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Editorial

The hepatic extracellular matrix

II. Ontogenesis, regeneration and cirrhosis

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Abstract. The unique nature of the hepatic extracellular matrix (ECM) is predicted by the special configuration of the space of Disse. Whereas other epithelial organs have two basement membranes (BM) and a substantial ECM interposed between endothelial and epithelial cells, the liver lobule has no BM and only an attenuated ECM, consisting mostly of fibronectin (FN), some collagen type I, and minor quantities of types III, IV, V, and VI. This configuration, together with the abundant fenestrations and gaps of the sinusoidal endothelial cells, seems ideally suited to facilitate the rapid bidirectional exchange of macromolecules normally taking place between plasma and hepatocytes. During organogenesis, the liver anlage is vascularized by continuous capillaries with BM, but by day 13.5 of development (in the rat) the vessels in the immediate proximity of hepatocytes become fenestrated, lacking specialized junctions and BM, suggesting that the hepatocytes produce signals capable of modulating the endothelial phenotype. In regeneration, hepatocyte proliferation precedes vascular proliferation resulting in the formation of hepatocyte clusters that, temporarily, lack sinusoids. Eventually, vascular proliferation follows and the normal hepatocyte-vascular relationships are restored. During this period laminin synthesis by Ito cells is prominent. As soon as hepatocytes become stable, secretion of the sinusoid phenotype-maintaining factors resumes and laminin synthesis and secretion terminates. The interplay between extracellular matrix and liver cells is essential for normal homeostasis and its modification results in derranged hepatic function.

Key words: Basement membrane – Cirrhosis – Collagen – Fibrosis – Matrix

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Introduction

Although the extracellular matrix (ECM) (Rojkind and Ponce-Noyola 1983; Martinez-Hernandez 1984; Cunningham 1987a, b; Schuppan 1990) is only a small component of the liver (Rojkind and Martinez-Palomo 1976; Seyer et al. 1977; Seyer 1980; Rojkind et al. 1983), it has a crucial role, providing a structural framework and maintaining the hepatocyte differentiated state. This role of the hepatic ECM has been dramatically demonstrated in cell culture, where the hepatocyte phenotype is dependent on the nature of the ECM upon which it is cultured (Rojkind et al. 1980; Reid and Jefferson 1984; Bissel et al. 1987; Schuetz et al. 1988). The ECM modulates repair in many tissues (Kurkinen et al. 1980; Martinez-Hernandez 1985a), including the liver (Abe et al. 1984; Martinez-Hernandez 1985a). Therefore, defining the ECM distribution in the normal liver, its phenotypic expression in various reparative states, and the cells responsible for its synthesis in vivo is an important step in understanding its role in homeostasis and repair.

In these editorials we review the contributions of light and electron microscopic immunohistochemistry to our understanding of physiopathology of the hepatic ECM. In the first part we reviewed those components relevant to the liver, and described the localization of individual ECM components in normal adult liver. In this part, we review, hepatogenesis, and the hepatic response to injury: regeneration and cirrhosis. Comparing and contrasting the data from these studies provides new insights into the role of the ECM in hepatic function, ontogenesis and repair.

Fetal and neonatal liver

A great deal of the information available on hepatogenesis has been obtained from animal studies (Elias 1955; Bankston and Pino 1980; Shiojiri 1984; Barioz et al. 1988; Eyken et al. 1988). We will concentrate on murine hepatogenesis; the model studied in our laboratory.

