hepatic sinusoidal tone.

Several reviews on hepatic stellate cells have been published in recent years, considering general aspects [95, 99] or focusing on their biology [39], their role in fibrogenesis [15, 32, 41, 42], matrix degradation [2] and retinoid storage [51]. Current data on stellate cells is derived primarily from data from animal models, and information on stellate cells in human pathology is still scanty. It is interesting to note that there are some differences between rat and human stellate cells. They differ, in particular in their expression of intermediate filaments and other cellular markers. Most rat stellate cells express desmin [7, 123] and glial fibrillary acidic protein (GFAP) [36, 88, 89]. In normal human liver, stellate cells are devoid of desmin [108] while GFAP expression is limited to a small subpopulation of stellate cells [49]. The expression of α-smooth muscle actin varies between rats and human stellate cells, both in quiescent stellate cells and myofibroblast-like cells [7, 28, 92, 108, 109, 122], and there are subtle differences between rat and human stellate cells in culture [34]. It is the purpose of this review to summarize the present knowledge concerning the role of the hepatic stellate cell in human pathology. We will focus on available knowledge on stellate cells in human liver diseases and stress the differences with findings in the rat where necessary.

Identification of human hepatic stellate cells – heterogeneity of the stellate cell population

Hepatic stellate cells are not readily recognised in routinely (haematoxylin and eosin) stained sections. Certain staining techniques, electron microscopy or immunocytochemical markers are needed for their identification (Table 1). Histological techniques used are toluidine blue staining [13, 48, 53, 54, 55, 93], basic fuchsin staining [35] and oil-red-O [56], which all allow the detection of the characteristic lipid droplets (Fig. 2). A minority of cells possess no lipid droplets, however [112]. The cells can also be detected by the autofluorescent properties of vitamin A, which causes a rapidly fading blue-green fluorescence at 328 nm. This method can be used in rats and has been successfully applied in cases of vitamin A intoxication in humans [47, 106, 124] but in other conditions it is impractical in the human liver because of rapid fading of fluorescence and marked background fluorescence. Von Kupffer originally described the cells using a gold chloride method; this method has been modified [67, 116] but occasionally yields inconsistent results. Using gold chloride staining on human liver a higher number of positive cells was observed in the centrolobular areas [67]; positivity of this stain depends on the presence of vitamin A in the stellate cells [67, 116]. Cellular retinol-binding protein is another marker for stellate cells in human liver that relies on their vitamin-A storing capacity [91].

Electron microscopic features of stellate cells include the presence of lipid droplets that compress the nucleus

Table 1 Identification methods and markers for rat and human hepatic stellate and myofibroblast-like cells*

	Rat	
	"Quiescent" stellate cells	Myofibroblast-like cells
Fat stains (toluidine blue, basic fuchsin, oil-red-O)	+ (a minority of cells possess no lipid droplets)	–
Transmission electron microscopy	+	+
Methods based upon detection of vitamin A		
autofluorescence	+	–
gold chloride stain	+	–
cellular retinol-binding protein	unstudied	unstudied
Antibodies to cytoskeletal proteins:		
desmin	+ (in pericentral region up to 50% of cells are negative)	+
glial fibrillary acidic protein	+ (not expressed in periportal cells)	+ (expression less than in quiescent cells)
vimentin**	+	+
α-smooth muscle actin	+	+
Others: neural cell adhesion molecule	–	+

	Human	
	"Quiescent" stellate cells	Myofibroblast-like cells
Fat stains (toluidine blue, basic fuchsin, oil-red-O)	+ (a minority of cell possesses no lipid droplets)	–
Transmission electron microscopy	+	+
Methods based upon detection of vitamin A:		
autofluorescence	+***	–
gold chloride stain	+	–
cellular retinol-binding protein	+	unstudied
Antibodies to cytoskeletal proteins:		
desmin	–	+ (a small fraction of the cells are positive)
glial fibrillary acidic protein	+ (mainly expressed in cells around portal tract)	+ (expression greater than in quiescent cells)
vimentin**	+	+
α-smooth muscle actin	+ (different degrees of positivity reported, probably depending on technique)	+
Others: neural cell adhesion molecule	+ predominantly periportal	unstudied

* Myofibroblast-like cells are thought to be derived from stellate cells. For further details and references, see text
** vimentin is not a very useful marker as it is also expressed by Kupffer and endothelial cells
*** only succesfully applied in cases of vitamin A intoxication

(Fig. 3), a moderately developed rough endoplasmic reticulum, moderate amounts of cytoskeletal elements, small numbers of mitochondria and the presence of long processes underlying the endothelium [11, 12, 13, 48, 53–55, 57, 84, 93].

Immunohistochemically, stellate cells may be identified by antibodies to cytoskeletal proteins. In rats, desmin is a good marker for stellate cells [123], although up to 50% of centrolobular stellate cells are desmin negative [7]. In normal adult human liver, stellate cells are desmin negative [90, 108, 122], but may stain for α-smooth muscle actin (Fig. 4). Schmitt-Gräff and colleagues [108] found that stellate cells are only exceptionally α-smooth muscle actin positive in the normal human liver. α-

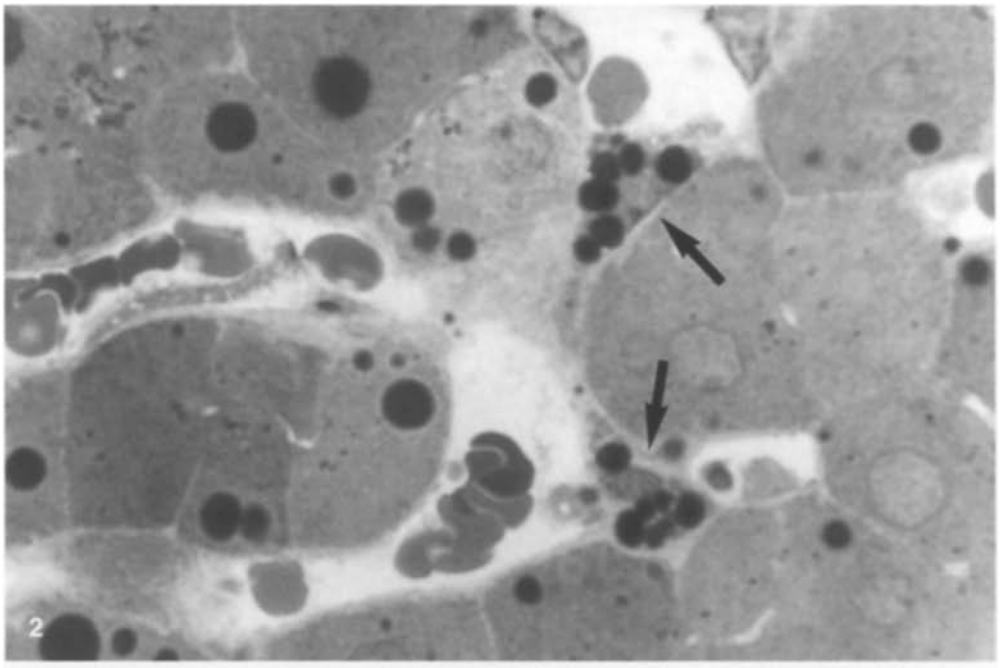


Fig. 2 Normal human liver. Stellate cells (*arrows*) are readily identified in toluidine blue-stained sections by the presence of characteristic lipid droplets (Toluidine blue, × 950)

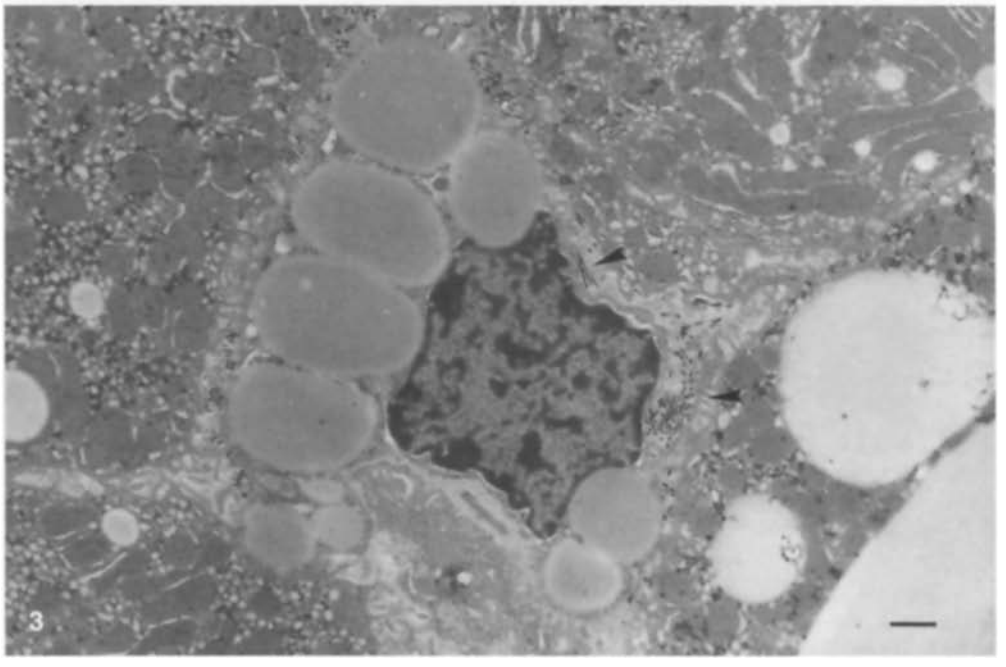


Fig. 3 Transmission electron microscopy of a stellate cell in normal human liver shows the vitamin A-containing lipid droplets that compress the nucleus; the remaining cytoplasm is scanty and few organelles are visible. A few collagen bundles are observed in the vicinity of the cell (*arrowheads*) (*Bar* 1 μm)

