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Distribution and Origin of the Basement Membrane Component Perlecan in Rat Liver and Primary Hepatocyte Culture

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Basement membranes contain three major components (ie collagen IV, laminin, and the heparan sulfate proteoglycan termed perlecan). Although the distribution and origin of both collagen IV and laminin have been well documented in the liver, perlecan has been poorly investigated, so far. We have studied the distribution and cellular origin of perlecan in rat livers in various conditions as well as in hepatocyte primary culture. By immunolocalization in both adult and 18-day-old fetal liver, perlecan was found in portal spaces, around central veins, and throughout the lobule. Immunoelectron microscopy revealed its presence at the level of basement membranes surrounding bile ducts and blood vessels, and in the space of Disse discontinuously interacting with hepatocyte microvilli. Precursors of perlecan were detected in the rough endoplasmic reticulum of bile duct cells and both vascular and sinusoidal endothelial cells. Both hepatocytes and Ito cells were negative. Northern-blot analysis confirmed the lack of appreciable expression of perlecan in hepatocytes isolated from either fetal or adult livers. In 18-month-diethylnitrosamine-treated rat liver, perlecan was abundant in neoplastic nodules. Electron microscopic investigation revealed an almost continuous layer of perlecan in the space of Disse and intracellular staining in sinusoidal endothelial cells, only. Perlecan mRNAs were detectable in malignant nodules, and absent in hepatocytes from nontumorous areas. Hepatocytes expressed high levels of perlecan mRNAs only when put in culture. This expression was reduced in conditions that allow improvement of hepatocyte survival and function (ie addition of corticoids, dimethylsulfoxide or nicotinamide to the medium, or in coculture with liver epithelial cells from biliary origin). Immunolocalization by light and electron microscopy showed that deposition of the proteoglycan occurred in coculture, in basement membranelike structures located around hepatocyte cords. In vitro ***attachment assay of hepatocytes on purified perlecan substrate indicated that these cells may interact with the proteoglycan through integrins which belong to the β_1 family. These data suggest that deposition of perlecan in the space of Disse requires cellular cooperation. This article on perlecan, the third major component of hepatic basement membranes, shows a unique cellular origin in the liver and, as found for both collagen IV and laminin, an expression in adult hepatocytes when placed in culture. (Am J Pathol 1993, 142:199–208)***

Proteoglycans are macromolecules consisting of a core protein with covalently bound glycosaminoglycan side chains.[1,2] They are widely distributed either anchored in the cell membrane[3–5] or associated with extracellular matrices.[6,7] A large heparan sulfate proteoglycan termed perlecan[8] is the major proteoglycan in various basement membranes—the sheet-like extracellular matrices that underlie epithelial, endothelial, nerve, and fat cells. Perlecan extracted from the Engelbreth–Holm–Swarm (EHS) tumor has a large core protein (M_r = 400,000) with 3 or 4 heparan sulfate chains projecting from a globule at one end.[9,10] Smaller heparan sulfate proteoglycans have been isolated (eg from the glomerular

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basement membrane), but they are likely degradation or alternatively spliced forms.[11] The primary structure of the core protein of perlecan has been determined by cDNA sequencing and showed homologies to portions of the laminin A chain, the repeats characteristic to those of neural cell adhesion molecule (N-CAM), often found in the immunoglobulin super family, and low-density lipoprotein–receptorlike domains.[12,13] Perlecan has a variety of functions. It creates ionic charge barrier on the surface of basement membrane,[7] interacts with other basement membrane components,[14] and has been implicated in cell adhesion through plasma membrane-associated proteins.[15,16]

Despite the biological importance of perlecan, little is known about the cellular sources and biosynthesis of this basement membrane component in several tissues, including liver. The distribution of perlecan in several tissues does not strictly match with those of laminin and collagen IV,[17] thus suggesting that basement membrane components might originate from different sources and/or be uncoordinately expressed. Recent studies with immunoelectron microscopy and *in situ* mRNA hybridization of matrix proteins have shown that hepatocytes interact with small clusters of laminin and collagen IV that are produced by both adjacent endothelial cells and Ito cells.[18,19] Interestingly, hepatocytes may express collagen IV and/or laminin in both the developing liver and following a variety of injuries in the adult, as well as early in culture.[18,20–22]

We have studied the distribution and origin of perlecan in adult and fetal rat livers and in hepatocarcinoma induced by diethylnitrosamine treatment. We show that, whatever the situation, perlecan is produced by endothelial cells *in vivo*, and both Ito cells and hepatocytes are negative. However, when put in primary culture, hepatocytes become perlecan producers and interact *via* β_1-integrin.