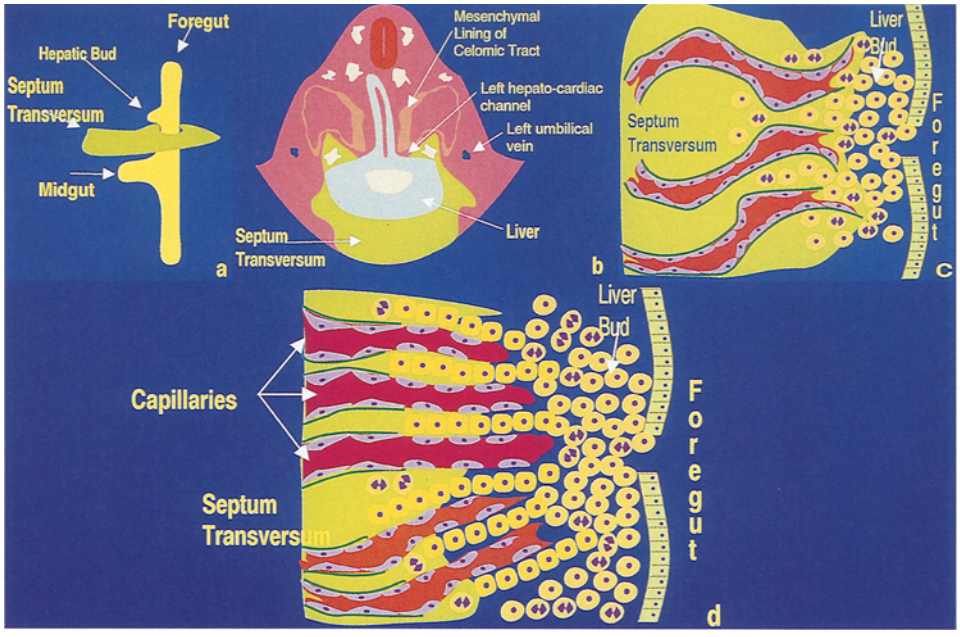

Fig. 1 a–e. Diagram of a proposed model of hepatic sinusoid formation; human at 25 days gestation or rat at 8.5–10.5 days of gestation. **a** A foregut derivative, the hepatic diverticulum consists of proliferating cell cords that penetrate the septum transversum. **b** With penetration into the septum, the vasculature present (derived from the umbilical and viteline vessels) is fragmented and resorbed. **c** The original vasculature is replaced by sinusoids derived from proliferating buds of endothelium (*arrows*) forms those septum transversum vessels bordering the hepatic diverticulum. **d, e** The continuous, non-fenestrated capillaries have been replaced by fenestrated sinusoids (day 13.5 in the rat). Sinusoidal endothelial cells, surround hepatocyte cords. At this date, hepatocytes and endothelial cells synthesize collagen types I, and IV, laminin, and fibronectin, and this components begin to deposit in the perisinusoidal space

In the rat, the hepatic diverticulum appears at approximately day 8.5 of gestation and acquires the adult architecture in approximately 3 weeks (1 week of neonatal life). All mammals begin hepatogenesis with the formation of a diverticulum from the caudal foregut, which extends into the mesodermal septum transversum of the splanchnic mesenchyme (Bloom 1926; Elias 1955; Shiojiri 1984). The liver anlage is surrounded by a capsule composed of a layer of coelomic mesothelial cells with their basement membrane (BM) and occasional cross-banded collagen fibres present beneath the BM, some coated by laminin. Within the boundaries of this capsule, clusters or cords of fetal hepatocytes are admixed with haematopoietic elements and surrounded by a layer of sinusoidal lining cells, derived from the umbilical and vitelline veins. Initially, in humans, hepatic sinusoids are reportedly lined by a continuous endothelium (Enzan et al. 1983) with well-formed intercellular junctions. Recent studies from our laboratories demonstrate a fenestrated, discontinuous sinusoidal phenotype by 13.5 days gestation. BM, however, have not been identified in the sinusoids at any developmental stage. The transformation of capillaries into sinusoids during hepatogenesis is depicted in Fig. 1.

Current evidence supports the concept that intrahepatic bile ducts differentiate from periportal hepatocytes (Bloom 1926; Elias 1955; Shiojiri 1984; Eyken et al. 1988). In rats, intrahepatic bile ducts become evident by 19.5 days of gestation surrounding portal vein radicles, first in the hilar region. Preceding the appearance of these ductules, hepatocytes surrounding the portal vein radicles begin to express a "bile duct-associated" keratin. These periportal hepatocytes eventually form the small periportal ductules (Eyken et al. 1988). These same cells are negative for keratin at earlier dates of gestation, while extrahepatic bile ducts are positive. These findings support a phenotypic switch from bile duct keratin-negative hepatocytes to bile duct keratin-positive hepatocytes, rather than penetration of the splanchnic mesenchyme by extrahepatic biliary cells to form the intrahepatic biliary system. As hepatogenesis progresses, the ductules become more prominent, stroma is deposited to form the portal triads, and central veins develop to form the characteristic hepatic lobule. By 1 to 2 weeks of neonatal life, the hepatic architecture is virtually that of the adult, containing only rare haematopoietic elements.

