REVIEW

# Hepatic fibrogenesis

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**Abstract**

**The main pathogenic mechanism of the progress of chronic hepatitis into cirrhosis is represented by fibrogenesis. Stopping this progress is the subject of much research and would**
**The hepatic stellate cells play a central role based on their ability to undergo activation following liver injury from any cause is important. On the basis of improved knowledge of the pathophysiology of fibrogenesis, the development of new therapeutic approaches will become possible.** ***(Fig. 3, Ref. 50.)***
**Key word: stellate cells, hepatic fibrosis, cirrhosis, cytokines.**

## Epidemiology of chronic liver diseases

The actual incidence and prevalence of chronic liver diseases and liver cirrhosis in the current population does not give an accurate picture. The chronic liver disease can stay clinically inert for a long period of time for the majority of patients. The final stage of chronic liver disease, i.e. liver cirrhosis, is frequently detected late or even post mortem. The sensitivity of the diagnosis of liver cirrhosis ante mortem is approximately 60% (Dufour et al, 1993). In 1988, Italy had the same mortality rate from cirrhosis as is now present in Slovakia, e.g. approximately 3% of total mortality (Capocaccia et al, 1990). In the period 1991—1993, the first study in northern Italy was carried out which was oriented towards discovering the prevalence of chronic liver diseases in the greater population. This "Dionysos study" disclosed a much higher prevalence of chronic liver diseases for the current population than had been assumed previously. Chronic liver disease was confirmed in 17.5%, liver cirrhosis in 1.1% and hepatocellular carcinoma in 0.07% of examined persons (Bellentani et al, 1994).

Liver cirrhosis represents the ninth most frequent cause of death in the USA, and represented 1.2% of deaths in the year 1988 (Dufour et al, 1993). Cirrhosis is the third most frequent cause of death in men 35—45 years of age in the USA. It is the leading non-tumor cause of death among the diseases of the digestive tract. In the last decade, a reduction of mortality from cirrhosis was recorded in the USA, which was simply explained by the availability of liver transplantation. Mortality in USA is higher than in Canada and in England, but it represents approximately only one third of the mortality in France. France has the highest mortality from cirrhosis (35 per 100 000 citizens), followed by Austria, Italy and Germany. The statistics monitoring the mortality of liver cirrhosis in Slovakia for the last 40 years (1953—1994) indicate a 10-fold growth of mortality for men and a 4-fold growth of mortality for women (Szántová et al, 1997). Mortality from chronic liver diseases in Slovakia contributed to total mortality in the year 1988 by 2—2.4%, and in the year 1990, it increased to 3.1% (Szántová et al, 1997).

The importance of cirrhosis is not only in the fact that many die, but also in the fact that the mortality from cirrhosis is increasing and the majority of the patients are adults still in productive age groups. The identification of patients with risk factors can increase the number of established diagnoses of cirrhosis. The main risk factors for cirrhosis include: chronic infection with virus hepatitis B or C, chronic excessive consumption of alcohol, increased accumulation of iron in primary hemochromatosis and increased accumulation of copper from Wilson's disease.

The results of different studies indicate that alcohol represents the cause of perhaps 50% of all cirrhosis worldwide. Naturally, there are large geographic differences. In states with a traditional high alcohol consumption, alcoholic cirrhosis represents

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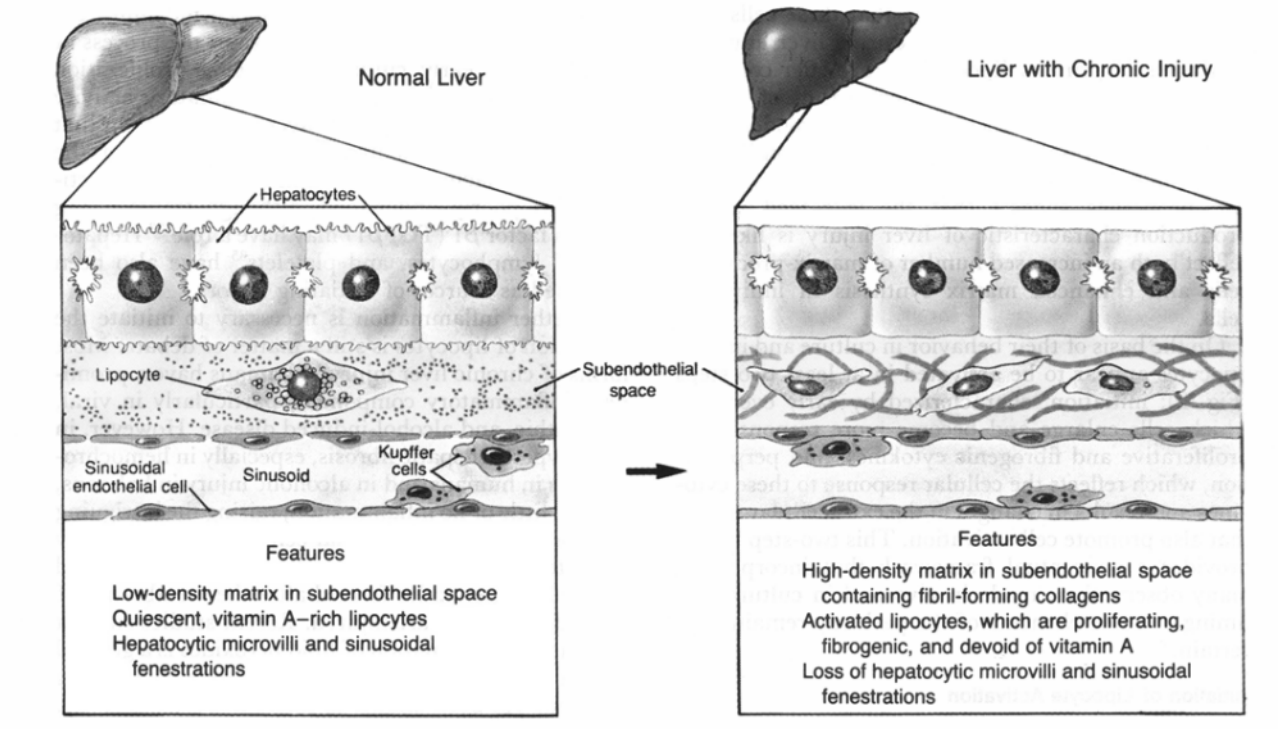