Materials and Methods

Reagents

Perlecan was purified from the EHS tumor and antibodies raised against the core protein of perlecan as described.[23] Perlecan cDNA was a 2.2 kb (BPG-7) Eco-RI fragment cloned from a EHS cell library.[12] Albumin cDNA was from murine origin.[24] Monoclonal anti-human β_1-integrin antibodies were kindly provided by Dr. K. Yamada, National Institute of Dental Research (Bethesda, MD).

Liver Sample and Cell Cultures

Normal adult livers were obtained from male Sprague–Dawley rats weighing 180 to 200 g and fetal livers from 18-day-old gestation Sprague–Dawley rats. Hepatocytes were isolated by the two-step perfusion collagenase method[25] and plated in a mixture of 75% minimum essential medium (MEM) and 25% medium 199 containing 10 μg/ml bovine insulin, 0.2% bovine serum albumin, and 10% fetal calf serum. After 4 hours, the medium was removed and the cells subsequently maintained in serum-free medium containing (or not) 7×10^{-6} M hydrocortisone hemisuccinate and supplemented with either 2% dimethylsulfoxide (DMSO) or 25 mM nicotinamide.[26,27] Co-cultures were set up 4 hours after hepatocyte seeding by adding rat liver epithelial cells (RLECs) derived from 10-day-old Fisher rats in the complete medium used to plate hepatocytes containing 10% fetal calf serum.[28,29] Serum-free medium supplemented with 7×10^{-6} M hemisuccinate hydrocortisone was renewed after 24 hours and every day thereafter. For Northern-blot analysis, cocultured hepatocytes were selectively detached from RLECs by incubation in a calcium-free HEPES-buffered collagenase solution (pH 7.6) for 10 minutes.[29]

Hepatocytes from diethylnitrosamine-treated rats were obtained as previously described.[30] Briefly, female Sprague–Dawley rats received three doses of diethylnitrosamine (25 mg/kg) 24 hours, 48 hours, and 72 hours after a two-thirds hepatectomy. After 18 months, both malignant nodules and hepatocytes from nontumorous areas were isolated by collagenase perfusion and collected separately after passage through gauze and low-speed centrifugations.

Fixation and Immunohistochemistry

Normal adult and diethylnitrosamine-treated rat livers were rapidly washed by perfusion with phosphate-buffered saline (PBS) for 1 minute, then perfused with a 4% paraformaldehyde solution buffered with 0.1 M sodium cacodylate (pH 7.4) for 15 minutes, then cut in small pieces. Rat fetal livers were cut in fragments and immediately immersed in the same fixative solution for 4 hours. Samples were washed with PBS, soaked in PBS containing 10% glycerol for 1 hour and then frozen in liquid nitrogen-cooled isopentane. Eight μ cryostat sections were routinely prepared.

Cultured hepatocytes were briefly washed with PBS for 1 minute, then fixed with a 4% paraformaldehyde solution buffered with 0.1 M sodium cacodylate (pH 7.4) for 45 minutes. Cells were washed three times, 15 minutes each, in PBS at 4 C then processed for perlecan immunolocalization.

Perlecan was visualized using either indirect immunofluorescence or immunoperoxidase