To facilitate the discussion of the hepatic ECM localization it is helpful to divide hepatogenesis into two stages, based on the development of the intrahepatic bile ducts. During stage 1, the liver consists of cords or clusters of hepatocytes surrounded by sinusoidal endothelial cells. Portal vein radicles are scattered throughout the hepatic parenchyma. This stage, in rats, continuous until day 18.5. During stage 2 (days 18.5 of gestation to 14 post-natal days), first the portal vein radicles become more prominent. These vessels are surrounded by a thin layer of ECM, and immediately adjacent, small ductules, the presumptive bile dutcs form. Finally, the features characteristic of the adult liver become evident

including the formation of portal triads, hepatocyte plates, and central veins in the early days of neonatal life.

"Stage 1" of hepatogenesis. The mesothelial cells of the hepatic capsule are separated from adjacent ECM by a continuous BM containing collagen type IV and laminin. The mesothelial cells also contain BM antigens within the cisternae of the endoplasmic reticulum, indicating their synthesis by these cells. Beneath the BM, collagen type I fibres and bundles are coated by fibronectin (FN). FN is more abundant than collagen type I in this region. The mesothelial cells contain neither collagen type I nor FN.

The space of Disse has only a tenuous ECM. FN, laminin, and collagen types I and IV are all present in the space of Disse. In contrast to the adult, laminin and FN are more prevalent in these early stages of hepatic development than collagen type IV and collagen type I is the least prevalent sinusoidal ECM component.

Electron immunohistochemistry of fetal chicken liver has demonstrated that both hepatocytes and sinusoidal lining cells are capable of the synthesis of ECM components (Barioz et al. 1988), with antigenic determinants identified in the synthetic organelles of those cells. Similarly, our preliminary studies have demonstrated that rat fetal hepatocytes and sinusoidal cells are also capable of some ECM synthesis.

"Stage 2" of hepatogenesis. The ECM phenotype of the hepatic capsule is unchanged from the prior stage. FN, laminin, and collagen types I and IV remain in the space of Disse, with laminin and FN being more prominent than collagen type IV. The portal vein radicles are surrounded by a BM containing laminin and collagen type IV. Collagen type I and FN are also present in the periportal regions. On day 19.5, the presumptive bile ducts identified in the periportal region contain an abundance of intracellular laminin and collagen type IV. These components remain demonstrable in ductular cytoplasm until, at least, the end of the first week of post-natal life. In contrast, the ductular cells contain neither collagen type I nor FN, although these ECM components surround the ductules defining the eventual portal regions. During this stage there is a definitive remodeling of the existing hepatic structure. The ECM of the space of Disse decreases its laminin content, the portal spaces with bile ductules, hepatic artery, and portal vein radicles become defined, and haematopoietic elements become inconspicuous. The adult architecture and ECM phenotype become apparent by 1 week of gestation. After this date, evidence of ECM synthesis becomes inconspicuous. Interestingly, in the neonatal rat, all laminin chains, except A, are present in the perisinusoidal space. As the animal matures, the reactivity for B1, B2, S, and M laminin chains decreases so that by 6–8 weeks post-partum no laminin is detectable in the perisinusoidal space.

Regenerating liver

Unlike chronic injury (cirrhosis), subsequent to an acute injury, the liver is capable of a complete regeneration (Fig. 2), thus restoring its structural and functional integrity. It is worth noting that the result of regeneration following partial hepatectomy is not the growth of a new liver segment at the resection margin of an old, quiescent liver remnant. Rather, the entire remaining liver undergoes proliferation, until the hepatocyte cell mass is re-established and subsequently the typical hepatocyte-vascular relationships are restored. Thus, in this regard, post-hepatectomy regeneration is similar to that following toxic or viral liver injury.