**Fig. 1. Capillarization of the sinusoid (adapted from Bissell, 1990).**

as much as 90% of all cirrhosis (France, some American states). On the contrary, in Islamic countries it represents only approximately 11%. In Europe, the present figure is from 30% to 40%. Since the end of the World War II, the total alcohol consumption has increased and increasing rates of alcoholism have become a worldwide problem. During the last 40 years, alcohol consumption increased in one third of European countries 4-fold, including Slovakia.

Slovakia is one of the countries with the highest alcohol consumption. The substantial majority of consumption of hard alcohol and its steep growth after the year 1965, with an equally steep growth of the consumption of beer during this period, includes Slovakia among the so-called combined hard alcohol — beer countries. In the analysis of the type of alcohol consumed since the year 1994, the highest is the consumption of hard alcohol (5,06 l of pure alcohol/capita/year), compared to beer consumption (2,95 l of pure alcohol/capita/year) and wine (Szántová et al, 1995).

**Definition of fibrogenesis and fibrosis**

For the purpose of evaluating the development and progress of chronic liver diseases, we determined the activity (grading) and stage of chronic liver diseases. The activity (grading) reflects the degree of necrotic-inflammation processes. The stage (staging) reflects the stage of the proliferation processes, this means the scope of fibrotic injury of the liver parenchyma. The majority of chronic liver diseases of different etiology (toxic, virus, parasitary, autoimmune) are combined with the development of liver fibrosis.

Liver connective tissue consists of two basic components: *specialized cells and intercellular mass (the so-called extracellular matrix).* The cellular component consists of fibroblasts. Components of the extracellular matrix can be divided into fibrous structures and shapeless interfibrous mass. The extracellular liver matrix (ECM) consists of three different groups of macro-molecules:

— collagens (type I, III, IV and VI),

— non-collagen glycoproteins (fibronectin, laminin, entactin/nidogen, tenascin, undulin),

— proteoglycans (perlecan, syndecan, decordin). Glycosaminglycans represent a part of proteoglycans (hyaluronic acid, chondroitinsulphates, dermatansulphate) (Shuppan et al, 1992).

Hepatic fibrosis is a reversible, increased accumulation of the components of the extracellular matrix in the liver parenchyma as a reaction to chronic damage. These fibrotic changes during chronic liver diseases are caused by a loss of the homeostatic mechanisms, which under physiological circumstances control the creation and deposition of matrix (fibrogenesis) and also the degradation and removal of matrix (fibrolysis). In chronic liver damage, the excessive creation of matrix is continuously stimulated, but this excessive fibrotic reaction of the liver is not necessary. Because of the high regenerative activity of hepatocytes, fast and complex replacement of damaged (necrotic) parenchyma and the complete recovery of the damaged liver is possible.

Disse's subendothelial space is determined, on the one hand, from the nonluminal surface of endothelial cells of sinusoids, and on the other hand, its border is represented by the membrane

of hepatocytes with microvilli. With damage to the liver, changes are soon created in the liver sinusoids. This transformation of liver sinusoids is called *"capillarization of sinusoids"*. During this process, the structure of sinusoids will approximate the structure of capillaries located elsewhere in the body. This process includes a reduction of the number and the size of pores in the endothelial cells of the sinusoids, a development of the right basal membrane and accumulation of components of the extracellular matrix in the subendothelial Disse's space, so-called perisinusoidal fibrosis (Bissell, 1990) (Fig. 1). Below the endothelial cells, the matrix proteins will increase, including the components of the basal membrane, type IV collagen, fibronectin and laminin. These components of the extracellular matrix are organized into a structure similar to the basal membrane. Collagen of types I, III, V and VI, tenascin and undulin also accumulate in Disse's space.

