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Invited Review

The hepatic perisinusoidal stellate cell

N. Kawada
Department of Internal Medicine, Osaka City University Medical School, Osaka, Japan

Summary. Hepatic stellate cell (also referred to as Ito cell, fat-storing cell, perisinusoidal cell, lipocyte) is one of the sinusoid-constituent cells that play multiple roles in liver pathophysiology. Although identification of the stellate cell had taken about 100 years because of the misconception caused by the discoverer von Kupffer, Wake made a great contribution to the "re"discovery of the cell in 1971. Establishment of the isolation of hepatic non-parenchymal cells from rats by Knook has made it possible to uncover the metabolic function of individual cells. Now, the stellate cell function is expanding from a retinol (fat)-storing site to a center of extracellular matrix metabolism and mediator production in the liver. Function as a liver specific pericyte has also been elucidated. Transition of the stellate cells from the vitamin A-storing phenotype to "activated" or "myofibroblastic" cells that produce a large amount of type I collagen and transforming growth factor β triggers the progress of liver fibrosis in the course of hepatic inflammation. Communication of the stellate cells with the other hepatic constituent cells and invading inflammatory cells is also an important factor that regulates the local pathological reaction. Analysis of cellular and molecular aspects of the stellate cell activation would lead to the establishment of a novel therapeutic strategy against the progress of liver fibrosis in human liver disease.

Key words: Stellate cell, Fibrosis, Cytokine, Smooth muscle α-actin, Myofibroblast

History of the stellate cells

In 1876, the stellate cells ("Sternzellen") in the liver were first described by an anatomist von Kupffer, who was interested in the development of nerve fiber in the liver (Kupffer, 1876). By using a gold chloride method, he found black star-shaped cells in the liver lobule, which were located perisinusoidally, always attached to the sinusoids and to the parenchymal cells. However, in 1898, von Kupffer changed his opinion incorrectly and suggested that the gold chloride reactive Sternzellen were endothelial cells of the sinusoids with phagocytic activity (Kupffer, 1898). This misconception has led to a great confusion in the liver histology until Wake re-discovered the cells in 1971 (Wake, 1971). Wake, by employing gold impregnation method, the silver impregnation method, fluorescence, and electron microscopy, revealed that the Sternzellen first described by von Kupffer are quite different from the phagocytic liver endothalial cells and macrophages (Kupffer cells), and are identical to the "fat-storing cells" described by Ito in 1951 (Ito and Nemoto, 1952) and also to "interstitial cells" described by Suzuki (1958) (Suzuki, 1958). Now, all pericytes, fat-storing cells, interstitial cells, perisinusoidal cells, lipocytes, Ito cells, and vitamin A-storing cells are recognized to be identical to the stellate cells (Wake, 1980).

Offprint requests to: Norifumi Kawada, M.D., The Third Department of Internal Medicine, Osaka City University Medical School, 1-5-7, Asahimachi, Abeno, Osaka 545, Japan

Anatomy of the stellate cells

The stellate cells are located in the space of Disse in close contact with hepatocytes and sinusoidal endothelial cells (Wake, 1980, 1988; Wake et al., 1991). Their three-dimensional structures consist of a cell body with prominent lipid particles and several dendritic processes. The most conspicuous feature of the stellate cells is the presence of lipid droplets in the cytoplasm. The diameter of lipid droplets in rats is about 2 μm. There are two types of lipid droplets: membrane-bound and non-membrane-bound (Wake, 1980). The former are smaller than the latter in size. These lipid droplets are important in the hepatic storage of retinyl esters. A well-developed granular endoplasmic reticulum and Golgi complex have often been reported in stellate cells, and suggest an active protein synthesis and secretion from the cells. Cytoplasmic matrix is rich in microtubles and intermediate (10 nm) filaments. The stellate cells produce several types of cytoskeletal proteins such as desmin, vimentin, actin and tubulin. Mitochondria of the stellate cells are smaller in size and number than those of parenchymal cells (Wake, 1980). There are two different types of processes, the perisinusoidal (subendothelial) and the interhepatocellular processes (Wake, 1988). The perisinusoidal processes branch out from the cell body

and direct along the sinusoid with numerous finger-like secondary branches. These secondary branches encircle the endothelial tube. The interhepatocellular processes, which are much fewer, traverse the hepatic cord. After reaching the neighboring sinusoids, the processes branch out similarly into a number of perisinusoidal processes. One stellate cell embraces two or more neighboring sinusoids (Wake, 1988).

The stellate cells are distributed rather regularly in the liver lobule (Kupffer 1876; Wake, 1980; Giampieri et al., 1981). According to a stereological examination, hepatocytes account for 78% of parenchymal volume in the rat liver and the nonparenchymal cells account for 6.3% (2.8% endothelial cells, 2.1% Kupffer cells, and 1.4% stellate cells) (Blouin et al., 1977). Studies performed in rabbit liver have shown that the average spreading area of an individual stellate cell is approximately 120 μm. In human liver, stellate cells are disposed along the sinusoids with a nucleus-to-nucleus distance of 40 μm, indicating that the sinusoids are equipped with stellate cells at certain fixed distances. Although the total number of stellate cells constitutes a small percentage of the total number of liver cells, their spatial disposition and extension may be sufficient to cover the entire hepatic sinusoidal microcirculatory network (Bronfenmajer et al., 1966; Blouin et al., 1977; Giampieri et al., 1981).