In mammals, hepatic regeneration is completed well within a 2 week period (Alison 1986; Michalopoulos 1990). The most often studied model of regeneration is that following partial (60%) hepatectomy (Harkness 1952; Sell and Ruoslahti 1982; Fausto and Shank 1983; Ivanetich et al. 1986; Martinez-Hernandez et al. 1991), as described by Higgins and Anderson in 1931. Subsequent to partial hepatectomy the hepatocytes undergo brisk mitosis, reaching its pinnacle at 48 h. Mitoses can be first identified by 24 h in the periportal and pericentral regions. The effect of this hepatocyte proliferation is the loss of the typical plate-like architecture and the formation of clusters containing 8–10 hepatocytes without intervening sinusoids or ECM. These events result in the loss of the unique hepatocyte-vascular relationship. Hepatocytes in the normal liver form plate-like structures in such a manner that, at least, two facets of each hepatocyte are in contact with the space of Disse. Therefore, metabolites have a relatively short and unimpeded diffusion path between plasma and the hepatocyte cell membrane. During regeneration, with the formation of the hepatocyte clusters, this relationship is lost, even hepatocytes at the edge of the clusters have only one facet in contact with the space of Disse. Hepatocytes in the central portions of the clusters are only in contact with adjacent hepatocytes; therefore, metabolites must diffuse now along a protracted and encumbered path before reaching these cells. This hepatocyte/sinusoidal rearrangement probably explains the biochemical and immunohistochemical studies reporting a loss of ECM during hepatic regeneration (Sell and Ruoslahti 1982; Rojkind et al. 1983). Rather than a loss of ECM, there is simply a decrease in the ratio ECM/cell mass (Martinez-Hernandez et al. 1991).

By 48 h, the rate of mitosis reaches its maximum, as evidenced by the increased mitotic figures and increased thymidine incorporation (Fausto 1984; Ivanetich et al. 1986; Martinez-Hernandez 1988; Michalopoulos 1990; Martinez-Hernandez et al. 1991). It has also been demonstrated that the Ito cells (lipocytes or corner cells), are also proliferating and reach a maximum activity at 48 h post-hepatectomy (Tanaka et al. 1990; Martinez-Hernandez et al. 1991). As regeneration continues, the normal hepatic architecture begins to reemerge. An essential event is the vascularization of the hepatocyte clusters. By 48 h the insinuation of small vessels with adjacent ECM into the hepatocyte clusters becomes evi-

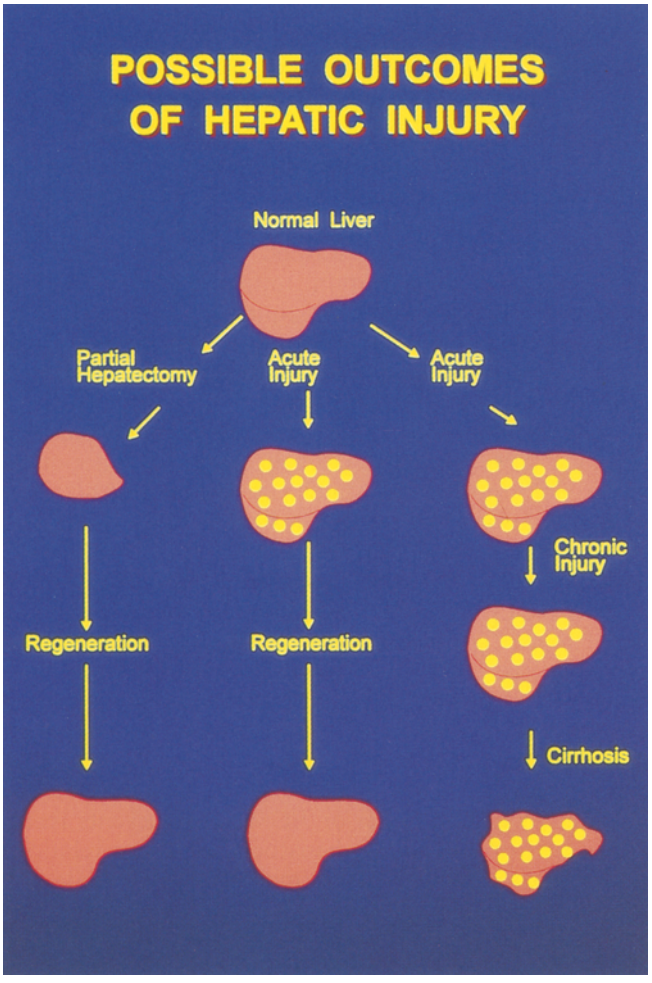

Fig. 2. The liver has a great capacity for regeneration. Destruction (or removal) of up to 90% of liver tissue will be followed by complete regeneration of a normal liver. In contrast, chronic injury results in cirrhosis