Increased accumulation of the fibrotic tissue in Disse's space, where the fibrogenesis is initiated at the beginning, is not beneficial. It causes a deterioration of the supply of oxygen and nutritional substances to the hepatocytes, which will lead to a necrosis of hepatocytes with consequent further intensification of the process of fibrogenesis. Capillarization of the sinusoids limits the normal exchange of substances between plasma and hepatocytes and therefore represents the main cause for the deterioration of the hepatic function in the developed liver fibrosis, or cirrhosis. Simultaneously, through specific membrane receptors — integrines, interactions between hepatocytes and pathologic components of ECM occur, which can lead to a modification of the genetic program of the hepatocytes, with a deterioration of their function (Shuppan and Gressner, 1999). Deposition of collagen in Disse's space leads to a narrowing of the lumen of the liver sinusoids and an increase of the intrahepatic vascular resistance, which contributes to the development of portal hypertension. The final irreversible stage of fibrosis is liver cirrhosis, which represents a diffusion type of septal fibrosis.

Fibrogenesis represents a multifactorial process of creation and deposition of matrix, with the result being the creation of fibrosis. Fibrogenesis represents a complex pathobiochemical reaction with the participation of parenchyma and non-parenchyma liver cells, inflammatory cells, the underlying extracellular matrix and paracrine/autocrine/juxtacrine signal transfer mechanisms. Fibrogenesis is influenced by many factors, which influence its start and duration.

Liver fibrogenesis culminates in the development of fibrosis characterized by:

a) 3 to 6-fold increase of the majority of molecules of the extracellular matrix (ECM) of the collagenous as well as non-collagenous nature,

b) disproportionate elevation of some individual components of ECM, including several types of collagens, proteoglycans and structural glycoproteins,

c) small changes in the microcomposition of specific ECM molecules, for instance, the degree of hydroxylation of collagen alpha chains, the degree of sulfation of Glycosaminglycans ,

d) redistribution of ECM in the injured liver leading to subendothelial deposition of connective tissue in Disse's space (perisinusoidal fibrosis).

Because the early stages of the cascade of fibrogenesis are initiated in the subendothelial Disse' s space, efforts to identify cellular sources in which components of the extracellular matrix are created were aimed at these sources. It was discovered that stellate cells (denoted also as Fat storing cells — FSC, lipocytes, perisinusoidal lipocytes, vitamin A-storing cells, ITO cells, Stellate cells, portal fibroblasts) are the main cellular source responsible for fibrogenesis (Friedman, 1993; Li and Friedman, 1999) and partially also for fibrolysis (Arthur et al, 1989). Stellate cells are located perisinusoidally in Disse's spaces, have a stellate shape and in physiologic circumstances represent the place of storage for vitamin A and fat. Any damage to the liver can cause an activation of the stellate cells (Hrušovský and Gočár, 1999).

Liver fibrosis is created not only as a consequence of the changes in the secretion of matrix, but also from changes in its degradation, which means a loss of the dynamic functional balance between fibrogenesis and fibrolysis. The matrix metalloproteinases (MMP) and their specific inhibitors (TIMP — tissue inhibitor of metalloproteinases) as well as enzymes which activate some latent metalloproteinases (stromelysin, plasmin, membrane type of metalloproteinase) (Arthur, 1995) participate in the degradation of the hepatic ECM.

**Hepatic fibrosis, cirrhosis, clinical consequences**

When the damage to the liver does not cause a collapse of necrotic parts or destroy the liver architecture, a clinically unimportant cure with a defect occurs. The reduced mass of cells represents a stimuli for the regeneration of hepatocytes, but also for an active synthesis and degradation of collagen while these processes are in balance. Prograding damage leads to the prograding fibrosis, which accompanies the chronic active hepatitis, relapsing alcohol hepatitis, the accumulation of iron or copper and so on.

With a continuation of pathological stimuli a diffuse destruction of hepatocytes, nodular regeneration of liver parenchyma cells and diffusion propagation of connecting tissue occurs that leads to a disorganization of the lobular and vascular architecture of the liver and *cirrhosis*. Three parallel processes participate: 1) hepatocellular necrosis, 2) inflammation changes outgoing from the mezenchyma leading to the proliferation of the connective tissue, 3) nodular conversion of parenchyma based on preserved parts of the liver tissue (Brodanová and Kordač, 1993). This process is diffuse, usually irreversible and, as a rule, permanently progressive. Strips of connective tissue can be soft but also broad — septal fibrosis, when they replace complete areas of the liver tissues and then contain very different structures (artery, veins, bile ducts and different cells). Direct communication between the portal system and the system of liver veins occurs. The portal blood bypasses the areas of the previously functional hepatocytes. Entire groups of hepatocytes suffer from reduced oxygen and succumb to necrosis, which maintains the vicious cycle. The liver becomes an obstacle for blood flow from