smooth muscle actin is, however, positive in embryonic and infant liver. Using a slightly different technique, Yamaoka et al. [122] and Enzan et al. [28] found α-smooth muscle actin positivity in the stellate cells in normal human liver (Fig. 4). Not all stellate cells were positive, however, and positive cells were unevenly distributed in the lobules [28] with centrolobular preponderance [122]. The reaction product was present in the peripheral cytoplasm and the cell processes and the α-smooth muscle actin positive cells showed no signs of myofibroblastic differentiation [28]. Like most cells of mesenchymal origin, human and rat stellate cells express vimentin [1, 18]. Because vimentin is also found in Kupffer and endothelial cells [1], it is a less useful marker. Rat stellate cells contain GFAP, the major component of the intermediate filaments in astrocytes [36, 88, 89]. The GFAP-expressing stellate cell subpopulation partially differs from the desmin-expressing subpopulation: desmin is preferentially expressed in periportal stellate cells whereas GFAP-positive stellate cells are more evenly distributed throughout the lobule but are absent in a narrow periportal zone [89]. GFAP expression diminishes after chronic carbon tetrachloride (CCl_4) exposure [88, 89]. In normal human liver, GFAP expression is limited to a small subpopulation of stellate cells located at the edge of, or around, the portal tracts. In cirrhosis, GFAP expression

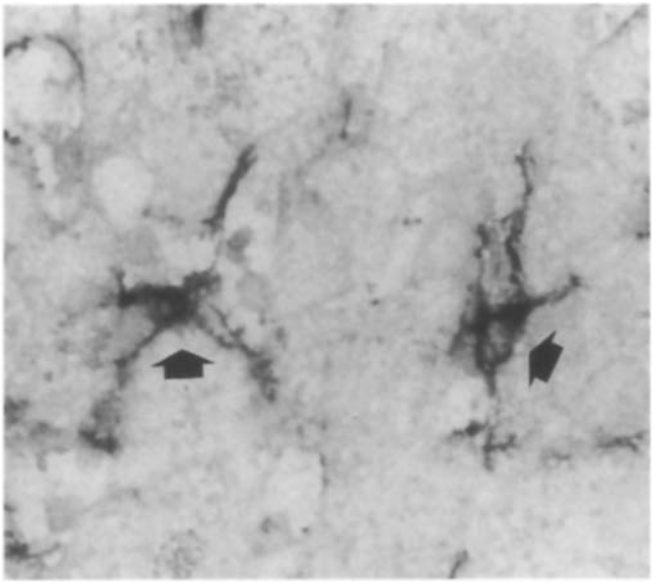
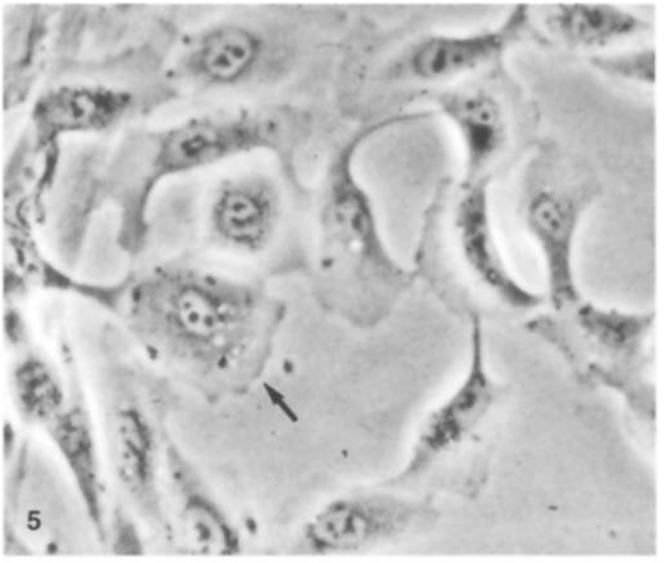


Fig. 4 In normal human liver, α-smooth muscle actin staining reveals positivity of stellate cells (*thick arrows*). Not all stellate cells in human liver are α-smooth muscle actin positive, however. Note the cytoplasmic extensions. (× 125)



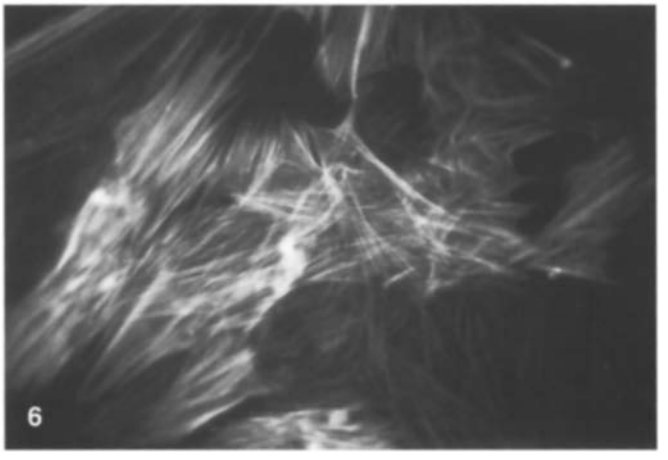


Fig. 5 Phase contrast microscopy of human hepatic stellate cells that have been in culture for 2 weeks, (× 450). The stellate cells acquire a "fibroblastic" appearance; a limited number of lipid droplets is still visible (*arrow*). (From [115] with permission of the publisher, courtesy of Dr. A.M.B.C. Tiggelman, Academic Medical Center, University of Amsterdam)

Fig. 6 α-smooth muscle actin expression by human hepatic stellate cells that have been cultured for 2 weeks (× 560). (From [115] with permission of the publisher, courtesy of Dr. A.M.B.C. Tiggelman, Academic Medical Center, University of Amsterdam)

is enhanced with positive cells lying at the edge of regenerative nodules or in clusters in the nodules [49]. Finally, stellate cells express neural cell adhesion molecule (N-CAM) [66, 87]. In normal human liver expression is predominantly periportal [87]. In normal rat liver little N-CAM positivity is detectable, but in fibrotic liver marked N-CAM expression is present in a subpopulation of myofibroblast-like cells [66].

It is interesting that stellate cells, which are often referred to as "liver-specific pericytes" because they exhibit striking similarities with vascular smooth-muscle cell-like pericytes in other organs [95], posses markers of cells of astroglial (GFAP) and neural (N-CAM) lineage. Furthermore, their pattern of intermediate filament expression is striking. Rat hepatic stellate cells posses at least three intermediate filaments (desmin, vimentin and GFAP), and many stellate cells in culture express all three intermediate filaments concurrently [14]. This combination of intermediate filament expression is unique for the stellate cell [14]. Moreover, the simultaneous expression of three intermediate filaments in one cell seems to be rare, and even the simultaneous expression of two intermediate filaments is unusual in quiescent, differentiated adult cells. Vimentin is expressed by human hepatic stellate cells [18], but desmin and GFAP are only detected in a small subpopulation of human hepatic stellate cells. It is therefore tempting to suggest that human hepatic stellate cells possess at least one other, at present uncharacterized, intermediate filament.