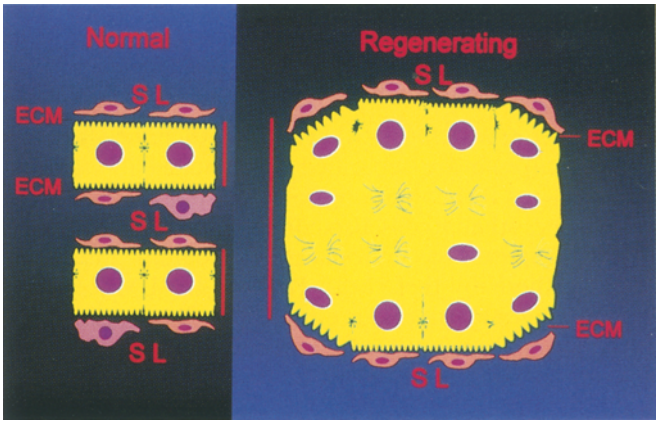

Fig. 3. In the normal liver, each hepatocyte has, at least, one facet adjacent to a sinusoid. Regenerating nodules often contain hepatocytes that are 2–4 cells away from a perisinusoidal space. This transient arrangement creates the impresion of a decrease in ECM

dent. Given the lack of ECM within the clusters, the gradual invasion by sinusoids can easily be followed using ECM markers. By 6 to 8 days post-hepatectomy the normal hepatic plate-like architecture is restored. Some controversy remains as to whether the hepatocytes are capable of differentiation into bile ducts, or a hepatic stem cell forms the terminal biliary ductules.

The distribution of most of the matrix components studied is identical to that in the normal liver. Collagen types I, III, and VI are present in the hepatic capsule; surrounding large vessels; in the interstitium of portal triads, and as occasional, thin, delicate bundles in the space of Disse; and surrounding central veins. Collagen type III and VI are more prevalent in Disse's space, than is collagen type I. Collagen type IV has a similar distribution to that reported in the normal liver (Martinez-Hernandez 1984) and is present in all BM zones, in-

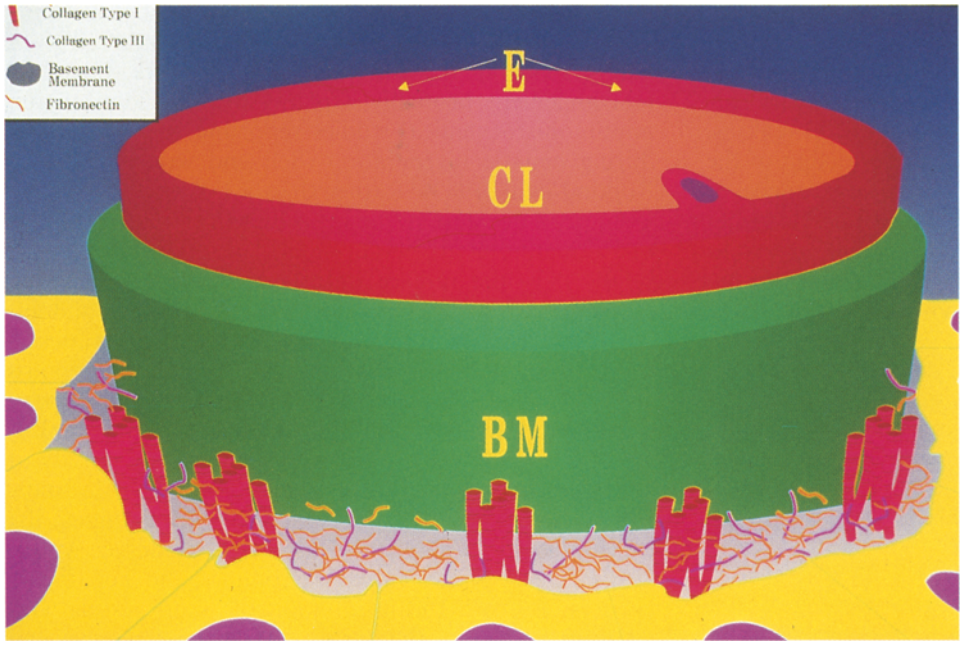

Fig. 4. Diagram of a capillary in cirrhotic liver (compare with Fig. 5, part I of this series). The endothelium lacks fenestrations, has specialized junctions and is encased by a continuous basement membrane