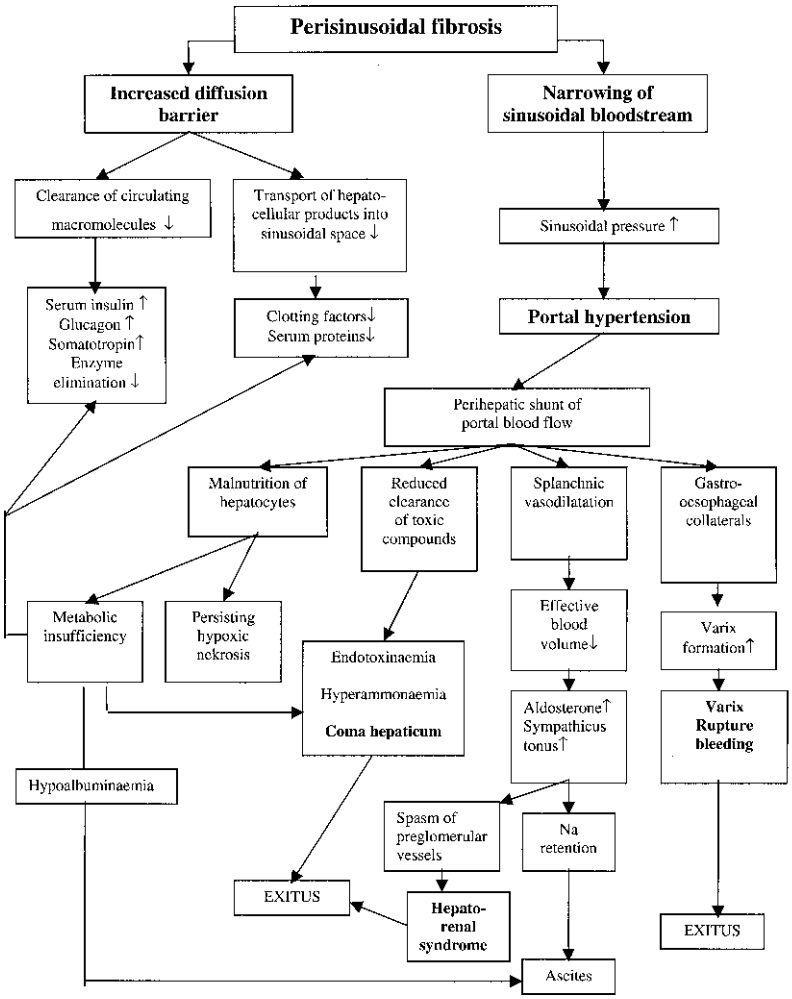


**Fig. 2. Major functional consequences of extracellular matrix deposition in the perisinusoidal space of Disse (adapted from Gressner and Schuppan, 1999).**

the portal vein into the lower vena cava. The following factors contribute to this: perisinusoidal fibrosis, high contractility of myofibroblastes in sinusoids (Marečková a Horký, 2001) oppression of the vena centralis by nodular regenerats of hepatocytes and fibrotic changes, transmission of a 10-fold higher arterial pressure from a.hepatica by anastomoses in the fibrous strips directly into the v. centralis. In the cirrhotic liver the share of blood supply from a. hepatica is increased and the speed of the blood flow in v. portae is substantially reduced, currently below 10 cm/s, and in extreme situations the blood flow in v. portae can be reversed. Also the portal hypertension develops. Collateral circulation created by porto-caval anastomoses (Szántová et al, 1999) open.

Perisinusoidal fibrosis represents a deposition of collagen and of other components of the extracellular matrix into the subendothelial Disse's space, which leads to a narrowing of the lumen of the liver sinusoids with increased intrahepatic vascular resistance. Therefore it is considered to be the principal cause of portal hypertension. The perisinusoidal fibrosis heavily limits the diffusion of nutritional substances between hepatocytes and the blood in the sinusoids and reduces the removal of toxins of the digestive tract with clinical consequences (Fig. 2).

For an assessment of the degree of significance of the fibrotic process in the liver besides the histological changes, serum markers of fibrogenesis can also be used, for instance hyaluronic acid, N-terminal peptide of procollagen III, 7S domain of procollagen type IV (Szántová and Kupčová, 1999; Schuppan et al, 1999; Turecký 1985), which do not require an invasive examination. For the assessment of the function of the liver parenchyma, a decisive role is played by the failure of the proteosynthetic (Kupčová et al, 1985, 1993, 1994, 1996) and biotransformational functions of the liver (Kupčová et al, 1992, 1994, 1996, 1999; Kupčová, 1999).

## Changes in the extracellular matrix during the development of liver fibrosis and cirrhosis

In fibrotic and cirrhotic livers, the total content of collagen is increased. The early change is the elevation of collagen III, which is followed by a predominance of collagen I. The col-

lagen of type I represents approximately 60—70% of the entire collagen in the cirrhotic liver. Collagen IV, V and VI are also located in the fibrosis septum. In the fibrotic septum and the limiting lamella, fibronectin, tenascin and vitronectin are also multiplied. The fibrotic septum also contains laminin, undulin, entactin and von Willebrand factor.

For liver cirrhosis, the growth of glycosaminglycans has also been proven: hyaluronic acid, dermatansulphates and chondroitinsulphates, which prevail over heparansulphate in comparison to a normal liver. Glycosaminglycans are components of complexes of proteoglycans, which represent the interfibrous mass of the connective tissue.