The patterns of intermediate filament expression illustrate the existence of considerable heterogeneity within the stellate cell population, both in rat and human. This has been documented for desmin [7, 108], GFAP [88, 89], N-CAM [87] and α-smooth muscle actin expression [28, 108] and for retinoid content, size and number of droplets, and arborisation [11, 12, 41, 48, 112, 119]. This heterogeneity often has a zonal distribution [7, 41, 87, 89]. The cause and functional significance of the heterogeneity are unclear but it may be related to the exertion of specific functions by subpopulations of stellate cells. The zonal character of stellate cell heterogeneity has been related to the "streaming liver" concept, in which hepatocytes and accompanying sinusoidal cells move from portal (stem cell compartment) to central regions during their life span [125]. In this hypothesis, stellate cells might be dynamic during their life span [41]. Another hypothesis [41] relates stellate cell heterogeneity to the gradient in matrix composition

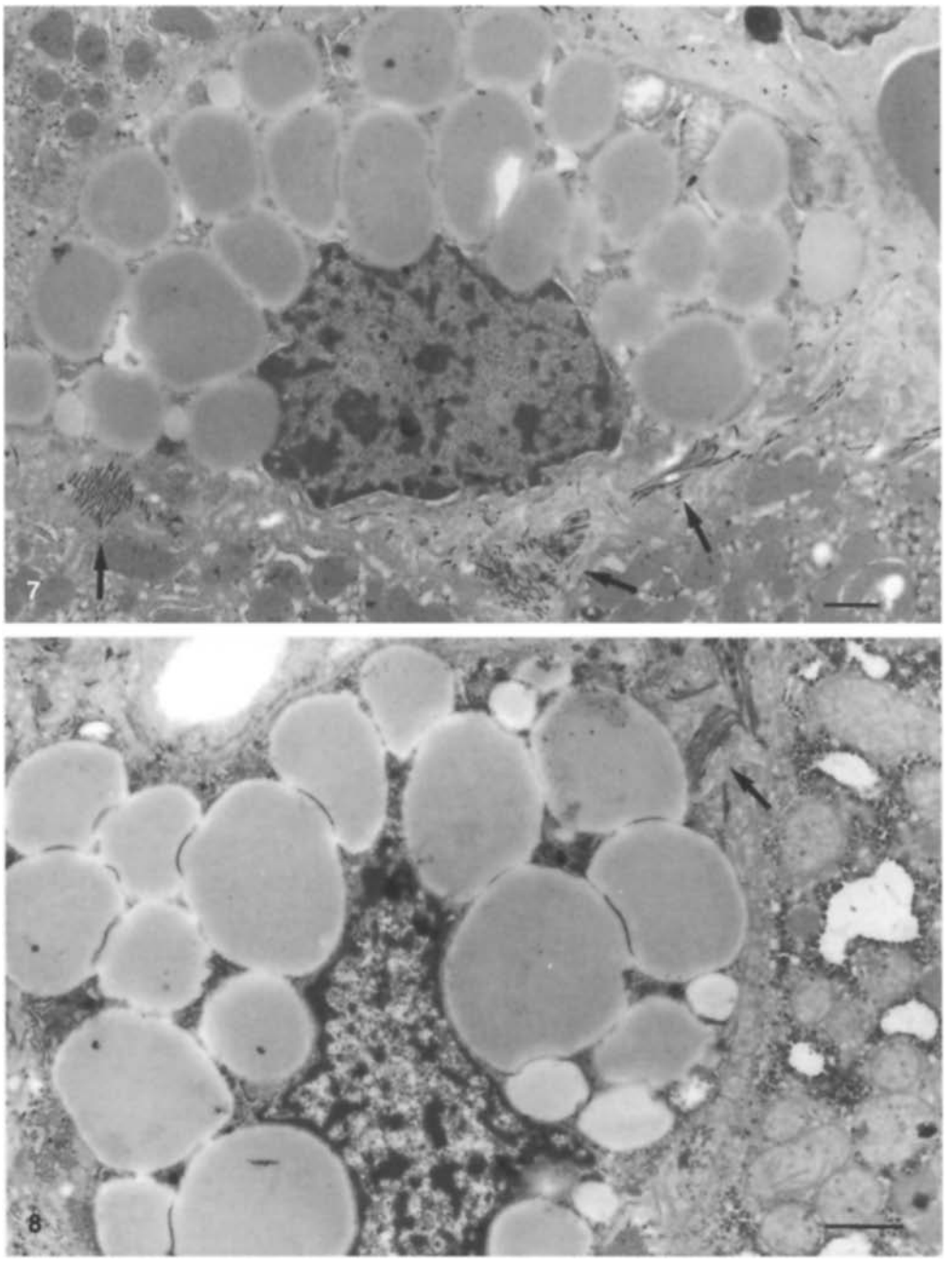


Fig. 7 Marked increase in the number of lipid droplets in stellate cells in vitamin A intoxication. Collagen bundles are observed in the vicinity of the cell (*arrows*). (Transmission electron microscopy, *bar* 1 μm)

Fig. 8 Stellate cell in early alcoholic liver disease. Note the similarities with **Fig. 7**, with marked increase in lipid droplets that compress the nucleus and each other. Collagen bundles are observed in the vicinity of the cell (*arrows*). (Transmission electron microscopy, *bar* 1 μm)

which is believed to exist from portal tracts to central veins [100].

Methods to isolate, purify and culture human stellate cells from wedge sections of human liver have been developed [34]. Cultured on plastic, the cells spread within 48–72 h; they express procollagens I and III, collagen IV and laminin. After culture on plastic for more than 7 days, cells acquire the "activated" phenotype (Fig. 5) with increased spreading and diminished retinoid content, and start proliferating [34]. Cells in early culture lack desmin, but cells in 7-day culture are desmin positive [34] and express α-smooth muscle actin (Fig. 6) [114, 115]. Culture on plastic thus mimicks in vivo activation, as is the case for rat stellate cells [24, 37].

Stellate cells in acute liver disease

In acute liver disease, the stellate cell population expands beyond the space of Disse in areas of liver injury. This has been observed in centrolobular necrosis following circulatory failure [67, 108], subacute massive hepatic necrosis [67], acute viral hepatitis [60] and paracetamol-induced liver necrosis [78]. Expansion of the stellate cell population is accompanied by the appearance of transi-

tional stellate cells–cells that acquire certain features of myofibroblastic differentiation. They contain a reduced number of lipid droplets and develop an extensive, dilated rough endoplasmic reticulum and a prominent Golgi apparatus; they become surrounded by abundant interstitial collagen bundles [60, 69]. In acute viral hepatitis A, many transitional stellate cells are observed in areas of focal necrosis [60]. Schmitt-Gräff et al. [108] found α-smooth muscle actin positive cells around areas of perivenular necrosis and collapse in patients with acute heart failure and shock surviving longer than 48 h. Using the same marker, Mathew et al. [78] found an expansion of the stellate cell population in moderate to severe (but not mild) paracetamol-induced liver necrosis. They also found an expansion of the Kupffer cell population, with a strong correlation between the size of these two populations [78]. After recovery, the number of stellate cells returned to normal.

These findings suggest that in areas of acute liver injury, an expansion of the stellate cell population occurs, accompanied by the appearance of transitional cells and pronounced α-smooth muscle actin expression. It is thought that transitional stellate cells play a role in extracellular matrix remodelling and tissue repair after recovery from injury [60]. Findings in paracetamol-induced liver necrosis in man closely parallel observations on the stellate cell and Kupffer cell populations in acute liver injury in animals [38, 52, 64] and suggest cooperation between stellate and Kupffer cells during the response to acute liver injury [78]. It is not clear to what extent the accumulation of α-smooth muscle actin positive stellate cells results from proliferation or transition of "quiescent" stellate cells, or to what extent the subsequent disappearance of the cells is caused by retrograde transition to quiescent cells or loss of activated cells through apoptosis [78]. It is interesting to note that during acute liver injury, several changes occur in the stellate cells which are also observed during chronic liver injury (appearance of transitional cells, pronounced α-smooth muscle actin positivity). At this stage, however, these changes are reversible and liver histology returns to normal.

Stellate cells in chronic liver disease

In chronic inflammation of the liver, stellate cells are found outside the space of Disse as in acute liver diseases. In areas of inflammation, stellate cells seen in close proximity to damaged parenchymal cells, Kupffer cells and various inflammatory cells. The stellate cells differentiate into transitional cells and transform further into cells that acquire a morphology similar to myofibroblasts, showing complete (or nearly complete) loss of lipid droplets, extensive microtubules, intermediate filaments and bundles of actin filaments with local condensations [48, 55, 69, 84, 93]. The cells are termed "myofibroblast-like cells" because they seem closely related, but not identical, to myofibroblasts [37, 72, 113]. Myofibroblast-like cells express α-smooth muscle actin, and some of the cells also express desmin [28, 90, 108, 122].

Differentiation of stellate cells into myofibroblast-like cells has been studied in human alcoholic and viral liver disease as well as in a number of other conditions. In early alcoholic liver injury a diminution of quiescent stellate cells is observed in acinar zone 3 [55] and in the midzone of the hepatic lobule [93] with a corresponding increase in activated cells. In alcoholic hepatitis, activated cells are found in small foci of fibrosis in association with inflammation and hepatocellular degenerative changes [93]. α-smooth muscle actin positive cells are mainly found in the perivenular zone [108] and myofibroblast-like cells are a common cell type in perivenular fibrosis [86]; transitional cells are also observed. In alcoholic hepatitis with "chicken-wire" fibrosis, α-smooth muscle actin positive stellate cells are found surrounding hepatocytes [108] and cell processes in the perisinusoidal spaces are associated with the net-like fibrosis, sometimes linking up with the perivenular lesion [86].