Interlobular heterogeneity of hepatic constituent cells is now well recognized. The heterogeneous zonal distribution of various enzymes is reported in parenchymal cells (Gumucio, 1989; Jungermann, 1989). Fenestration, the thin portions and the size of the endothelial cells vary among zones (Wisse et al., 1985). Kupffer cells (Sleyster and Knool, 1982; Bouwens et al., 1986) and pit cells (Kaneda and Wake, 1983) are more numerous in periportal regions. Lysosomes of Kupffer cells in the periportal region are more developed than those in the central region, indicating the active phagocytic activity in Kupffer cells in the periportal region. Wake demonstrated the heterogeneity of stellate cells as well (Wake and Sato, 1993). Stellate cells in the portal area store abundant vitamin A and extend encompassing processes that show intense desmin immunoreactivity. In midzone, the cells are conspicuously dendritic in appearance, each process being more elongated and attenuated with numerous thorn-like microprojections, whereas their desmin immunoreactivity and vitamin-A storage are reduced. In centrilobular area, the stellate cells have an irregular branching pattern, the branches being studded with long thorn-like microprojections. Few or no lipid droplets are present in the cytoplasm of these cells. Desmin-negative stellate cells were also demonstrated in the centrilobular area (Ballardini et al., 1994).

Isolation and culture of the stellate cells

Recent dramatical progress in the cell biology of the stellate cells has depended on the development of isolation and culture of stellate cells from mouse, rat, and human liver. Knook et al. in 1982 showed the isolation procedure of the hepatic non-parenchymal cells for the first time (Knook et al., 1982). The isolation procedure is based on the separation of each hepatic constituent cell by perfusing the liver with digestive enzymes such as collagenase and pronase. To get a homogenous population of the stellate cells, several different technical approaches have been performed using centrifugal elutriation rotor (De Leuw et al., 1984), stractan (Friedman and Roll, 1987), metrizamide and nycodenz (Blomhoff and Berg, 1990). All these purification procedures are based on the buoyancy of the stellate cells due to their large fat content. The final yield of the stellate cells obtained from a single rat liver will vary according to the age, body weight and nutritional status of the animal. The highest yield is usually obtained using older rats maintained on a normal diet, or younger rats fed on a vitamin-A-supplemented diet. About $2\text{-}3\text{x}10^7$ of stellate cells with a purity of more than 95% can be isolated from a rat using these isolation procedures.

Isolated stellate cells can be maintained in a primary culture on uncoated or substratum-coated plastic culture plates in an usual cell culture medium (Fig. 1A). The medium should be supplemented with a serum such as fetal calf serum or newborn calf serum for a longer culture (Friedman and Roll, 1987). However, as described below, the culture triggers the "activation" or "myofibroblastic transformation" of the stellate cells. Extracellular environment, such as extracellular matrix and oxygen concentration, and growth factors presented in the serum may stimulate the activation although the precise molecular mechanism has not been well understood.

Desmin is a reliable marker for the identification of stellate cells in rat or mouse liver (Yokoi, et al., 1984; Tsutsumi, et al., 1987), but not the cells in human liver (Friedman et al., 1992). Hence, the human stellate cells would be desmin-negative. The other cytoskeletal marker for the stellate cells is smooth muscle isoform of α-actin (SM α-actin, Fig. 1B) (Ramadori et al., 1990; Enzan et al., 1994). This protein is expressed in the stellate cells of human liver but not in those of normal rat liver. However, the stellate cells of injured rat liver (Enzan et al., 1994) or of culture-induced myofibroblastic phenotype exhibit SM α-actin, indicating that SM α-actin is a good indicator for stellate cell activation (Ramadori et al., 1990; Rockey et al., 1992a). More recently, Kawada and Nakatani demonstrated the expression of heat shock protein 47 (HSP47, Fig. 2) (Kawada et al., 1996a-c) and neural cell adhesion molecule (NCAM) (Nakatani et al., 1996) in mouse and human stellate cells, respectively. NCAM would be a novel activation marker for rat stellate cells because it is neither expressed in normal rat liver nor quiescent cultured rat stellate cells (Knittel et al., 1996a).

Function of the stellate cells

1. Retinol metabolism

About 50-80 % of total retinol in mammals is stored in the liver under normal conditions (Blomhoff et al., 1990). Stellate cells contain about 30 nmol total retinol/10^6 cells. Parenchymal cells contain 0.5-0.8 nmol total retinol/10^6 cells. Endothelial cells and Kupffer cells contain negligible amounts of total retinol. Hence, 80-90% of the total retinol in liver is present in the stellate cells. Ninety eight percent of this is in the form of retinyl esters (Blomhoff et al., 1985; Batrens and Olson, 1987). Values for retinyl ester are between 12 and 65% of the total lipid mass in the cells. Another main component is triacylglycerol and comprises between 35 and 50% of the lipid mass. Minor amounts of un-esterified retinol, cholesteryl esters, cholesterol, free fatty acids, and phospholipids are found in the lipid droplets (Moriwaki et al., 1988).