During the development of fibrosis, the capacity of the degradation of the matrix is not eliminated, but is reduced during progressive disease.

**Mediators of liver fibrogenesis and the task of intercellular interactions**

During fibrogenesis there is an interaction of different liver cells not only in the physical cell-to-cell contact but also in chemical interactions. Cells produce peptides as well as non-peptide chemical mediators, which influence not only the neighboring cells, but also distant cells.

All cell types which are located or accumulate in the liver in diseased regions release several peptide mediators important for liver fibrogenesis — the so-called growth factors (Gressner, 1996). Many of these factors play a pleiothropic function (Nathan, 1991). The transforming growth factors alpha and beta (TGF-alpha, TGF-beta), the tumor necrosis factor alpha (TNF) and platelet derived growth factor (PDGF) are considered the main "fibrogenous" mediators released from these cells. Their influence activates stellate cells. TGF-beta is considered to be the most important cytokine in the fibrogenesis of the liver and other organs (Annoni et al, 1998; Castilla et al, 1991). TGF-beta not only stimulates the synthesis of ECM, but also inhibits the degradation of the matrix a) by a reduction of the synthesis of matrix metalloproteinases and stromalysin and b) by an increase of inhibitors of proteases, for instance: as inhibitors of the activator of plasminogene and inhibitors of metalloproteinases and $alpha_2$-macroglobulin. Other effects of TGF-beta, for example the stimulation of the chemotactical migration and motility of fibroblasts and monocytes, the increase of expression of other growth factors, receptors for growth factors and receptors for components of ECM, are also important in the process of fibrogenesis (Border, 1994). TGF-beta also participates in the suppression of the reproduction of hepatocytes, by which it reduces the regeneration processes and supports the reparation processes.

There are also nonpeptide mediators of fibrogenesis, which include: free oxygen radicals (VKR), eikozanoids, acetaldehyde and others (Gressner, 1996). All these peptide and non-peptide mediators create a highly complicated network with positive and negative feedback regulation.

**The central pathobiochemical event in fibrogenesis is the activation of stellate cells in the place of necro-inflammation, with their consequential change into myofibroblasts**

Myofibroblasts produce molecules of the extracellular matrix, which leads to a pathological increase in the connective tissue in liver with clinical consequences. Before fibrogenesis starts, the stellate cells must be activated (Bissell, 1992).

**Activation of stellate cells**

Stellate cells in a normal liver can be recognized by presence of intracellular vesicles containing vitamin A. These inactive mesenchymal cells are the primary depot of retinoids in the liver. For the cytoskeletonal phenotype, their orientation around the perimeter of the sinusoids and their relation to endothelium, these cells are analogous to perivascular cells (pericytes) of other organs, including mesangial cells in kidneys. Stellate cells of many animal species exprimate desmin, characteristic for muscle cells. Morphological studies of animals and human beings demonstrate that by progressive liver damage, these vitamin A rich cells are transformed into cells similar to fibroblasts, which have an active secretional apparatus for proteins and only a negligible amount of vitamin A.

Transformed stellate cells — *myofibroblasts* — produce molecules of the extracellular matrix (at least five types of collagen, heparansulphate, dermatan, chondroitinsulphate proteoglycans, laminin, cellular fibronectin, tenascin, decordin, and biglycan) as well as a broad spectrum of growth factors and proinflammatory cytokines (Brenner et al, 2000).

The activation of stellate cells and their consequent transformation happens at *sites of inflammation and necrosis of hepatic cells.* The start of the process of activation of the stellate cell in situ is the result of interactions between non-activated stellate cells on the one hand, and activated Kupffer cells, damaged parenchymal liver cells, thrombocytes, endothelial sinusoidal cells and changed components of the extracellular matrix on the other hand.

*The activation of stellate cells includes:*

a) stimulation of their cellular proliferation, and phenotypic transformation of the stellate cell into a myofibroblast, which produces components of ECM,

b) increased expression of nearly all gene coding proteins of the extracellular matrix,

c) development of contraction of the stellate cell, for example, after stimulation by an endothelin (Gressner, 1994; Rockey, 2000). Stellate cells can thus limit the blood flow in sinusoids and contribute to portal hypertension. The contraction of stellate cells leads to a contraction of the entire organ (the so-called end-stage wer disease).

Gressner (1994) assumes a *three-step cascade* activation of stellate cells (pre-inflammatory, inflammatory, and post-inflammatory steps). This includes stepwise interactions of the non-activated stellate cell with hepatocytes, Kupffer cells, endothelial sinusoidal cells and finally the myofibroblasts themselves (transformed stellate cells) (Fig. 3).