In alcoholic cirrhosis, a diminution of the number of quiescent stellate cells is found [13, 48, 84]. Bands of fibrous tissue contain mesenchymal cells immunoreactive for α-smooth muscle actin; a portion of these cells coexpresses desmin [108]. These cells are present in expanding septa and in the perisinusoidal space in cases of active cirrhosis; in inactive cirrhosis they are restricted to the periphery of the regenerative cell plates [108]. In the fibrous bands of alcoholic cirrhosis, myofibroblast-like cells usually comprise 50%–60% of the fibroblasts in the scar tissue [105].

In chronic active viral hepatitis, there is an increase in size, number and intensity of immunostaining of α-smooth muscle actin-positive stellate cells in areas of piecemeal necrosis. The cells are observed adjacent to the inflammatory infiltrate trapping clusters of hepatocytes [28, 108, 122]. The α-smooth muscle actin-positive cells have the ultrastructural features of myofibroblast-like cells [28]. Findings in postviral cirrhosis parallel those in alcoholic cirrhosis [8, 108].

In extrahepatic cholestasis, the stellate cell population is increased [13, 48]. In chronic large bile-duct obstruction and in early stages of primary biliary cirrhosis, α-smooth muscle actin positive stellate cells that have the ultrastructural features of myofibroblast-like cells are encountered predominantly in the periportal areas [108]. In chronic right-sided heart failure, α-smooth muscle actin positive stellate cells are found around terminal venules and cords of atrophic hepatocytes adjacent to engorged sinusoids [108].

All these data are consistent with the assumption that the appearance of α-smooth muscle actin and desmin expressing myofibroblast-like cells results, at least in part, from the phenotypic modulation of stellate cells [108]. It is thought that stellate cells are the most important cell type involved in active fibrogenesis in chronic liver disease and that most (if not all) of the myofibroblast-like cells in perivenular fibrosis, bridging fibrosis and cirrhosis originate from stellate cells. This does not exclude

that other cell types might play a secondary role in liver fibrogenesis. Studies in rats have demonstrated that fibroblasts in portal fields, the liver capsule and the so-called second-layer cells (that is, those fibroblasts located around the terminal branches of the central veins) contribute to liver fibrogenesis [10]. Their contribution may vary according to the cause of liver injury; in cholestatic liver injury after bile duct ligation portal fibroblasts are activated and extend into the parenchyma whereas this is not the case in CCl_4-acetone induced liver injury [26]. To what extent these findings apply to human pathology remains to be seen.

The differentiation of stellate cells to myofibroblast-like cells is regulated by an intricate network of intercellular communication. Stellate cell activation happens through interaction between stellate cells and activated Kupffer cells, damaged hepatocytes, platelets, endothelial and inflammatory cells. This occurs mainly through secretion of cytokines, although nonpeptide mediators such as reactive oxygen species, eicosanoids and acetaldehyde probably also play a role [41, 42]. Kupffer cell-conditioned medium enhances proliferation of stellate cells, increases extracellular matrix production and promotes transition of the quiescent into the myofibroblast-like phenotype [33, 43, 80, 110, 123]. The main factor responsible for these effects seems to be transforming growth factor(TGF)-β. Exposure of stellate cells to TGF-β increases extracellular matrix protein synthesis [5, 6, 18, 20, 21, 79, 80, 115, 120]. TGF-β inhibits proliferation of parenchymal and stellate cells, however [21, 30, 80, 81, 97]. Therefore, the presence of TGF-β in Kupffer cell medium explains the increased extracellular matrix synthesis but is not compatible with enhanced proliferation. Friedman and Arthur [33] have found that stellate cells in culture proliferate in response to platelet-derived growth factor (PDGF), but only after pretreatment with Kupffer cell medium. This finding suggests that stellate cell activation requires induction of PDGF receptors by Kupffer cell-derived factor(s). PDGF is a mitogen for human liver myofibroblast-like cells in culture; this effect is suppressible by retinoic acid, suggesting that, as in rats, retinoids contribute to the maintenance of the quiescent phenotype [22]. Cultured human myofibroblast-like cells release PDGF A- and B-chain in the culture medium, raising the possibility of an autocrine or short-loop paracrine effect of PDGF on stellate cells [76]. PDGF has little effect on collagen synthesis [104].

TGF-β is secreted in latent form and cellular activation of latent TGF-β requires binding to the insulin-like growth factor type II/mannose-6-phosphate (IGF-II/M6P) receptor [25]. Stellate cells bear TGF-β receptors [31]. Activated stellate cells contain IGF-II/M6P receptor mRNA and bear this receptor at the cell surface [23]. In co-culture of sinusoidal endothelial and activated stellate cells the receptor is required for activation of latent TGF-β [23]. In the diseased human liver, TGF-§ mRNA expression is found in stellate cells and in mesenchymal cells at sites of inflammation and scarring [9, 73].

Apart from TGF-β, Kupffer cells release a spectrum of other cytokines and mitogenic factors modulating the proliferation and phenotype of stellate cells. Interleukin(IL)-1α stimulates stellate cell proliferation in a concentration-dependent manner while inhibiting collagen and non-collagen protein formation [80]. Tumour necrosis factor-α (TNF-α) has a similar mitogenic effect but inhibits collagen formation [80, 121]. Cultured Kupffer cells also express TGF-α which has a mitogenic effect on stellate cells [82]. Bachem et al. [6] postulated a paracrine loop in which TGF-α and TGF-β secreted by myofibroblast-like cells operate cooperatively to promote transition of quiescent and transitional stellate cells to myofibroblast-like cells. These authors demonstrated that differentiated stellate cells express significant amounts of TGF-α and TGF-β as well as the receptors for these growth factors. Conditioned medium of differentiated stellate cells stimulates the proliferation, transformation, and proteoglycan synthesis of primary stellate cell cultures. Similar results were obtained in co-culture experiments of primary stellate cells with differentiated stellate cells [6].

Other factors are probably involved in stellate cell activation. Parenchymal cells release a 60 kDa cytosolic protein that stimulates stellate cell proliferation [45]. This unidentified factor does not affect transition to the myofibroblast-like phenotype or synthesis of extracellular matrix proteins, and is supposedly released from hepatocytes by membrane damage or other forms of parenchymal cell injury. It provides the first mitogenic signal to stellate cells, prior to paracrine stimuli by Kupffer cells, inflammatory cells, and platelets [45]. Additionally, hepatocyte-conditioned medium potentiates the mitogenic effects of IGF-I and IGF-II 20-fold [46]. Acetaldehyde stimulates the synthesis of certain collagen types and the proliferation of myofibroblast-like cells [17, 19, 85], although proteoglycan synthesis remains unaffected [44]. Ethanol-induced lipoperoxidation stimulates collagen gene expression [94]. Finally, reactive oxygen species also seem involved in stellate cell activation [42].

Endothelial-stellate cell interactions probably operate through secretion of eicosanoids. Rat liver endothelial cells secrete thromboxane, prostaglandin(PG)E2 and PGD2, and low amounts of prostacyclin [101]. These prostanoids inhibit activation of platelets and promote vasodilation, possibly by reversing contraction of stellate cells. In addition, endothelial cells produce endothelin-1, a strong vasoconstrictor. Stellate cells, and especially myofibroblast-like cells, contract in response to endothelin-1 [58, 96, 98, 103].

Inflammatory cells, in particular T lymphocytes and natural killer cells, secrete interferon(IFN)-γ [117]. IFN-γ decreases proliferation of stellate cells and reduces expression of α-smooth muscle actin, collagen types I and IV, and fibronectin [44, 74, 102, 115]. Collagen synthesis by cultured human liver myofibroblast-like cells is enhanced by IL-1β and IL-4 [115]. Thrombin may also be involved in stellate cell activation and stimulates proliferation of stellate cells through proteolytic activation of

its receptor [77]. Human myofibroblast-like cells in culture produce IL-6; production is enhanced by TNF-α, lipopolysaccharide and IL-1β [114]. As IL-6 plays a key role in the regulation of the production of acute phase proteins by the liver, myofibroblast-like cells derived from stellate cells are thought to play a major role in the local production of acute phase proteins in the damaged liver [114]. Finally, human liver myofibroblast-like cells secrete monocyte chemotactic peptide-1; secretion is enhanced by IL-1α, IFN-γ, TNF-α and thrombin [75, 77]. This observation suggests that activated stellate cells can attract mononuclear cells within injured liver tissue, especially after stimulation by pro-inflammatory cytokines [95].