Before the role of stellate cells in liver retinol metabolism was established, hepatocytes were thought to be the exclusive site of retinol mobilization (Goodman, 1984). Rat parenchymal cells contain 70-110 ng retinol binding protein (RBP)/mg cell protein, whereas rat stellate cells contain 25-168 ng RBP/mg cell protein, indicating that more than 90% of hepatic RBP is present in parenchymal cells (Hendriks et al., 1988; Moriwaki et al., 1988). As essentially all retinol in plasma is bound to RBP, the above results indicate the exclusive role of

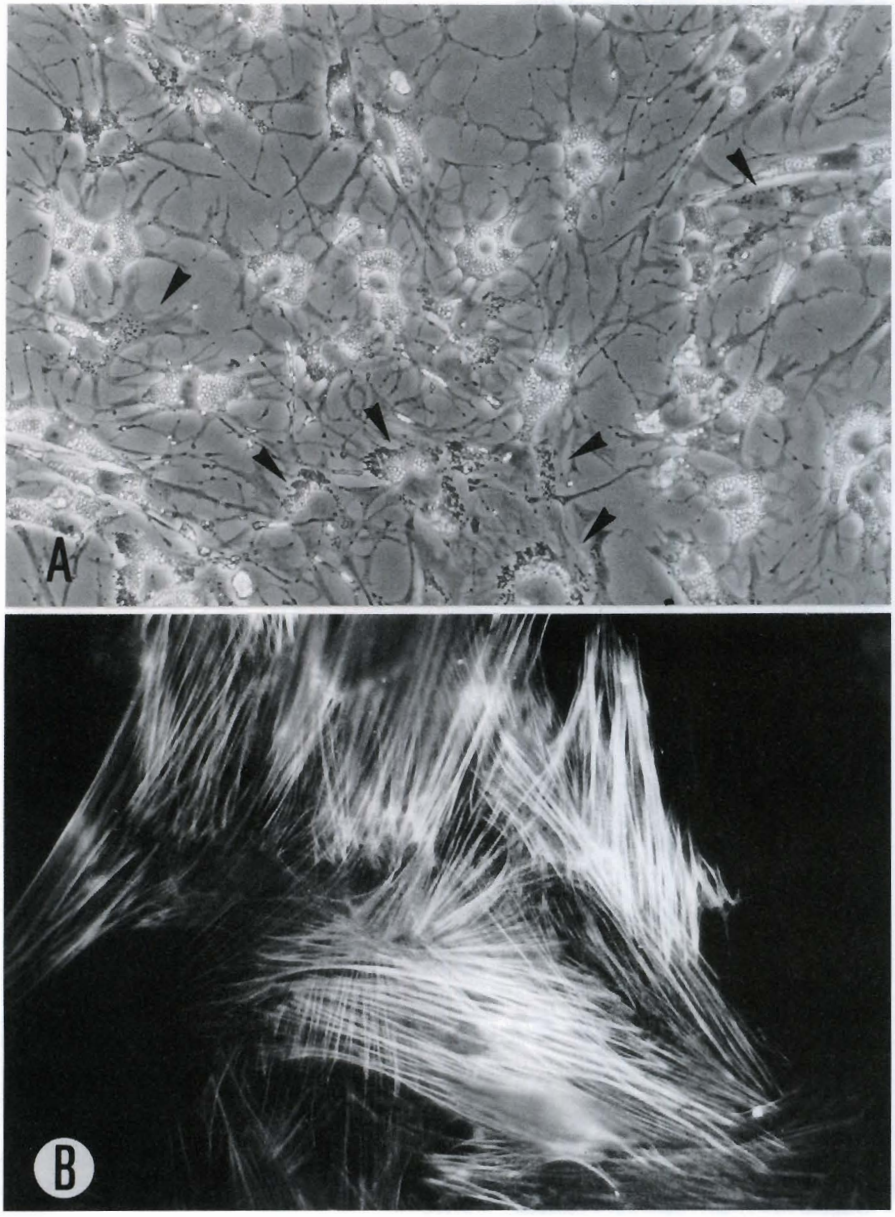

Fig. 1. Stellate cells in primary culture. **A.** Phase-contrast microscopic observation of stellate cells isolated from rat liver. They were cultured on a plastic dish in DMEM supplemented with 10% FCS for 2 days. Note that the cells contain several lipid particles and extend branching processes. Some of them start loosing droplets, enlarging in their size and having so-called 'membranous' processes (arrow heads). x 200. **B.** Immunofluorescent staining of smooth muscle α-actin in stellate cells. Stellate cells became 'activated' phenotype after the culture for 7 days. Apparent stress fibers of smooth muscle α-actin are composed in the cytoplasm. x 400

hepatocytes in retinol transport from the liver to plasma. The other proteins related to vitamin A metabolism including cellular retinol binding protein I (CRBP I), cellular retinoic acid-binding protein, retinyl palmitate hydrolase, acyl-coA:retinyl acyl transferase and lecithin:retinol acyl transferase (LRAT) are found in the stellate cells (Blaner, et al., 1985; Blomhoff et al., 1985). Although hepatocytes are quantitatively the greatest source of these proteins in the liver on a cell-protein basis, the concentration of many of these proteins are greater in the stellate cells than parenchymal cells. The incorporation of retinol-RBP complex secreted from