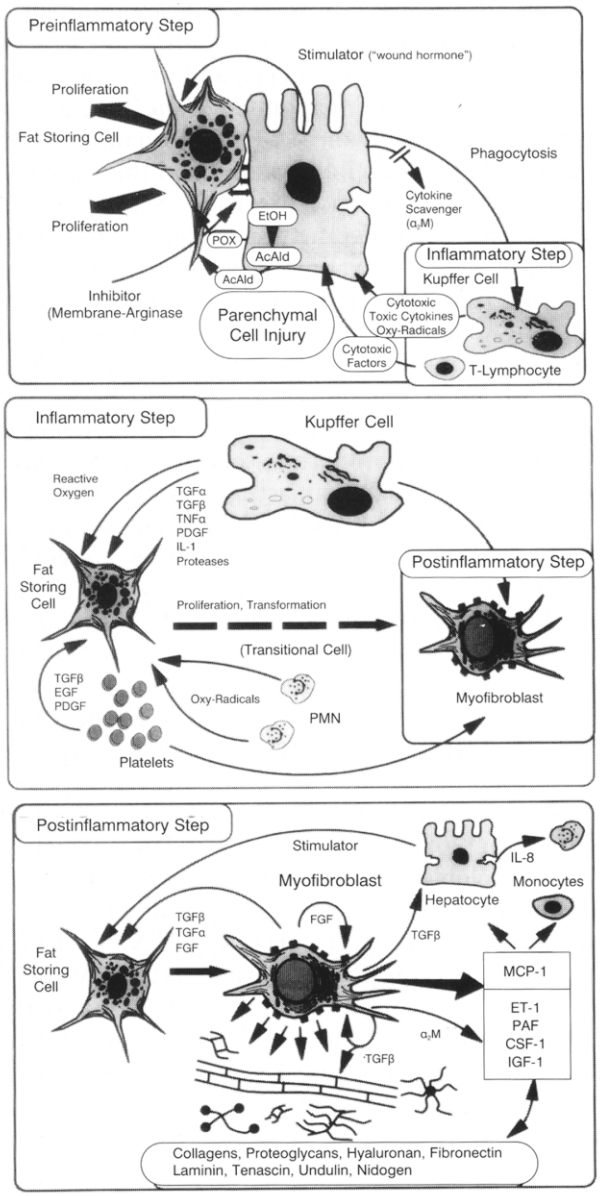


**Fig. 3. The three step cascade model of fat storing cell (FSC) alias stellate cell activation (adapted from Gressner, 1996). The preinflammatory step is initiated by damage of parenchymal cells (PC), which releases stimulators of FSC proliferation and decreases membrane-associated inhibitors like membrane arginase. In the inflammatory step, mitogens from activated Kupffer cells, and disintegrated platelets are potent stimulators of FSC proliferation and transformation into myofibroblasts. Activated Kupffer cells can in turn, via the release of proteases, toxic cytokines (TNF-alpha) and oxygen radicals; induce membrane damage in PC. In the postinflammtory step, the fully transformed FSC (myofibroblasts) release various cytokines and growth factors, which stimulate in a paracrine way the non-transformed FSC and in an autocrine lopp the myofibroblast itself.**

Abbreviations: alpha2-M — alpha2-macroglobulin, CSF-1 — colony stimulating factor-1, EGF — epidermal growth factor, ET-1 — endothelin-1, FGF — fibroblast growth factor, IGF-1 — insulin-like growth factor, IL-1 — interleukin-1, MCP-1 — monocyte chemotactic peptide, PAF — platelet activating factor, PDGF — platelet derived growth factor, PMN — polymorphonuclear leukocytes, TGF-alpha — transforming growth factor TGF-transforming growth factor-beta.

*The pre-inflammatory step* is initiated by damage to the hepatocyte. In this step, damage of the membrane of the hepatocyte, paracrine operating mitogenes are released (for instance lipid peroxides), which initiate the proliferation of stellate cells. Also the natural membrane-associated inhibitors of the activation of stellate cells are reduced, which will lead to an increased sensitivity of stellate cells to influence a broad spectrum of cytokines and growth factors which are released during the inflammatory stage. The first demonstration of the activation of stellate cells is the creation of receptors for proliferative and fibrogennous cytokines on their surface.

During the following *inflammatory step,* in the areas of necro-inflammation, different cytokines are released from the activated Kupffer cells (TGF-beta, TGF-alpha), disintegrated thrombocytes (TGF-beta, EGF-like factor, PDGF) and neutrophils. PDGF represents for stellate cells a strong proliferative cytokine. TGF-beta, a prototype of the profibrogen cytokine, strongly supports the transformation of stellate cells into myofibroblasts (Border, 1992).

During the *post-inflammatory step,* the fully transformed stellate cells (myofibroblasts) release various cytokines and growth factors, which stimulate in a paracrine way non-transformed stellate cells and in an autocrine loop the myofibroblast itself. Myofibroblasts stimulate autocrine themselves to their **own** activity, using TGF-alpha, TGF-beta and FGF-growth factor of fibroblasts, which they produce. By this mechanism, the post-inflammatory stage potentially contributes to the maintenance of the ongoing process of fibrogenesis, despite the termination of the operation of the triggering mechanisms (Bachem et al, 1992).