In conclusion, stellate cell activation occurs through intercellular communication with Kupffer cells, hepatocytes, platelets, endothelial cells and inflammatory cells. The observations described above have led to the development of the so-called three-step model of stellate cell activation [42]. In a pre-inflammatory phase, hepatocytic damage leads to release of a factor (or factors) leading to stellate cell proliferation. During the subsequent inflammatory phase, cytokines of activated Kupffer cells and damaged platelets are released; in particular TGF-β affects the differentiation of stellate cells in myofibroblast-like cells. In the post-inflammatory phase, myofibroblast-like cells are stimulated through autocrine and paracrine loops, leading to self-perpetuation of fibrogenesis [42].

In addition to their role in fibrogenesis and extracellular matrix synthesis, stellate cells also play a role in matrix degradation, through synthesis and secretion of extracellular matrix degrading metalloproteinases. A 72 kDa type IV collagenase/gellatinase (also called MMP-2 or gelatinase A) is present in human stellate cells [3, 83]. In freshly isolated cells mRNA levels are low; when the cells are cultured, expression increases with time [3]. MMP-2 expression is increased in fibrotic human liver [2]. By in situ hybridization, MMP-2 transcripts can be detected both in normal and fibrotic human liver in vimentin-positive, CD68 negative mesenchymal cells [83]. These cells are probably differentiated stellate cells. Cultured human myofibroblast-like cells may also express interstitial collagenase (also called MMP-1) RNA [83]. Interestingly, human stellate cells also express tissue inhibitor of metalloproteinase type 1 (TIMP-1), a potent inhibitor of metalloproteinases [61]. TIMP-1 mRNA expression is low or absent in freshly isolated cells but increases dramatically when stellate cells are activated to a myofibroblast-like phenotype in cell culture [61]. TIMP-1 expression is markedly increased in fibrotic human liver when compared with normal liver [2]; this may play a role in promoting fibrogenesis. Stellate cells thus appear to regulate extracellular matrix degradation at three levels: (i) at the transcriptional level, (ii) at the level of activation of latent enzyme, and (iii) by secretion of TIMPs [39]. The intriguing finding that stellate cells are involved in the synthesis of extracellular matrix as well as in its degradation exemplifies their suitability to play a key role in extracellular matrix remodelling during tissue repair after injury.

Stellate cells in vitamin A intoxication

In the early stages of chronic vitamin A intoxication, there is an increase in the number of stellate cells and an increase in the number of lipid droplets per cell [40, 69] (Fig. 7). Cells protrude into the sinusoidal lumen [69], and, in a further stage, perisinusoidal fibrosis follows [40, 59, 106, 124]. This can be accompanied by dilatation of the sinusoidal lumen [106], resulting in sinusoidal congestion [59, 106] and sometimes peliosis-like lesions [40, 124]. Central vein sclerosis can be found [59, 106]. The combination of stellate cell hyperplasia, perisinusoidal fibrosis and sinusoidal dilatation can lead to non-cirrhotic portal hypertension [40, 47, 106, 124]. Non-cirrhotic portal hypertension occurring early in the course of the disease is usually due to intoxication with massive doses, in which case portal hypertension is thought to result from obstruction of the sinusoids by hypertrophic and hyperplastic stellate cells [40, 47, 106]. Non-cirrhotic portal hypertension occurring after years of intoxication seems mainly to result from perisinusoidal fibrosis [40, 59].

In still later stages the disease progresses to bridging fibrosis and cirrhosis [40, 63, 65, 106]. In these stages, the pattern of accumulation of hyperplastic stellate cells appears to be related to the timing of vitamin A intake: recent intake is mainly associated with lobular hyperplasia [40], whereas in former intake hyperplastic stellate cells are mainly localized in portal spaces and fibrous septa [40, 63, 65]. At the electron microscopic level, the cells have the appearence of myofibroblast-like cells but still contain a high number of lipid droplets [12, 59]. Hence in vitamin A intoxication, the cells become activated to a myofibroblast-like cell without loss of vitamin-A containing lipid droplets, in contrast with what happens in other chronic liver diseases.

It is noteworthy that a similar accumulation of lipid droplets in stellate cells can also be found in several diseases not associated with vitamin A intoxication. This phenomenon has been described in cholestasis [12, 13], chronic pancreatitis [13], diabetes [68], acquired immune deficiency syndrome [27], primary biliary cirrhosis [91] and early alcoholic liver disease (Fig. 8) [12, 13, 48]. The pathogenesis and significance of this phenomenon remain to be established. Several authors have postulated that the increase in number of lipid droplets is due to accumulation of vitamin A in stellate cells [27, 91]. However, lipid droplets in stellate cells are composed of vitamin A and other lipids, mainly triglycerides [50]. As there is a decrease in liver vitamin A content in chronic liver disease, especially in alcoholic liver disease [70, 71], it seems more likely that accumulation of lipids other than vitamin A occurs.

Stellate cells and liver tumours

In focal nodular hyperplasia, abundant α-smooth muscle actin positive cells are observed in the stellate scar and in the fibrous septa. Some of these cells also express desmin. α-smooth muscle actin positive cells are also found in the nodular parenchyma adjacent to hepatocytes [108]. These cells have the morphology of transitional cells or myofibroblast-like cells [12, 16, 108] and may be responsible for fibrosis in the central scar [16].

In hepatocellular adenoma, α-smooth muscle actin positive cells are found in the fibrous capsule and in foci of haemorrhage [108], but are much less abundant than in focal nodular hyperplasia.

In hepatocellular carcinoma or metastatic carcinoma, accumulation of α-smooth muscle actin positive stellate cells is observed in areas of liver cell atrophy, congestion or inflammation adjacent to the tumour [29, 108]. Furthermore a striking proliferation of α-smooth muscle actin positive cells is seen in bands of fibrous tissue surrounding tumour masses [29, 108]. The cells are sometimes positive for vimentin [29] and a small number of these cells co-express desmin [108]. The cells are also found in the tissue spaces between endothelial cells and trabeculae of tumour cells. They have extending cytoplasmic processes and occasionally surround trabeculae of cancer cells [29]. Immunohistochemically and ultrastructurally, they closely resemble stellate cells that show myofibroblastic transformation [29].

Stellate cells thus seem to play a major role in fibrogenesis and capsule formation around liver tumours. Stellate cell activation probably occurs as a result of hepatocytic damage in the vicinity of the tumour. It is suggested that the stellate cells in non-necrotic cancer tissue produce the extracellular matrix in the tissue space and maintain the cancerous trabecular structure. After necrosis of cancer tissue they become activated and participate in fibrogenesis [29].

It is unknown whether the liver stellate cells themselves can undergo malignant transformation in humans. Spongiosis hepatis (also called spongiotic pericytoma), a benign lesion that can develop in the livers of rats and fish treated with hepatocarcinogens, is thought to be derived from the stellate cells [111]. It is possible that some human hepatic sarcomas arise out of the stellate cells, but at present no convincing case of a "stellate cell sarcoma" has been reported.

Conclusion and perspectives

The changes in the stellate cells which are observed in human liver diseases indicate that they play a central role in extracellular matrix remodelling after recovery from acute liver injury, in chronic liver disease and in fibrous capsule formation around liver tumours. To elucidate the pathophysiological role of stellate cells in human liver diseases, it is necessary to find specific differentiation markers to characterize the different phenotypic stages of the cell. The functional significance of the important heterogeneity within the stellate cell population should be explored further. The mechanisms that regulate stellate cell differentiation into myofibroblast-like cells also await more detailed elucidation. The contribution of other cells to fibrogenesis in normal and diseased human liver must be better defined. Stellate cells, and especially myofibroblast-like cells, contract in response to vasoactive substances [58, 96, 98, 103, 107], and future investigations should establish whether the stellate cell plays a role in the regulation of hepatic sinusoidal vascular tone in humans, and whether myofibroblast-like cells are involved in the pathogenesis of portal hypertension in cirrhosis. Knowledge concerning stellate cells expands rapidly and it can be expected that further study will ultimately lead to therapeutic interventions aimed at the stellate cells. Future studies are expected to lead to drugs that specifically influence fibrogenesis and extracellular matrix degradation by the stellate and myofibroblast-like cells. In patients with vitamin A intoxication, ways might be found to remove the excess of retinoids and limit liver damage. Finally, a better understanding of the vasomotor role of the cell might lead to therapeutic interventions in the field of liver haemodynamics and portal hypertension.

Acknowledgements The authors wish to thank Professor G. Klöppel and Professor E. Wisse for their continuous support and suggestions, Kit Van den Berg and Carine Seynaeve for technical help and Chris Derom for preparation of the photographs. We are indebted to Professor K. Wake, Department of Anatomy, Tokyo Medical and Dental University, for allowing us the use of Fig. 1. We thank Dr. A.M.B.C. Tiggelman, Academic Medical Center, University of Amsterdam, for providing pictures of human hepatic stellate cells in culture.