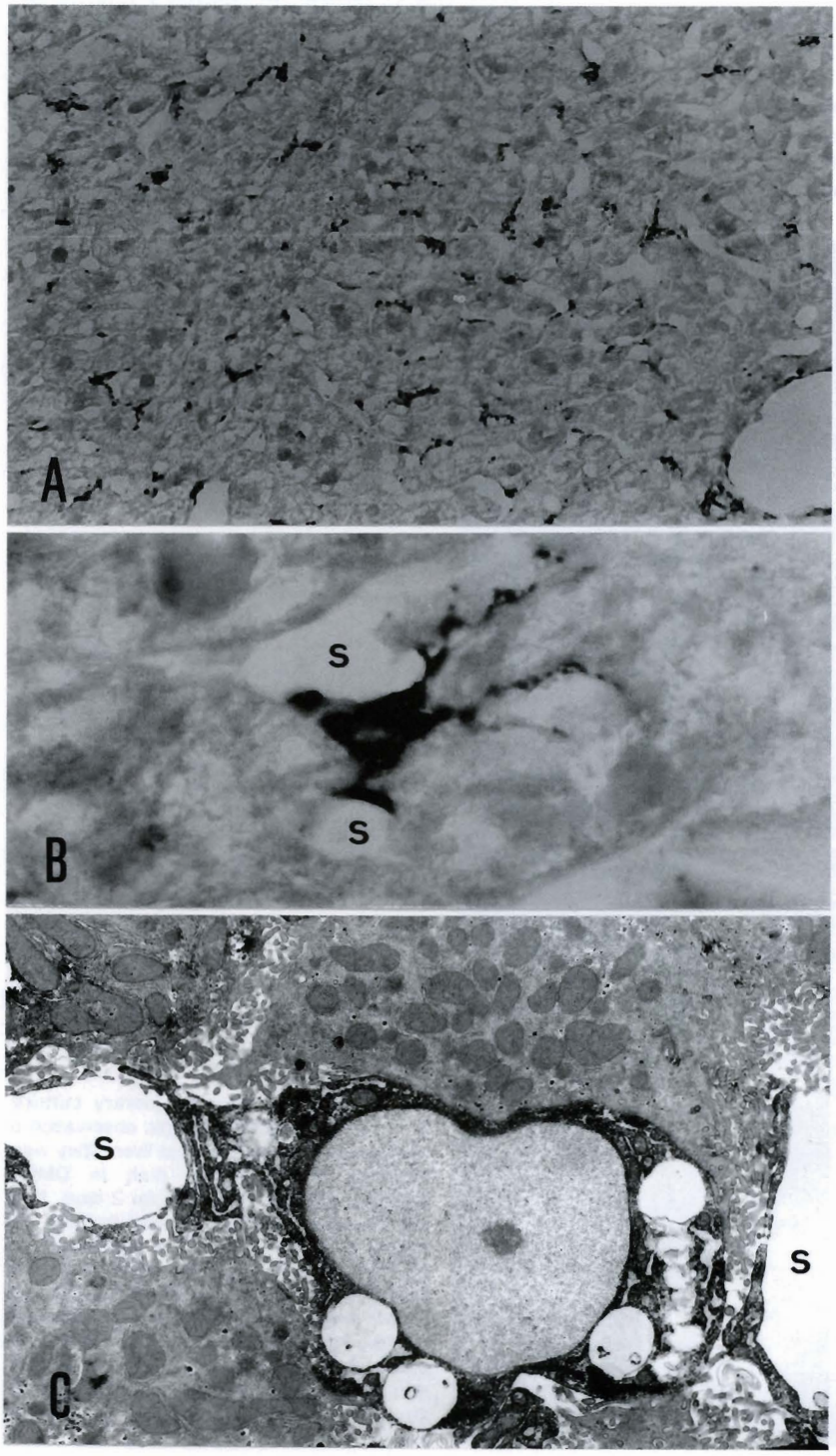

Fig. 2. Localization of stellate cells in the mouse liver. Folmalin-fixed mouse liver sections were stained for heat shock protein 47 (HSP47) using a specific antibody. Indirect immunoperoxidase staining. **A.** HSP47-positive cells are observed evenly in the liver lobule. They are spindle-shaped and localize in the peri-sinusoidal site. x 200. **B.** One perisinusoidal cell with dendritic process is stained for HSP47. Small lipid-like particles are also seen in the cytoplasm. The processes seem to be associated with several sinusoids (s). x 1000. **C.** Immunoelectronmicroscopy. HSP47-positive cells were confirmed as stellate cells. Typical lipid particles, which might contain vitamin A, are seen. The stellate cell localizes in the Disse space and closely contacts hepatocytes and sinusoid (s). The stellate cell is associated with two different sinusoids. x 6,400

hepatocytes by stellate cells through RBP receptors has been shown (Blomhoff et al., 1988; Senoo et al., 1993). Stellate cells also take up some retinyl esters, and retinol in a non-energy- and non-RBP-dependent manner (Matsuura et al., 1993). Retinol taken up by the stellate cells is bound to CRBP I and esterified in the presence of LRAT and the resulting retinyl esters are stored in the stellate cell lipid droplets (Norum and Blomhoff, 1992). Whether retinol is transfered from the stellate cells to the plasma directly has not been determined. In addition, the transport of retinol from stellate cells to hepatocytes has not been well studied. Ikeda demonstrated that rat hepatocytes in primary culture can incorporate retinoids released from the stellate cells using a gene transfection technique (Ikeda et al., 1996). Such an experiment will reveal an uncovered metabolic pathway of retinol in the liver.