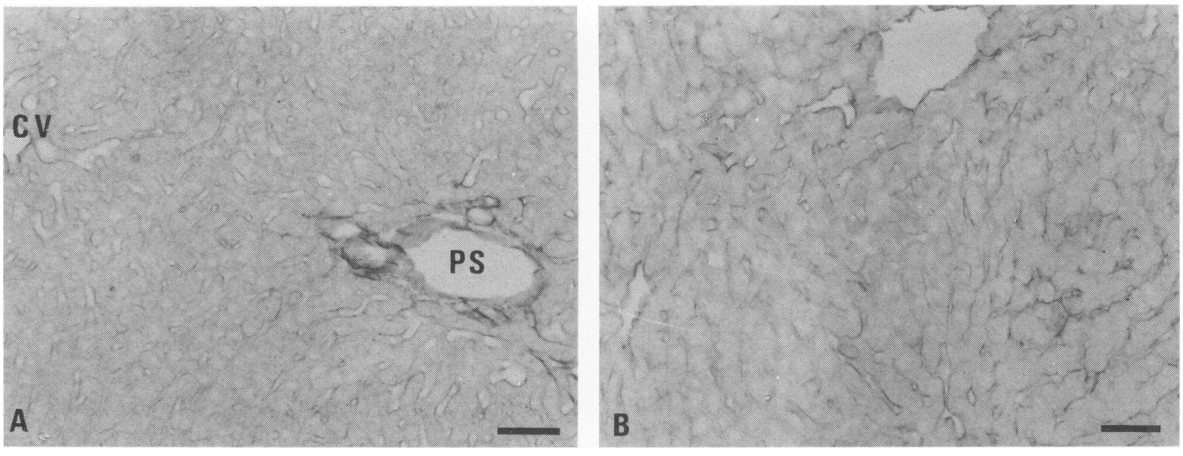

Figure 1. *Immunoperoxidase localization of perlecan in normal adult* (A) *and 18-month-diethylnitrosamine–treated rat livers* (B). *PS, portal space; CV, central vein. Bars = 100 μ (×110).*

method.[31] Briefly, cryostat sections and cultured cells were incubated with 0.2% saponin in PBS at room temperature for 1 hour prior to their incubation with anti-perlecan antibodies for 1 hour. After three washes in PBS, samples were incubated with either fluorescein- or peroxidase-labeled sheep anti-rabbit immunoglobulins. All antibody solutions and washing solutions contained 0.2% saponin. For electron microscopic examination, immunoperoxidase-labeled samples were subsequently postfixed with 2.5% glutaraldehyde buffered with 0.1 M sodium cacodylate (pH 7.4) for 4 minutes at 4 C then incubated in 0.2 M glycine (pH 10) for 15 minutes. Staining was visualized with 3,3′-diaminobenzidine/H_2O_2. Then, samples were postfixed in 1% osmium tetroxide buffered with 0.1 M cacodylate buffer for 30 minutes, dehydrated in ethanol, and embedded in Epon. Controls were first incubated with normal immunoglobulins, or directly placed in the medium used to reveal peroxidase activity.

Northern-Blot Analysis

Total RNAs were extracted by the guanidium-thiocyanate cesium chloride method.[32] The parietal yolk sac cell line, isolated from a rat carcinoma known to produce high levels of perlecan, was used as a positive control for Northern-blot hybridization with perlecan cDNA probe. RNAs (10 μg per lane) were resolved by electrophoresis on a 1% agarose slab gel buffered with 10 mM sodium phosphate (pH 7.4) containing 1.1 M formaldehyde and transferred onto nitrocellulose sheets. Filters were hybridized in 3 × standard saline citrate (SSC), 0.2% polyvinylpyrrolidone, 0.2% Ficoll, 0.1% sodium dodecyl sulfate (SDS), 0.1% bovine albumin, and 50% formamide. The filters were washed twice with 3 × SSC, 0.1% SDS for 2 minutes at room temperature, and then twice with 1 × SSC, 0.1% SDS for 20 minutes at 65 C. The hybridized filters were exposed to Kodak X-Omat film at −80 C.

Inhibition of Hepatocyte Adhesion

Perlecan (2 μg in 100 μl of serum-free MEM) was coated on 0.32 cm^2 well tissue-culture plates at 37 C in a 5% CO_2 atmosphere. After 2 hours, 3% bovine albumin in MEM was added to a final concentration of 1.5% for a further 30 minutes to prevent unspecific binding of cells. Medium was removed and plates were immediately used for attachment assay. Prior to

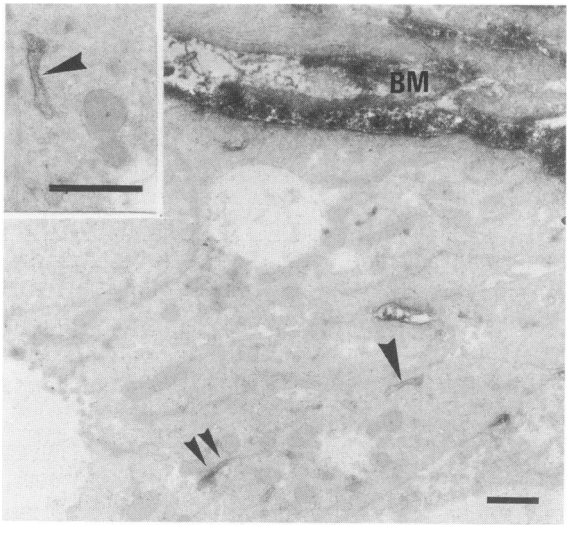

Figure 2. *Electron microscopic immunolocalization of perlecan in normal adult liver. Perlecan is present extracellularly in basement membrane (BM) and intracellularly in the rough endoplasmic reticulum (arrows) of cells that form a canal of Hering. Bars = 1 μ (×7,350; inset, ×12,700).*