References

1. Ahmed Q, Hines JE, Harrison D, Burt AD (1991) Expression of muscle-associated cytoskeletal proteins by human sinusoidal liver cells. In: Wisse E, Knook DL, McCuskey RS (eds) Cells of the hepatic sinusoid, vol 4. The Kupffer Cell Foundation, Leiden, pp 203–206
2. Arthur MJP (1994) Matrix degradation in the liver. In: Surrenti C, Casini A, Milani S, Pinzani M (eds) Fat-storing cells and liver fibrosis. Kluwer, Dordrecht, pp 110–127
3. Arthur MJP, Stanley A, Iredale JP, Rafferty JA, Hembry RM, Friedman SL (1992) Secretion of 72 kDa type IV collagenase/gelatinase by cultured human lipocytes: analysis of gene expression, protein synthesis and proteinase activity. Biochem J 287:701–707
4. Aterman K (1986) The parasinusoidal cells of the liver: a historical account. Histochem J 18:279–305
5. Bachem MG, Riess U, Melchior R, Sell KM, Gressner AM (1989) Transforming growth factors (TGF α and TGF β1) stimulate chondroitin sulfate and hyaluronate synthesis in cultured rat liver fat-storing cells. FEBS Lett 257:134–137
6. Bachem MG, Meyer D, Riess U, Melchior R, Sell KM, Bittinger A (1991) Paracrine stimulation of fat-storing cells via soluble mediators produced by myofibroblast-like cells. In: Wisse E, Knook D (eds) Cells of the hepatic sinusoid, vol 3. The Kupffer Cell Foundation, Leiden, pp 164–170
7. Ballardini G, Groff P, DeGiorgi LB, Schuppan D, Bianchi FB (1994) Ito cell heterogeneity: desmin-negative Ito cells in normal rat liver. Hepatology 19:440–446

8. Bathal PS (1972) Presence of modified fibroblasts in cirrhotic livers in man. Pathology 4:139–144
9. Bedossa P, Peltier E, Terris B, Franco D, Poynard T (1995) Transforming growth factor-beta 1 (TGF-β1) and TGF-β1 receptors in normal, cirrhotic, and neoplastic human livers. Hepatology 21:760–766
10. Bhunchet E, Wake K (1992) Role of mesenchymal cell populations in porcine serum-induced rat liver fibrosis. Hepatology 16:1452–1473
11. Bioulac-Sage P, Lafon ME, LeBail B, Balabaud C (1988) Perisinusoidal and pit cells in liver sinusoids. In: Bioulac-Sage P, Balabaud C (eds) Sinusoids in human liver: health and disease. Kupffer Cell Foundation, Leiden, pp 39–62
12. Bioulac-Sage P, Lafon ME, LeBail B, Boulard A, Dubuisson L, Quinton A, Lamouliatte H, Saric J, Balabaud C (1988) Ultrastructure of sinusoids in liver disease. In: Bioulac-Sage P, Balabaud C (eds) Sinusoids in human liver: health and disease. Kupffer Cell Foundation, Leiden, pp 223–278
13. Bronfenmajer S, Schaffner F, Popper H (1966) Fat-storing cells (lipocytes) in human liver. Arch Pathol Lab Med 82:447–453
14. Buniatian G, Gebhardt R, Schrenk D, 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
15. Burt AD (1993) Cellular and molecular aspects of hepatic fibrosis. J Pathol 170:105–114
16. Callea F, Mebis J, Desmet VJ (1982) Myofibroblasts in focal nodular hyperplasia of the liver. Virchows Arch [A] 396: 155–166
17. Casini A, Cunningham M, Rojkind M, Lieber CS (1991) Acetaldehyde increases procollagen type I and fibronectin gene transcription in cultured rat fat-storing cells through a protein synthesis-dependent mechanism. Hepatology 13: 758–765
18. Casini A, Pinzani M, Milani S, Grappone C, Galli G, Jezequel AM, Schuppan D, Rotella CM, Surrenti C (1993) Regulation of extracellular matrix synthesis by transforming growth factor β1 in human fat-storing cells. Gastroenterology 105:245–253
19. Casini A, Galli G, Salzano R, Rotella CM, Surrenti C (1993) Acetaldehyde-protein adducts, but not lactate and pyruvate, stimulate gene transcription of collagen and fibronectin in hepatic fat-storing cells. J Hepatol 19:385–392
20. Casini A, Ceni E, Salzano R, Schuppan D, Milani S, Pellegrini G, Surrenti C (1994) Regulation of undulin synthesis and gene expression in human fat-storing cells by acetaldehyde and transforming growth factor-β1: comparison with fibronectin. Biochem Biophys Res Commun 199:1019–1026
21. Davis BH (1988) Transforming growth factor beta responsiveness is modulated by the extracellular collagen matrix during hepatic Ito cell culture. J Cell Physiol 136:547–553
22. Davis BH, Coll D, Beno DW (1993) Retinoic acid suppresses the response to platelet-derived growth factor in human hepatic Ito-cell-like myofibroblasts: a post-receptor mechanism independent of raf/fos/jun/egr activation. Biochem J 294:785–791
23. DeBleser PJ, Jannes P, Buul-Offers SC van, Hoogerbrugge CM, Schravendijk CFH van, Niki T, Rogiers V, Van den Brande JL, Wisse E, Geerts A (1995) Insulinlike growth factor-II/mannose 6-phosphate receptor is expressed on CCl_4-exposed rat fat-storing cells and facilitates activation of latent transforming growth factor-β in cocultures with sinusoidal endothelial cells. Hepatology 21:1429–1437
24. DeLeeuw A, McCarthy S, Geerts A, Knook D (1984) Purified rat liver fat-storing cells in culture divide and contain collagen. Hepatology 4:392–403
25. Dennis P, Rifkin D (1991) Cellular activation of latent TGF-β requires binding to the cation-independent mannose-6-phosphate/insulin-like growth factor type II receptor. Proc Natl Acad Sci USA 88:580–584
26. Desmoulière A, Tuchweber B, Gabbiani G (1995) Role of the myofibroblast differentiation during liver fibrosis. J Hepatol [Suppl 2] 22:61–64
27. Dupon M, Kosaifi T, LeBail B, Lacut Y, Balabaud C, Bioulac-Sage P (1991) Lipid-laden perisinusoidal cells in patients with acquired immunodeficiency syndrome. Liver 11:211–219
28. Enzan H, Himeno H, Iwamura S, Saibara T, Onishi S, Yamamoto Y, Hara H (1994) Immunohistochemical identification of Ito cells and their myofibroblastic transformation in adult human liver. Virchows Arch 424:249–256
29. Enzan H, Himeno H, Iwamura S, Onishi S, Saibara T, Yamamoto Y, Hara H (1994) α-smooth muscle actin-positive perisinusoidal stromal cells in human hepatocellular carcinoma. Hepatology 19:895–903
30. Fausto N, Mead JE (1989) Regulation of liver growth: protooncogenes and transforming growth factors. Lab Invest 60:4–13
31. Friedman S (1992) The molecular basis for Kupffer cell/lipocyte interactions. In: Gressner A, Ramadori G (eds) Molecular and cell biology of molecular fibrogenesis. Kluwer, Dordrecht, pp 385–392
32. Friedman SL (1993) The cellular basis of hepatic fibrosis: mechanisms and treatment strategies. N Engl J Med 328: 1828–1835
33. Friedman SL, Arthur MJ (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 84:1780–1785
34. Friedman SL, Rockey DC, McGuire RF, Maher JJ, Boyles JK, Yamasaki G (1992) Isolated hepatic lipocytes and Kupffer cells from normal human liver: morphological and functional characteristics in primary culture. Hepatology 15:234–243
35. Gampieri MP, Jezequel AM, Orlandi F (1981) The lipocytes in normal human liver: a quantitave study. Digestion 22:165–169
36. Gard L, White F, Dutton G (1985) Extra-neural glial fibrillary acidic protein (GFAP) immunoreactivity in perisinusoidal stellate cells of rat liver. J Neuroimmunol 8:359–375
37. Geerts A, Vrijsen R, Rauterberg J, Burt A, Schellinck P, Wisse E (1989) In vitro transition of fat-storing cells to myofibroblast-like cells parallels marked increase of collagen synthesis and secretion. J Hepatol 9:59–68
38. Geerts A, Lazou JM, DeBleser P, Wisse E (1991) Tissue distribution, quantitation and proliferation kinetics of fat-storing cells in carbon tetrachloride-injured rat liver. Hepatology 13:1193–1202
39. Geerts A, DeBleser P, Hautekeete ML, Niki T, Wisse E (1994) Fat-storing (Ito) cell biology. In: Arias IM, Boyer JL, Fausto N, Jakoby WB, Schachter DA, Schaffritz DA (eds) The liver: biology and pathobiology, 3rd edn. Raven Press, New York, pp 819–838
40. Geubel AP, DeGalocsy C, Alves N, Rahier J, Dive C (1991) Liver damage caused by therapeutic vitamin A administration: estimate of dose-related toxicity in 41 cases. Gastroenterology 100:1701–1709
41. Gressner AM (1994) Perisinusoidal lipocytes and fibrogenesis. Gut 35:1331–1333
42. Gressner AM (1995) Cytokines and cellular crosstalk involved in the activation of fat-storing cells. J Hepatol [Suppl 2] 22:28–36
43. Gressner A, Zerbe O (1987) Kupffer cell-mediated induction of synthesis and secretion of proteoglycans by rat liver fat-storing cells in culture. J Hepatol 5:299–310
44. Gressner AM, Althaus M (1988) Effects of ethanol, acetaldehyde, and lactate on proteoglycan synthesis and proliferation of cultured rat liver fat-storing cells. Gastroenterology 94:797–807
45. Gressner A, Lofti S, Gressner G, Lahme B (1992) Identification and partial characterization of a hepatocyte-derived fac-