cluding: vascular, muscular, mesothelial, neural, and epithelial. In addition, collagen type IV maintains its prevalence in the space of Disse. FN is present in the capsule; surrounding large vessels; in the interstitium of portal triads; as discontinuous deposits along Disse's space; and surrounding central veins in the regenerating liver. The formation of hepatocyte clusters in the periportal and pericentral zones creates the appearance of a loss ECM in those areas (Fig. 3), as was initially reported (Sell and Ruoslahti 1982). During the early stages of regeneration, laminin appears along the space of Disse in a pattern similar to that of the developing liver. Furthermore, laminin antigens are found in the cytoplasm of many Ito cells. The maximum laminin staining in Ito cells is at 48 h post-hepatectomy, at the point of maximum proliferation for this cell type. Electron microscopy demonstrates that, at least, some of the perisinusoidal laminin is actually deposited in the extracellular space. The presence of the isotypes M, B1, and B2 as well as M, S, and B2 in development and regeneration suggests that these variants have a role in ontogenetic and regenerative processes.

During the latter stages of post-hepatectomy induced regeneration, mitotic activity returns to the usual low levels, the normal hepatocyte-vascular relationships return, the overall hepatic architecture is restored, and distribution of ECM antigens approaches that of the normal liver. Nevertheless, some intracellular laminin remains in non-parenchymal cells even at 8 days following hepatectomy.

Cirrhosis

The liver responds to chronic injury with a stereotypic set of reactions that has been termed cirrhosis. The type of injury (toxic, viral, immune, etc.) may modulate, to some extent, the initial changes (i.e. macronodular vs. micronodular cirrhosis), but regardless of the aetiological agent, all forms of cirrhosis have at some stage, hepatocyte necrosis, regenerating nodules, fibrosis, and decreased plasma clearance (Anthony et al. 1978; Huet et al. 1986; MacSween et al. 1987; Ohnishi et al. 1989; Martinez-Hernandez and Martinez 1991). It is worth emphasizing that cirrhosis is not synonymous with excessive accumulation of ECM (fibrosis), but the former, in addition, denotes a radical change in the structure of the hepatic lobule and hepatocyte-vascular relationships. The following description is based on studies on carbon tetrachloride-induced cirrhosis, although there may be some differences in time-frame, degree of necrosis, extent of ECM deposition etc., the general pattern seems applicable to most cirrhotic processes. To facilitate description of these changes the evolution of cirrhosis can be arbitrarily divided in three stages: early, septal fibrosis and cirrhosis.

Early stages. In terms of the ECM, the first detectable response of the liver to chronic injury is increased FN deposition in the space of Disse. FN begins to accumulate in the central regions of the hepatic lobule and extend, radially, towards portal regions. Soon after increased extracellular FN deposition becomes noticeable, FN can be detected within secretory organelles of hepatocytes. At this stage, no other cells contain detectable intracellular FN, indicating that hepatocytes are the cells responsible for the synthesis and deposition of FN in the space of Disse. Some time after FN deposition is obvious, increased deposition of collagen type I takes place. Like FN, collagen type I deposition starts in the central regions and from there extends towards the portal triads, and like FN, collagen type I can be demonstrated within secretory organelles of hepatocytes and Ito cells. It seems that collagen type I deposition follows FN deposition, not only in time, but also in location, suggesting that FN may act as an organizer of the ECM in the space of Disse. By the time collagen type I becomes prominent, an increase in collagen type IV becomes evident, with the clusters of collagen type IV normally present in the space of Disse becoming more extensive and beginning to form a continuous lining. At the transition point towards the next stage, some laminin begins to appear in the space of Disse in the centrilobular regions.

Septal fibrosis. The distinctive feature at this stage is the formation of delicate bands of ECM extending in a spoke-like pattern from the centrilobular regions. By immunohistochemistry, these bands contain FN, collagen types I, III, V, and VI. The general sequence remains one of FN deposition, followed in time and space by deposition of collagen types I, III, V, and VI. At any given stage FN is present at the septal front, with deposits of collagen type I trailing behind. Simultaneously, with the formation of these septa, the accumulation of ECM in the space of Disse continues. The phenotype of the Ito cells undergoes changes, such as a decrease in the number of lipid vacuoles, increased prominence of the rough endoplasmic reticulum, and formation of abundant cell membrane infoldings. These changes correspond with the transformation of the Ito cells into cells actively secreting, at least, collagen types I, III, IV, and laminin. The secretory activity of the Ito cells, combined with the synthetic activity of the injured hepatocytes, results in the accumulation of ECM in the space of Disse. A continuous lining of collagen type IV begins to appear focally, with discrete laminin clusters becoming more prominent and extensive.