2. Role as a liver-specific pericyte

Because the stellate cells encircle the contraluminal surface of sinusoidal endothelial cells with their well branching cytoplasmic processes, Wake hypothesized that the stellate cells may act as a liver-specific pericyte and regulate sinusoidal blood flow by their contractility (Wake, 1988; Wake et al., 1992). Liver perfusion experiments demonstrated that the resistance of portal vein system increases in correspondence to the addition of extracellular nucleotides and eicosanoids indicating that there is a contractile machinery in port-sinusoidal network (Fisher et al., 1987; Haussinger et al., 1988). Smooth muscle cells consisting of the portal venules are possible candidates, but precise contracting points could not be identified by such a perfusion experiment. Existence of presinusoidal sphincter has been postulated (McCuskey, 1966, 1971, 1988). The contraction of sinusoidal endothelial cells or their fenestra may participate in the regulation of the portal resistance (Gatmaitan and Arias, 1993). In 1992, Pinzani (Pinzani et al., 1992a) and Kawada (Kawada et al., 1993) independently demonstrated the contraction of the stellate cells using human and rat stellate cells, respectively. Pinzani showed that thrombin and angiotensin II induced diminution of cell surface area and the concomitant increase of intracellular calcium concentration of human stellate cells in culture under microscopic observation. Kawada demonstrated the contraction of rat stellate cells by using a sensitive silicone rubber membrane method that was originally used to detect the motility of fibroblasts. In this method, the increase of traction force of cultured stellate cells in response to eicosanoids and endothelin 1 (Kawada et al., 1993) was revealed by the development of wrinkling of a silicone rubber membrane that can be observed easily with microscopy. The contraction of stellate cells could be detectable as early as 30 seconds after the addition of the ligands. This is a most sensitive method in detecting the stellate cell contraction because it reveals the contractility of quiescent stellate cells as well (Fig. 3). In contrast, Rockey and Housset reported that only

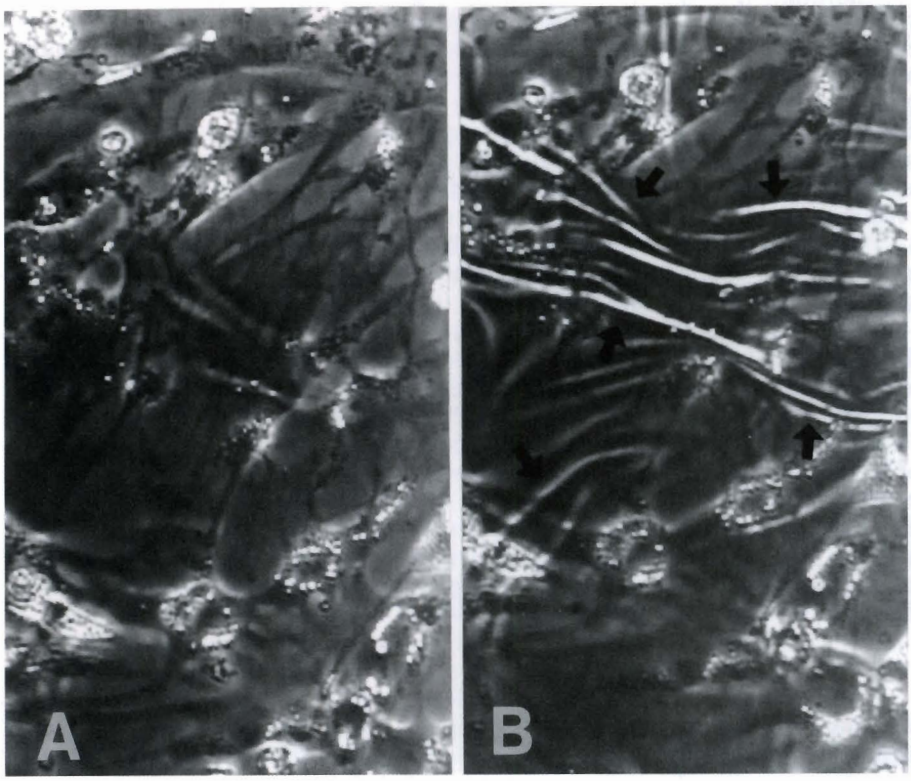

Fig. 3. Detection of stellate cell contraction by a silicone-rubber-membrane method. Isolated stellate cells were cultured on a silicone-rubber-membrane in DMEM supplemented with 10% FCS for 4 days. Then, the cells were stimulated with 1 nM ET-1 and observed under the microscope connected to a video-camera system for 1 h. Note that wrinkles (arrows) of the membrane produced by the traction force of the stimulated stellate cells are seen just 1 min after the addition of ET-1. x 200