tor promoting proliferation of cultured fat-storing cells (parasinusoidal lipocytes). Hepatology 16:1250–1266
46. Gressner A, Brenzel A, Voszmeyer T (1993) Hepatocyte-conditioned medium potentiates insulin-like growth factor (IGF) 1 and 2 stimulates DNA synthesis of cultured fat-storing cells. Liver 13:86–94
47. Guarascio P, Portmann B, Visco G, Williams R (1983) Liver damage with reversible portal hypertension from vitamin A intoxication: demonstration of Ito cells. J Clin Pathol 36:769–771
48. Hautekeete ML, Geerts A, Seynaeve C, Lazou JM, Klöppel G, Wisse E (1993) The human fat-storing cell studied using light and transmission electron microscopy. In: Knook DL, Wisse E (eds) Cells of the hepatic sinusoid, vol 4. The Kupffer Cell Foundation, Leiden, pp 263–267
49. Hautekeete ML, Niki T, Van den Berg K, Delvaux G, Geerts A (1996) A fraction of stellate cells in human liver express glial fibrillary acidic protein (GFAP). J Hepatol [Suppl 1] 25:112
50. Hendriks HFJ, Brekelmans PJAM, Buytenhek R, Brouwer A, DeLeeuw AM, Knook DL (1987) Liver parenchymal cells differ from the fat-storing cells in their lipid composition. Lipids 22:266–273
51. Hendriks HFJ, Bosma A, Brouwer A (1993) Fat-storing cells: hyper- and hypovitaminosis A and the relationships with liver fibrosis. Semin Liver Dis 13:72–79
52. Hines JE, Johnson SJ, Burt AD (1993) In vivo responses of macrophages and perisinusoidal cells to cholestatic liver injury. Am J Pathol 142:511–518
53. Hopwood D, Nyfors A (1976) Effect of methotrexate therapy in psoriatics on the Ito cells in liver biopsies, assessed by point-counting. J Clin Pathol 29:689–703
54. Horn T, Lyon H, Christoffersen P (1986) The blood-hepatocyte barrier: a light microscopical, transmission- and scanning electron microscopic study. Liver 6:233–245
55. Horn T, Junge J, Christoffersen P (1986) Early alcoholic liver injury: activation of lipocytes in acinar zone 3 and correlation to degree of collagen formation in the Disse space. J Hepatol 3:333–340
56. Horn T, Junge J, Nielsen O, Christoffersen P (1988) Light microscopical demonstration and zonal distribution of parasinusoidal cells (Ito cells) in normal human liver. Virchows Arch [A] 413:147–149
57. Horvath E, Kovacs K, Ross RC (1973) Liver ultrastructure in methotrexate treatment of psoriasis. Arch Dermatol 108:427–428
58. Housset C, Rockey DC, Bissel DM (1993) Endothelin receptors in rat liver: lipocytes as a contractile target for endothelin-1. Proc Natl Acad Sci USA 90:9266–9270
59. Hruban Z, Russell RM, Boyer JL, Glagov S, Bagheri SA (1974) Ultrastructural changes in livers of two patients with hypervitaminosis A. Am J Pathol 76:451–461
60. Inuzuka S, Ueno T, Torimura T, Sata M, Abe H, Tanikawa K (1990) Immunohistochemistry of the hepatic extracellular matrix in acute viral hepatitis. Hepatology 12:249–256
61. Iredale JP, Murphy G, Hembry RM, Friedman SL, Arthur MJP (1992) Human hepatic lipocytes synthesize tissue inhibitor of metalloproteinases-1: implications for regulation of matrix degradation in liver. J Clin Invest 90:282–287
62. Ito T, Nemoto M (1952) †ber die Kupfferschen Sternzellen und die "Fettspeicherungszellen" ("fat-storing cells") in der Blutkapillarenwand der menschlichen Leber. Okajimas Folia Anat Jpn 24:243–258
63. Jacques EA, Buschmann RJ, Layden TJ (1979) The histopathologic progression of vitamin-A induced hepatic injury. Gastroenterology 76:599–602
64. Johnson SJ, Hines JE, Burt AD (1992) Macrophage and perisinusoidal cell kinetics in acute liver injury. J Pathol 166:351–358
65. Jorens PG, Michielsen PA, Pelckmans PA, Fevery J, Desmet VJ, Geubel AP, Rahier J, Van Maercke YM (1992) Vitamin A abuse: development of cirrhosis despite cessation of vitamin A: a six-year clinical and histopathologic follow-up. Liver 12:381–386
66. Knittel T, Aurisch S, Neubauer K, Eichhorst S, Ramadori G (1996) Cell-type-specific expression of neural cell adhesion molecule (N-CAM) in Ito cells of rat liver: upregulation during in vitro activation and in hepatic tissue repair. J Hepatol [Suppl 1] 25:113
67. Kobayashi Y, Fujiyama S (1981) Pathological study on gold impregnation of fat-storing cells in human liver. Acta Pathol Jpn 31:65–74
68. Latry P, Bioulac-Sage P, Echinard E, Gin H, Boussarie L, Grimaud JA, Balabaud C (1987) Perisinusoidal fibrosis and basement membrane-like material in liver of diabetic patients. Hum Pathol 18:755–760
69. LeBail B, Bioulac-Sage P, Senuita R, Quinton A, Saric J, Balabaud C (1990) Fine structure of hepatic sinusoids and sinusoidal cells in disease. J Electron Microsc Tech 14:257–282
70. Leo MA, Lieber CS (1982) Hepatic vitamin A depletion in alcoholic liver injury. N Engl J Med 307:597–601
71. Leo MA, Sato M, Lieber CS (1983) Effect of hepatic vitamin A depletion on the liver in humans and rats. Gastroenterology 84:562–572
72. Maher J (1989) Fat-storing cells and other myofibroblasts: one cell or two? Hepatology 9:903–904
73. Malizia G, Brunt EM, Peters MG, Rizzo A, Broekelmann TJ, McDonald JA (1995) Growth factor and procollagen type I gene expression in human liver disease. Gastroenterology 108:145–156
74. Mallat A, Preaux AM, Blazejewski S, Rosenbaum J, Dhumeaux D, Mavier P (1995) Interferon alfa and gamma inhibit proliferation and collagen synthesis of human Ito cells in culture. Hepatology 21:1003–1010
75. Marra F, Valente AJ, Pinzani M, Abboud HE (1993) Cultured human liver fat-storing cells produce monocyte chemotactic protein-1: regulation by proinflammatory cytokines. J Clin Invest 92:1674–1680
76. Marra F, Choudhury GG, Pinzani M, Abboud HE (1994) Regulation of platelet-derived growth factor secretion and gene expression in human liver fat-storing cells. Gastroenterology 107:1110–1117
77. Marra F, Grandaliano G, Valente AJ, Abboud HE (1995) Thrombin stimulates proliferation of liver fat-storing cells and expression of monocyte chemotactic protein-1: potential role in liver injury. Hepatology 22:780–787
78. Mathew J, Hines JE, James OFW, Burt AD (1994) Non-parenchymal cell responses in paracetamol (acetaminophen)-induced liver injury. J Hepatol 20:537–541
79. Matsuoka M, Tsukamoto H (1990) Stimulation of hepatic lipocyte collagen production by Kupffer cell-derived transforming growth factor β: implication for a pathogenetic role in alcoholic liver fibrogenesis. Hepatology 11:599–605
80. Matsuoka M, Pham N, Tsukamoto H (1989) Different effects of interleukin-1 α, tumour necrosis factor α, and transforming growth factor β 1 on cell proliferation and collagen formation by cultured fat-storing cells. Liver 9:71–78
81. Meyer DH, Bachem MG, Gressner AM (1990) Transformed fat storing cells inhibit the proliferation of cultured hepatocytes by secretion of transforming growth factor beta. J Hepatol 11:86–91
82. Meyer DH, Bachem M, Gressner A (1991) Bidirectional effects of Kupffer cells on hepatocyte proliferation in vitro. FEBS Lett 283:150–154
83. Milani S, Herbst H, Schuppan D, Grappone C, Pellegrini G, Pinzani M, Casini A, Calabró A, Ciancio G, Stefanini F, Burroughs AK, Surrenti C (1994) Differential expression of matrix-metalloproteinase-1 and -2 genes in normal and fibrotic human liver. Am J Pathol 144:528–537
84. Minato Y, Hasumura Y, Tackeuchi J (1983) The role of fat-storing cells in Disse space fibrogenesis in alcoholic liver disease. Hepatology 3: 559–566