Cirrhosis. The distinctive feature of this stage is the formation of discrete nodules of regenerating hepatocytes, surrounded by fibrous septa. These septa join and intertwine, dividing the hepatic parenchyma into multiple nodules containing clusters of regenerating hepatocytes. The dense fibrous septa represent the final stage of the fibrous bands appearing in the previous stage. They are composed mainly of collagen type I fibres, some collagen types III and VI, and abundant FN. As in previous stages, at the front of the septa, FN deposition precedes deposition of other ECM components. Radical changes take place in the hepatocyte-vascular relationships. The endothelial cells in many vessels are no longer fenestrat-

ed; furthermore, the cytoplasm of adjacent cells overlaps and tight junctions form at these points. In addition to these changes in endothelial cells, the increased deposition in the space of Disse of collagen type IV and laminin, initiated in the previous stage, culminates with the formation of a continuous endothelial BM. This BM is identifiable by conventional electron microscopy, with a lamina rara and densa, containing collagen type IV and laminin. Occasionally, even pericytes can be found completely surrounded by an endothelial BM. The culmination of all these changes, is the transformation of the hepatic sinusoid into a continuous capillary (capillarization). In addition to the endothelial BM, collagen type I, FN, collagen types III, V, and VI are also increased in the space of Disse, resulting in a wider and denser space. In the late stages of the cirrhotic process, hepatocyte groups, or occasionally even individual hepatocytes, become surrounded by a continuous BM containing laminin and collagen type IV. Laminin and collagen type IV can be demonstrated in the endoplasmic reticulum of endothelial cells, vascular smooth muscle cells, bile ductular cells, and pericytes; while hepatocytes continue to secrete FN and collagen type I. The consequence of the cirrhotic process is a complete transformation of the hepatic architecture. Whereas in the normal hepatic lobule hepatocytes have, at least, one cell aspect adjacent to the space of Disse, no continuous barriers between plasma and hepatocytes, and a definite metabolic portal to central gradient exists; the cirrhotic nodule has capillaries (Fig. 4) with continuous barriers, many hepatocytes are more than one cell away from the perivascular space, and the portal to central gradient no longer exists.

Summary. The unique nature of the hepatic ECM is predicated by the special configuration of the space of Disse. Whereas other epithelial organs have two BM and a substantial ECM interposed between endothelial and epithelial cells, the liver lobule has no BM and only an attenuated ECM, consisting mostly of FN, some collagen type I, and minor quantities of types III, IV, V, and VI. This configuration, together with the abundant fenestrations and gaps of the sinusoidal endothelial cells, seems ideally suited to facilitate the rapid bidirectional exchange of macromolecules normally taking place between plasma and hepatocytes. Some authors (Friedman et al. 1985; Bissell et al. 1987) have coined the term basement membrane-like or unconventional basement membrane, to describe the ECM in the space of Disse. It would seem that this terminology can only serve to confuse the issues, absence of continuous filtration barriers in the normal lobule, and obscure a dominant feature of the cirrhotic process: BM formation in the space of Disse. It would be important to understand the exact mechanisms regulating the special features of the space of Disse. For instance, the lack of BM could be due to a repression of the BM genes, resulting in deficient synthesis and secretion of BM components, such as entactin (Wewer et al. 1992) or to an increased activity of the genes regulating the catabolism of BM components (collagenases and other proteolytic enzymes), or a combination of both mechanisms (decreased secretion and increased catabolism). The presence of some free (not associated with laminin, entactin, or perlecan) collagen type IV in the space of Disse, suggests that some BM components are expressed and secreted. It is worth noting that two BM (endothelial and epithelial) are missing, suggesting that a signal is present capable of inhibiting the formation of BM by two different cell lines. The changes in the late stages of cirrhosis, clearly demonstrate that both, endothelial cells and hepatocytes, have the potential to form distinct BM and that part of the response to chronic injury is the attainment of this potential.

The studies in the developing liver provide some insight on the formation of sinusoids. The liver anlage is vascularized by vessels of the septum transversum. The vessels present in the septum transversum, are continous capillaries with BM. However, by day 13.5 of development (in the rat) the vessels in the immediate proximity of hepatocytes have fenestrated endothelium, and lack specialized junctions and BM, they have become sinusoids. This transformation of the endothelium, in the immediate proximity of hepatocytes, suggests that the hepatocytes produce a signal capable of modulating the endothelial phenotype. It would be crucial to define if this signal is a soluble (cytokine) or insoluble (ECM) factor.