activated stellate cells having SM α-actin can induce the contraction of a hydrated collagen gel (Rockey, 1995; Housset et al., 1993). The silicone rubber membrane method additionally revealed the relaxation of stellate cells in response to prostaglandin E2 (Kawada et al., 1992), prostacycline (Kawada et al., 1992), donors of nitric oxide (Kawada et al., 1993), and adrenomedullin (Kawada and Inoue, 1994). The relaxation of the stellate cells was always accompanied by the disruption of actin stress fibers. Analysis of second messenger system operating for the cell contraction revealed that stellate cell contraction is mediated by the increase of intracellular calcium concentration and the stimulation of inositol phosphate metabolism (inositol tris-phosphate production) (Pinzani et al., 1992a,b; Kawada et al., 1993; Takemura et al., 1994) and cell relaxation by cAMP or cGMP (Kawada et al., 1992, 1993; Takemura et al., 1994). Recent findings by Bauer using invital fluorescent microscopic observation indicated that such a contractility of stellate cells also works in vivo perfused rat liver stimulated with endothelins (Bauer et al., 1994; Zhang et al., 1995). Suematsu suggested a crucial role of carbon monoxide produced by hepatocytes and Kupffer cells in the relaxation of stellate cells, and thereby the dilatation of sinusoids (Suematsu et al., 1995).

As suggested by Rockey and Housset, the contractility of the stellate cells seems to increase concomitantly with the expression of SM α-actin (Housset et al., 1993; Rockey 1995) although direct evidence is still lacking. In injured and fibrotic livers, the stellate cells surrounded by abundant extracellular matrix materials express SM α-actin. Hence, the increased contractility of the stellate cells may lead to the rigidity and shrinkage of the tissues, resulting in the chronic disturbance of sinusoidal microcirculation. It is therefore important to find out a method to suppress the expression of SM α-actin. Rockey demonstrated that interferon γ is potent in inhibiting the protein and the mRNA expression for SM α-actin in stellate cells of the rat (Rockey et al., 1992b; Rockey and Chung, 1994). Mallat showed that interferon α and β are also effective (Mallat et al., 1995a). Kawada (Kawada et al., 1996a) and Windmier (Windmeier and Gressner, 1996) showed that phosphodiesterase inhibitor methylxanthines, such as 3-isobutyl-1-methylxanthine, caffeine, theophilline, and pentoxifylline inhibited the protein expression. Kawada further demonstrated that nitric oxide is involved in interferon γ and lipopolysaccharide-mediated inhibition of SM α-actin expression in rat stellate cells (Kawada et al., 1996b). Further study will be needed, however, to understand the precise molecular mechanism of the protein expression in activated stellate cells in order to establish a medical treatment that contributes to maintain the microcirculation in the liver with chronic inflammation.

3. Extracellular matrix metabolism

Until 1988, collagen in normal liver was thought to be generated by hepatocytes. This concept seems to have come from the experimental results showing that hepatocytes in primary culture produce collagen (Sakakibara et al., 1976; Hata et al., 1980; Diegelmann et al., 1983). However, routinely prepared parenchymal cell fraction may contain about 10% stellate cells (Arenson et al., 1988). Maher showed that the main cell type within the rat parenchymal cell culture that displayed type I collagen by immunohistochemistry is the stellate cells (Maher et al., 1988). Furthermore, by using in situ hybridization, Milani demonstrated that stellate cells contain procollagen mRNA, whereas neither normal nor fibrotic rat livers showed any hybridization signal from parenchymal cells (Milani et al., 1990). Therefore, it seems that stellate cells are the principle cellular source of collagen in the liver. Liver fibrosis is characterized by the accumulation of extracellular matrix components, especially collagen types I, III and IV, fibronectin, laminin and proteoglycans in the space of Disse, to produce the so-called 'capillarisation' of the sinusoid, followed by the formation of connective tissue septa (Ogawa et al., 1985, 1986; Popper and Udenfriend, 1970; McGee and Patrick, 1972). Several investigations suggested that the stellate cell is mainly responsible for the accumulation of these matrix substances (Schuppan, 1990).

In conditions of chronic liver damage, as well as following prolonged culture on plastic, stellate cells undergo a process of activation from quiescent "storing" phenotype to the highly productive "myofibroblast-like" phenotype (Freidman, 1993; Gressner, 1995; Pinzani, 1995). This transformation is characterized by a gradual loss of intracellular vitamin A droplets, a significant increase in myofilaments and dense bodies associated with an increased expression of SM α-actin (Ramadori et al., 1990), cell enlargement with 'membranous' processes (Wake et al., 1992) associated with a loss of star-like cell appearance with finger-like projections, proliferative attitude, and a general activation of the synthetic properties of the cell. Increased production of collagen types I and III happen during this transformation (Maher et al., 1988; Milani et al., 1990). A gradual phenotypic transformation is also observed in an in vivo shift in predominance from stellate cells to myofibroblastic cells following liver injury. Studies performed in recent years have indicated some important aspects potentially related to the initiation of stellate cell activation. A first important element concerns the disruption of the normal extracellular matrix (ECM) pattern that follows liver tissue injury and acute inflammation. Loss of adhesion with the various elements constituting the basal membrane-like ECM of the space of Disse is likely to determine a marked increase in the proliferative and synthetic properties of stellate cells. When rat stellate cells are isolated and cultured on an EHS matrix gel containing laminin and resembling the normal liver subendothelial matrix, the cells do not undergo activation (Friedman et al., 1989). In contrast, proliferation of stellate cells is promoted when cultured on type I collagen, the predominant