Despite persuasive proofs of the presence of necro-inflammation for the creation of fibrosis, it is not completely clear if under any circumstances fibrogenesis must follow only after necro-inflammation. Non-peptide low-molecular chemical compounds like acetaldehyde, free oxygen radicals and lactate belong to the non-inflammatory mediators of fibrogenesis. These are involved in the process of the activation of stellate cells.

**The role of Kupffer cells in the activation of stellate cells**

The Kupffer cells represent approximately 80% of all fixed tissue macrophages in the body and therefore play a key role in the functioning of the mononuclear-phagocyte system. Besides the processes of phagocytosis, macrophages play an important role also for specific immunity — for the presentation of antigen, for the production of numerous immunoregulatory substances (cytokines), and for reparation processes. In many processes of tissue damage and wound healing in an organism, the tissue macrophages actively participate. They produce mediators, which are able to activate the mesenchymal cells (fibroblasts) and thus support fibrogenesis. Macrophages in the liver — the Kupffer cells — behave similarly. It has been detected that during liver damage, Kupffer cells are activated and proliferate. Activated Kupffer cells release many cytokines, growth factors, which have in vitro a stimulating effect on proliferation and the activation of stellate cells (TGF-alpha, TGF-beta, TNF) and fibrogenesis (TGF-beta).

The ability of Kupffer cells to activate stellate cells has been proven also by studies in vitro (Friedman and Arthur, 1989). How are the Kupffer cells themselves activated during liver damage? The answer is not clear. An attractive hypothesis is that the damaged hepatocytes release signals, which activate the Kupffer cells.

Kupffer cells can be activated in several ways, for instance by the endotoxin, which they contact at the transmission of blood from the insides through the liver sinusoids. For alcohol hepatitis an increased endotoxaemia has been proven and for the histological examination of liver, we find a massive infiltration of neutrophils. According to Thurman et al (1997), with the consumption of alcohol, the permeability of the wall of the digestive tract will increase for the endotoxin, which is transmitted by the blood circulation to Kupffer cells in the liver and activates them. As we have already mentioned above, the activated Kupffer cells release mediators (TGF-alpha, TGF-beta, TNF, PDGF, IL-1, free oxygen radicals), which activate stellate cells and consequent fibrogenesis. The Kupffer cells also release inflammatory mediators (IL-1, IL-8, IL-6, TNF), which are responsible for the accumulation of neutrophils in liver. Accumulated neutrophils in the liver release proteolytic enzymes from their granules (elastases, protease, collagenases) and also free oxygen radicals which cause tissue damage and the destruction of hepatocytes. By blocking the above mentioned cascade of events, for instance by the sterilization of the intestine using antibiotics (a reduction of endotoxaemia) or by a chemical destruction of Kupffer cells, we can prevent alcohol damage to rat livers after high doses of alcohol (Adachi et al, 1994, 1995). For patients with alcohol hepatitis, the immune-modulation treatment has the goal of limiting the extent of the immunologic stimulation with the purpose to minimize the scope of damage.

**The role of hepatocytes in the activation of stellate cells**

Stellate cells and hepatocytes (HC) are in vivo in close contact, which creates the precondition that the soluble mediators as well as the direct membrane contact could be of importance in the regulation of activation of the stellate cell (Wake, 1993). New data demonstrates that HC produce a broad spectrum of important profibrogenous and promitogennous cytokines. Mitogenes, derived from HC, interact additionally with factors produced by the activated Kupffer cells. It has been found that the hepatocytes contain a cytosolic protein, which is released during their damage. This cytosolic protein, the "wound hormone" is able to stimulate the proliferation of stellate cells.

The components of the extracellular matrix which are located pericellularly in the surrounding stellate cells and parenchymal liver cells (for instance fibronectin, laminin, collagen IV, proteoheparansulphate ) mediate the intercellular communication between both types of cells. Above all, the proteoglycansulphate, associated with the membrane of the liver cell, is an important physiologic regulator of the mitotic activity of neighboring stellate cells in a normal liver. Damaged HC, which have less pericellular heparansulphate, are not capable of contact inhibition of stellate cells. The stellate cells will thereby become sensitive to the growth factors produced by present and infiltrating inflammation cells.

Another potentially important way of cell interaction of hepatocyte is the metabolization of some profibrogenous xenobiotics into fibrogenous substances directly in the hepatocyte. These fibrogenous substances activate the transformation of stellate cells into myofibroblasts. This is, for instance, the metabolism of ethanol which itself does not perform fibrogenously. The product of its oxidation, acetaldehyde (and lactate), created in HC, effectively stimulates the synthesis of certain types of collagen, fibronectin as well as the proliferation of transformed stellate cells (Loiterer et al, 1992). Beside this, by ethanol or by the accumulation of iron induced generation of free oxygen radicals with the consequential lipid peroxidation also stimulates fibrogenesis.