During regeneration, hepatocyte proliferation precedes vascular proliferation resulting in the formation of hepatocyte clusters that, temporarily, lack sinusoids. Eventually, vascular proliferation follows and the normal hepatocyte-vascular relationships are restored. During this period laminin synthesis by Ito cells is prominent. It is tempting to speculate that proliferating hepatocytes stop secreting those factors that maintain the sinusoidal phenotype, allowing secretion of laminin (a part of the capillary phenotype). As soon as hepatocytes become stable, secretion of the sinusoid phenotype-maintaining factors resumes and laminin synthesis and secretion terminates. The role of laminin in this process is unknown, but perhaps its growth promoting properties may be important in the vascularization of the hepatocyte clusters.

Cirrhosis can be viewed as the hepatic adaptation to chronic injury. Most epithelial cells respond to chronic injury by increased production of ECM in general and BM in particular. Irradiation of the kidneys results in thickened BM (Martinez-Hernandez and Amenta 1983). Implantation of potassium permanganate crystals within the testis results in thickened BM of the seminiferous tubules with a clear gradient, the closer the tubule is to the crystal the thicker the BM (Martinez-Hernandez and Amenta 1983). Therefore, the increased deposition of ECM, and even the formation of BM can be viewed as the response of hepatocytes to injury. It is conceivable that formation of BM (with selective permeability) is one mechanism by which cells attempt to insulate themselves from an injurious agent. In the case of the liver, this insulation may be beneficial for the individual hepatocytes, but, by interfering with the bidirectional exchange of molecules between plasma and hepatocytes,

has a deleterious consequence for the organism as a whole.

The cellular origin of the ECM deposited during the cirrhotic process has been the subject of some controversy (Friedman et al. 1985; Tanaka et al. 1990). In terms of the non-BM components, it has been established in different systems, using various methodologies that hepatocytes, Ito cells, myofibroblasts, and fibroblasts secrete, at least, collagen type I (Kent et al. 1976; Dunn et al. 1979; Diegelmann et al. 1983; Rojkind et al. 1983; Martinez-Hernandez 1984; Friedman et al. 1985; Jefferson et al. 1985; Clement et al. 1986; Ala Kokko et al. 1987; Takahara et al. 1988; Friedman 1990; Gressner and Bachem 1990). Therefore, these four cell types contribute to ECM deposition. Clearly in large fibrous septa fibroblasts and myofibrobasts are the main contributors to ECM deposition. Within cirrhotic nodules, fibroblasts are rare, some authors (Friedman et al. 1985; Tanaka et al. 1990) argue that the Ito cell is the main contributor to nodular ECM deposition. It is clear that the ratio, hepatocytes/Ito cells, is overwhelmingly in favour of hepatocytes. Therefore, the burden of precise quantitation is on those proposing a cell other than the hepatocyte as the main contributor to the nodular ECM. In terms of BM, both endothelial and Ito cells secrete collagen type IV and laminin (Martinez-Hernandez 1984, 1985a, b). The extent of their relative contributions has not been established. It is likely that the few hepatocyte BM present in the late stages of cirrhosis are the secretory product of hepatocytes.

A considerable number of vessels in the cirrhotic nodule become capillaries. What triggers this change? It is clear that in most forms of cirrhosis, hepatocytes, not endothelial cells, are the target of the injury. Such is the case in chronic alcoholic injury, haemochromatosis, α1-antitrypsin deficiency, hepatitis B, etc. It is also the case in carbon tetrachloride induced cirrhosis. Carbon tetrachloride itself is not toxic, but a product of its metabolism by hepatocytes is a toxin (Casini and Farber 1981). Therefore, the injurious effect is exerted first and foremost on hepatocytes. This being the case, it would seem that hepatocyte injury results in the production of a new signal, that transforms the endothelial phenotype, from that of sinusoid, into that of continuous capillary. Alternatively, (and perhaps more likely) hepatocyte injury results in a block on the production of the signal, continuously produced by normal hepatocytes, that maintains endothelial cells in the sinusoidal phenotype. An important issue is the reversibility of hepatic fibrosis and the irreversibility of hepatic cirrhosis. It would be important to define the exact point of no return and the factor(s) determining irreversibility. However, at the present time we lack enough data even to venture a hypothesis regarding this crucial point.

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