matrix component in injured liver (Davis et al., 1987). Another important aspect is related to the exposure of stellate cells to soluble mediators. Following liver injury, several cell types could be involved in the synthesis and release of soluble factors such as platelet-derived growth factor (PDGF) and transforming growth factor β (TGFβ) that modulate a biological role of stellate cells. Among hepatic constituent cells, Kupffer cells are a focus of study. When exposed to conditioned medium from cultured Kupffer cells in vitro, stellate cells undergo activation, resulting in enhanced proliferation and fibrogenesis (Armendariz-Borunda et al., 1989; Freidman and Arthur, 1989). Although the existence of a 'lipocyte-stimulating factor' has been hypothesized, the precise nature of the factor(s) is still poorly understood. TGFβ1 would be one of them (Matsuoka et al., 1989; Matsuoka and Tsukamoto, 1990). Following parenchymal injury, sinusoidal endothelial cells and hepatocytes may also contribute to stellate cell activation by releasing soluble mediators. In addition, reactive oxygen species derived from damaged hepatocytes, Kupffer cells, infiltrating monocytes or granulocytes may trigger stellate cell activation because antioxidants such as vitamin E protect against CCl_4-induced chronic liver damage and fibrotic transformation of the liver tissue, and the production of collagen mRNA in cultured stellate cells (Parola et al., 1992a,b; Lee et al., 1995).

4. Matrix turnover

Many cell types including the stellate cells synthesize the metallo group of matrix-degrading proteinases (matalloproteinases or MMPs). The MMP family are zinc- and calcium-dependent endopeptidases. These enzymes are able to degradate the major macromolecules of extracellular matrix, including fibrillar and non-fibrillar collagens. To date, ten members of the metalloproteinase family have been cloned. The interstitial collagenase have the most specific substrate profile of the MMP family. They cleave the native helix of fibrillar collagens at a single site, Gly-Ile and Gly-Leu, to yield products of 25% and 75% of the original molecule (Murphy et al., 1991). The stromelysins have a broad substrate profile and in addition they may activate both procollagenase and progelatinase B. The gelatinases have a similar structure but are distinct gene products. They have activity against collagen types IV, V, VII and X. In addition, they may act in synergy with interstitial collagenase (Matrisian, 1990, 1992). Metalloelastase and membrane type (MT)-MMP are the most recently described membrane of the MMP family. Metalloelastase is a secretory product of murine macrophages. MT-MMP is expressed on the surface of metastatic pulmonary carcinoma cells and activates progelatinase (Sato et al., 1994).

Activated MMPs can be inhibited by general protease scavengers, such as α2-macroglobulin (Enghild et al., 1989). However, specific inhibition of activated metalloproteinases occurs by interaction with the tissue inhibitors of metalloproteinases, TIMPs (Murphy et al., 1991; Matrisian, 1990, 1992). To date, three TIMPs have been cloned: TIMP-1, TIMP-2 and TIMP-3 (Broone et al., 1990; Pavloff et al., 1992; Denhardt et al., 1993). Each TIMP is the product of a separate gene though there is some 40% amino-acid sequence homology between TIMP-1 and TIMP-2. TIMP-1 and TIMP-2 are not stored intracellularly, but are secreted into the extracellar space (Denhardt et al., 1993). Here, they have inhibitory activity against every activated metallo-poteinase.

The normal basement membrane-like matrix in the space of Disse consists predominantly of type IV collagen, laminin and proteoglycan with lower amounts of fibrillar collagen (types I and III). In early liver injury, this becomes replaced with increasing quantities of fibrillar collagen (Popper and Udenfried, 1970; McGee and Patrick, 1972; Ogawa et al., 1985, 1986; Schuppan, 1990). At this stage, MMPs with activity against type IV collagen such as gelatinase A and B and stromelysin represent those members of the MMP family. In more advanced fibrosis and cirrhosis, there is a predominant increase in collagen types I and III (Popper and Udenfried, 1970; McGee and Patrick, 1972; Ogawa et al., 1985, 1986; Schuppan, 1990). At this point, TGFβ1 play a crucial role in the progress of liver fibrosis. TGFβ1 upregulates the expression of TIMP-1, type I collagen and gelatinase A, while down-regulates interstitial collagenase generation (Knittel et al., 1996b; Nakatsukasa et al., 1990a). In addition, by enhancing the production of plasminogen activator inhibitor and down-regulating stromelysin synthesis, TGFβ1 down-regulates the interstitial collagenase activation cascade. In animal models of liver injury, TGFβ1 expression occurs simultaneously with the development of fibrosis (Nakatsukasa et al., 1990a,b). Expression of TGFβ1 and receptors for this cytokine are features of hepatic stellate cell activation (Friedman et al., 1994). Hence, TGFβ1 constitutes a cascade promoting fibrosis of the liver.