**Participation of sinusoidal endothelial cells and thrombocytes in the process of fibrogenesis**

The endothelial cells of sinusoids differ from other endothelial cells by the presence of pores. Because of these pores and the absence of the basal membrane, substances from the lumen of sinusoids penetrate into the perisinusoidal space and vice versa. With liver damage, pores in the endothelial cells are lost and the basal membrane is created as a consequence of the increased production of collagen and other components of the extracellular matrix. This transformation of sinusoids of the liver is called the "capillarization of sinusoids" and its consequence is represented even by the necrosis of the hepatocytes. During this process, the endothelial cells of sinusoids increase their volume and also the number of intracellular vesicles. It has been found that the endothelial cells of sinusoids start to produce IL-1, PDGF and fibronectin, which participate in the activation of the stellate cells (Rieder et al, 1992). Fibronectin, which is deposited in the subendothelial space, also has an unfavorable effect on the functioning of the hepatocyte.

TGF-beta is bound to the endothelial cells of sinusoids, and increases their synthesis and release of the inhibitor of the activator of plasminogene, by which the production of plasmin will be reduced. Plasmin represents the activator of metalloproteinases, which participate in the degradation of the matrix. This effect of TGF-beta on endothelial cells demonstrates that the endothelial cells are participating in fibrogenesis by the reduction of the degradation of the matrix (Rieder et al, 1993).

An increased trapping of thrombocytes together with neutrophils and macrophages is shown in necrotic areas. During the degranulation of thrombocytes, TGF-beta and pro-fibrogennous mediators; PDGF and EGP from alpha-granules of thrombocytes will be released. This indicates that the disintegrated thrombocytes can substantially participate in liver fibrogenesis. Thrombocytes, activated by these mediators, stimulate growth, the transformation of stellate cells and the synthesis of ECM in stellate cells.

**The role of the extracellular matrix in the process of fibrogenesis**

It has been indicated that the extracellular matrix plays an important role in intercellular communication, participates in the regulation of fibrogenesis and is important for the function of hepatocytes. All cells in the surroundings of sinusoids react to changes in the composition of ECM, which was created as a consequence of liver damage. There are cellular-matrix interactions. For instance, in the damaged endothelial cells, fibronectin will be created, which has the ability to activate the stellate cells and has an unfavorable effect on the functioning of the hepatocyte.

Studies on cellular cultures have demonstrated that in order to maintain the proper functioning of the hepatocyte, contact is necessary with the components of the "healthy" matrix, which is similar to the extracellular matrix of the normal liver. At the site of liver damage there is an exchange of the normal subendothelial matrix for the "ill" matrix, which contains mainly the interstitial collagen of the types I and III. This leads to interactions between hepatocytes and a pathologic matrix by integrines, which can modify the genetic programs of the cells. The result can be a deterioration of metabolic and synthetic functions of hepatocytes (for instance reduced creation of albumin, coagulant factors, metabolic dysregulation) as well as defenestration of endothelial cells. Integrines represent a large group of membrane receptors, which mediate these unfavorable matrix-cellular interactions (Schuppan and Gressner, 1999).

The effect of mediators of fibrogenesis can be influenced by cytokine trappers. Cytokine trappers also include components of the extracellular matrix such as fibronectin, collagens and nuclear proteins of proteoglycans.

Nuclear proteins of proteoglycans, which belong to the so-called family of small proteoglycans (biglycan, decordin, fibromodulin), play a role in molecular interactions and enable the bonding of proteoglycans with growth factors (cytokines), by which the bio-availability of cytokines is influenced. For instance, with the binding of the nuclear proteins biglycan and decordin with TGF-beta, the bio-availability of this important cytokine in the extracellular space will be reduced and proteoglycans inhibit the activation of stellate cells (Yamaguchi et al, 1990). A negative feedback regulation is assumed during which proteoglycans (decordin, biglycan, betaglycan) act as trappers of TGF-beta. As the TGF-beta stimulates the synthesis of proteoglycans in stellate cells, proteoglycans then participate in their own autoregulation. Injection administration of recombinant decordin was able to suppress the fibrogenesis in a rat model of glomerular sclerosis (Border et al, 1992).

In the last decade, substantial progress has been achieved in the understanding of liver damage and of the pathophysiology of fibrosis and fibrogenesis. Fibrosis, understood in the past as an irreversible answer to chronic damage, can now be seen as a dynamic and potentially reversible process. The antifibrotic effects of some substances such as interferons (Guido et al, 1996; Sobesky et al, 1999; Guerret et al, 1999), essential phospholipides, silymarin (Boigk et al, 1997), glycyrrhizine — extracted from the licorice root (Watanabe et al, 2001; Rossum et al, 2001; Tandon et al, 2001), ursodeoxycholic acid, S-adenozylmethionine, retinoids, relaxin, pentoxyfyline (Preaux et al, 1997), antagonists of TGF-beta, antagonists of PDGF, soluble receptors for TNF (Czaja et al, 1995), antagonists of receptors for IL1, antagonists of ET-1 receptors, indolinones, influencing the PDGF receptors (Sun et al, 2000), different inhibitors of the synthesis of collagen, etc., are presently studied intensively in the experimental setting and verified in clinical studies. New knowledge from the pathophysiology of hepatic fibrosis creates the possibility for the development of new drugs for the treatment of fibrosis, which will likely be oriented towards the neutralization of the activated stellate cells (Friedman, 1999; Gressner and Schuppan, 1999).

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