85. Moshage H, Casini A, Lieber CS (1990) Acetaldehyde selectively stimulates collagen production in cultured rat liver fat-storing cells but not in hepatocytes. Hepatology 12:511–518
86. Nakano M, Worner TM, Lieber CS (1982) Perivenular fibrosis in alcoholic liver injury: ultrastructure and histologic progression. Gastroenterology 83:777–785
87. Nakatani K, Seki S, Kawada N, Sakaguchi H, Kawakita N, Yanai A, Kitada T, Sakai Y, Yamada T, Masuichi H, Kuroki T, Kobayashi K, Kaneda K (1995) Expression of neural cell adhesion molecule (N-CAM) on the stellate cells of human liver. In: Wisse E, Knook DL, Wake K (eds) Cells of the hepatic sinusoid, vol 5. The Kupffer Cell Foundation, Leiden, pp 267–269
88. Neubauer K, Knittel T, Aurisch S, Fellmer P, 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
89. Niki T, DeBleser PJ, Xu G, Van den Berg K, Wisse E, Geerts A (1996) Comparison of glial fibrillary acidic protein and desmin staining in normal and CCL_4-induced fibrotic rat livers. Hepatology 23:1538–1545
90. Nouchi T, Tanaka Y, Tsukada T, Sato C, Marumo F (1991) Appearance of α-smooth-muscle-actin-positive cells in hepatic fibrosis. Liver 11:100–105
91. Nyberg A, Berne B, Nordlinger H, Busch C, Eriksson U, Lööf L, Vahlquist A (1988) Impaired release of vitamin A from liver in primary biliary cirrhosis. Hepatology 8:136–141
92. Ogawa K, Suzuki J, 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
93. Okanoue T, Burbige EJ, French SW (1983) The role of the Ito cell in perivenular and intralobular fibrosis in alcoholic hepatitis. Arch Pathol Lab Med 107: 459–463
94. Parola M, Pinzani M, Casini A, Albano E, Poli G, Gentilini A, Gentilini P, Dianzani MU (1993) Stimulation of lipid peroxidation or 4-hydroxynonenal treatment increases procollagen-alpha 1 (I) gene expression in human liver fat-storing cells. Biochem Biophys Res Commun 194:1044–1050
95. Pinzani M (1995) Hepatic stellate (Ito) cells: expanding roles for a liver-specific pericyte. J Hepatol 22:700–706
96. Pinzani M, Failli P, Ruocco C, Casini A, Milani S, Baldi E, Giotti A, Gentilini P (1992) Fat-storing cells as liver-specific pericytes. J Clin Invest 90:642–646
97. Pinzani M, Gentilini A, Caligiuri A, DeFranco R, Pellegrini G, Milani S, Marra F, Gentilini P (1995) Transforming growth factor-β1 regulates platelet-derived receptor β subunit in human liver fat-storing cells. Hepatology 21:232–239
98. Pinzani M, Milani S, DeFranco R, Grappone C, Caligiuri A, Gentilini A, Tosti-Guerra C, Maggi M, Failli P, Ruocco C, Gentilini P (1996) Endothelin-1 is overexpressed in human cirrhotic liver and exerts multiple effects on activated hepatic stellate cells. Gastroenterology 110:534–548
99. Ramadori G (1991) The stellate cell (Ito cell, fat-storing cell, lipocyte, perisinusoidal cell) of the liver. Virchows Arch [B] 61:147–158
100. Reid LM, Fiorino AS, Sigal SH, Brill S, Holst PA (1992) Extracellular matrix gradients in the space of Disse: relevance to liver biology. Hepatology 15:1198–1203
101. Rieder H, Meyer Zum Buschenfelde K, Ramadori G (1992) Functional spectrum of sinusoidal endothelial liver cells. J Hepatol 15:237–250
102. Rockey D, Maher J, Jarnagin W, Gabbiani G, Friedman S (1992) Inhibition of rat hepatic lipocyte activation in culture by interferon-γ. Hepatology 16:776–784
103. Rockey DC, Housset CN, Friedman SL (1993) Activation-dependent contractility of rat hepatic lipocytes in culture and in vivo. J Clin Invest 92:1795–1804
104. Ross R, Raines EW, Bowen-Pope DF (1986) The biology of platelet-derived growth factor. Cell 46:155–169
105. Rudolph R, McClure WJ, Woodward M (1979) Contractile fibroblasts in chronic alcoholic cirrhosis. Gastroenterology 76:704–709
106. Russel RM, Boyer JL, Bagheri SA, Hruban Z (1974) Hepatic injury from chronic hypervitaminosis A resulting in portal hypertension and ascites. N Engl J Med 291:435–440
107. Sakamoto M, Ueno T, Kin M, Ohira H, Torimura T, Inuzuka S, Sata M, Tanikawa K (1993) Ito cell contraction in response to endothelin-1 and substance P. Hepatology 18:978–983
108. 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
109. Schmitt-Gräff A, Chakroun G, Gabbiani G (1993) Modulation of perisinusoidal and cytoskeletal features during experimental hepatic fibrosis. Virchows Arch [A] 422:99–107
110. Shiratori Y, Geerts A, Ichida T, Kawase T, Wisse E (1986) Kupffer cells from CCl_4-induced fibrotic livers stimulate proliferation of fat-storing cells. J Hepatol 3:294–303
111. Stroebel P, Mayer F, Zerban H, Bannasch P (1995) Spongiotic pericytoma: a benign neoplasm deriving from the perisinusoidal (Ito) cells in rat liver. Am J Pathol 146:903–913
112. Sztark F, Dubroca J, Latry P, Quinton A, Balabaud C, Bioulac-Sage P (1986) Perisinusoidal cells in patients with normal liver histology: a morphometric study. J Hepatol 2:358–369
113. Takase S, Leo M, Nouchi T, Lieber C (1988) Desmin distinguishes cultured fat-storing cells from myofibroblasts, smooth muscle cells and fibroblasts in the rat. J Hepatol 6:267–276
114. Tiggelman AMBC, Boers W, Linthorst C, Brand HS, Sala M, Chamuleau RAFM (1995) Interleukin-6 production by human liver (myo)fibroblasts in culture: evidence for a regulatory role of LPS, IL-1β and TNF α. J Hepatol 23:295–306
115. Tiggelman AMBC, Boers W, Linthorst C, Sala M, Chamuleau RAFM (1995) Collagen synthesis by human liver (myo)fibroblasts in culture: evidence for a regulatory role of IL-1β, IL-4, TGF-β and IFN gamma. J Hepatol 23:307–317
116. Tozuka S, Hasumura Y, Takeuchi J (1985) Histochemical characteristics of fat-storing cells in alcoholic liver disease: a study by the gold chloride method using needle biopsy of the liver. Am J Clin Pathol 83:47–52
117. Trinchieri G, Perussia B (1985) Immune interferon: a pleiotropic lymphokine with multiple effects. Immunol Today 6:131–136
118. Von Kupffer C (1876) †ber Sternzellen der Leber. Briefliche Mittheilung an Professor Waldeyer. Arch Mikr Anat 12:353–358
119. Wake K (1971) "Sternzellen" in the liver: perisinusoidal cells with special reference to storage of vitamin A. Am J Anat 132:429–462
120. Wake K, Kishiye T, Yamamoto H, Bhunchet E, Sakamoto Y, Sato T (1993) Kupffer cells modulate the configuration of perisinusoidal stellate cells. In: Knook DL, Wisse E (eds) Cells of the hepatic sinusoid, vol 4. The Kupffer Cell Foundation, Leiden, pp 157–160
121. Weiner F, Giambrone M, Czaja M, Shah A, Annoni G, Takahashi S, Eghbali M, Zern M (1990) Ito-cell gene expression and collagen regulation. Hepatology 11:111–117
122. Yamaoka K, Nouchi T, Marumo F, Sato C (1993) α-Smooth-muscle actin expression in normal and fibrotic human livers. Dig Dis Sci 38:1473–1479
123. 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
124. Zafrani ES, Bernuau D, Feldmann G (1984) Peliosis-like ultrastructural changes of the hepatic sinusoids in human chronic hypervitaminosis A: report of three cases. Hum Pathol 15:1166–1170
125. Zajicek G, Ariel I, Arber N (1988) The streaming liver III: littoral cells accompany the streaming hepatocyte. Liver 8:213–218