5. Production of mediators

The stellate cells themselves are a good source of mediators in the liver. To date, the generation of insuline-like growth factor-1 (Pinzani et al., 1990), TGFβ1 (Friedman, 1993; Pinzani, 1995; Nakatsukasa et al., 1990a,b; Friedman et al., 1992), TGFα (Bachem et al., 1992), interleukin 6 (Tiggelman et al., 1995), manocyte colony stimulating factor (Pinzani et al., 1992b), monocyte-chemotactic peptide-1 (Marra et al., 1993), hepatocyte growth factor (HGF) (Schirmacher et al., 1992), platelet activating factor (Pinzani et al., 1994a), prostaglandin F2α (Athare et al., 1994), endothelin 1 (Pinzani et al., 1994b) and nitric oxide (Rockey and Chung, 1995; Helyer et al., 1994) has been reported in the stellate cells. All these mediators are involved in the regeneration of parenchymal cells, promotion of fibrogenesis of the liver parenchyma, and the regulation of sinusoidal microcirculation. ET-1 is a

most potent vasoconstrictor peptide produced mainly by endothelial cells (Yanagisawa et al., 1988). As ET-1 infusion into portal vein in perfused rat liver dramatically increases the perfusion pressure, porto-sinusoidal network seems to respond to ET-1 (Gondo et al., 1993; Tran-Thi et al., 1993). Recent investigations have revealed that stellate cells not only have receptors for ET-1 (Gondo et al., 1993), but also synthesize the peptide (Pinzani et al., 1994a,b). ET-1 mRNA is also detected in human liver tissues with fibrosis and cirrhosis, indicating a crucial role of this peptide in liver fibrosis (Pinzani et al., 1994a,b). Although the function of ET-1 as a possible mitogen for the stellate cells is confused (Mallat et al., 1995b), it can be speculated that the peptide triggers the development of microcirculatory disturbance and tissue shrinkage by inducing the contraction of the stellate cells (Rockey and Weisinger, 1996). On the other hand, the stellate cells produce nitric oxide (NO) (Helyer et al., 1994; Rockey and Chung, 1995). NO is known to be a gaseous metabolite of L-arginine by NO synthase (NOS) (Knowles and Moncada, 1994). There are three types of NOS, constitutive NOS (cNOS), inducible NOS (iNOS) and neural NOS (nNOS). Because the culture medium of unstimulated stellate cells contained a negligible amount of nitrate and nitrite, stable degradation products of NO, the cells seem not to have constitutive NO synthase (cNOS). However, when stimulated with interferon γ, TNFα, and lipopolysaccharide, the cells generated NO with a concomitant expression of iNOS mRNA and the resulting iNOS protein (Rockey and Chung, 1995). The role of NO derived from iNOS-expressing stellate cells is not known. Because NO is a potential relaxing factor for vascular smooth muscle cells and cultured stellate cells, NO may contribute to the maintenance of microcirculation in inflammatory sites.

Surpression of stellate cell activation. Therapeutic strategy for preventing liver fibrosis

As mentioned above, stellate cell activation accompanying proliferation, increase in the production of extracellular matrix materials, decrease in the synthesis of matrix metabolizing enzymes, generation of fibrotic mediators, and potentiation of the contractility, directly leads to the progress of liver fibrogenesis. Hence, it is reasonable to establish a specific, effective and less harmful strategy that depresses stellate cell activation. Hepatocyte protective effects of interferons and dexamethasone are clinically effective in the inhibition of liver fibrogenesis caused by virus infection, autoimmune or toxic drugs. Although drug targeting to the stellate cells or myofibroblasts in the liver would be feasible, this is presently not utilizable because cell type specific entry mechanism is not understood. Modulation of the expression of receptors for PDGF or TGFβ, application of antibodies against TGFβ, are of potential interest. Natural inhibitors like decorin may eventually prove useful in renal fibrosis caused by overproduction of TGFβ (Border et al., 1992). Rockey and Mallat reported that interferon γ is very potent in inhibiting stellate cell proliferation and matrix production (Rockey et al., 1992a,b; Rockey and Chung, 1994; Mallat et al., 1995a,b) although interferon γ cannot be utilized clinically because of its strong side effect. Application of vitamin A and the related compounds are reasonable because decrease of retinol content in the liver is related to liver injury and stellate cell activation (Senoo and Wake, 1985; Seifert et al., 1994). However, administration of retinoic acid does not seem to be practical because retinoic acid, but not retinol and retinyl palmitate, promotes TGFβ production by the stellate cells, thereby enhancing liver fibrogenesis in animal models (Okuno, personal communication). Antioxidants such as glutathione, N-acetylcystein and vitamin E are therapeutic candidates as oxidative stress triggers the activation of the stellate cells through activating transcription factors such as nuclear factor κB and c-Myb (Parola et al., 1992a,b; Lee et al., 1995). Agents that increase cellular level of cAMP, such as methyl-xanthines and pentoxifylline, are also candidates, although selective effect to the stellate cells could not be anticipated. Further studies should be done to establish a therapeutic strategy for preventing human liver fibrosis.

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