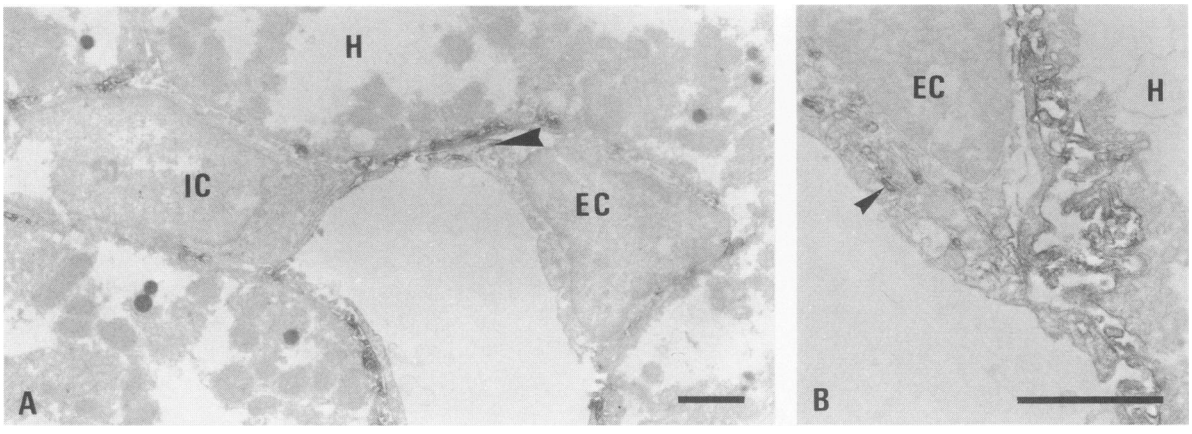

Figure 3. *Electron microscopic immunolocalization of perlecan in normal adult* (A) *and 18-day-old fetal liver* (B). *In the sinusoid, perlecan is present close to hepatocyte microvilli and intracellularly in the rough endoplasmic reticulum (arrow) of endothelial cells (EC). Both hepatocytes (H) and an Ito cell (IC) are negative. Bars = 1 μ* (A ×8,700; B, ×19,000).

their addition to perlecan-coated well tissue-plates, hepatocytes (20,000 cells/well) were incubated with various concentrations of monoclonal anti-β_1 integrin antibodies or Ca^{++}-Mg^{++}-free PBS for 10 minutes. Thirty minutes after hepatocyte seeding, media containing unattached cells were removed and plates were gently washed twice with 0.1 M PBS (pH 7.4). The percentage of attached cells was measured by determining the lactate dehydrogenase activity in both media and cell layers using the M.A.-kit Roche (LDH opt. DGKC). Because monoclonal antibodies were directed against human β_1-integrin, hepatocytes isolated from a normal adult human liver were used for this assay.

Results

Immunolocalization of Perlecan in Rat Liver

In the normal adult rat liver, immunoperoxidase analysis revealed that perlecan was abundant in portal spaces and formed an almost continuous layer in the sinusoids (Figure 1A). At the ultrastructural level, perlecan was detected in basement membranes surrounding bile ducts and blood vessels (Figure 2). In the space of Disse, electron-dense deposits were found close to hepatocyte microvilli (Figure 3A). Perlecan was localized intracellularly in the rough endoplasmic reticulum of vascular endothelial cells, bile duct cells, and cells that form the canal of Hering (Figure 2). In addition, sinusoidal endothelial cells, but neither hepatocytes nor Ito cells, also exhibited positive rough endoplasmic reticulum for perlecan (Figure 3). In controls, neither extracellular nor intracellular staining was observed, except in a few remaining blood cells that contained endogenous peroxidase (data not shown).

In 18-day-old rat fetal liver, perlecan was localized by immunofluorescence mainly around blood vessels and bile ducts (Figure 4). Perlecan formed a continuous layer around clusters of hepatocytes. Staining intensity was weaker in the fetus compared with that in the adult. Immunoelectron microscopy confirmed that perlecan interacted with hepatocytes (Figure 3B). Intracellular labeling was restricted to bile duct cells and vascular and sinusoidal endothelial cells (Figure 3B). Both hepatocytes and Ito cells were constantly negative.

After 18 months, diethylnitrosamine-treated rats developed neoplastic lesions and hepatocellular carcinoma. By light microscopy, perlecan was found in neoplastic nodules (Figure 1B) and around pseudoacinar structures. Electron microscopic investigation revealed an almost continuous layer of

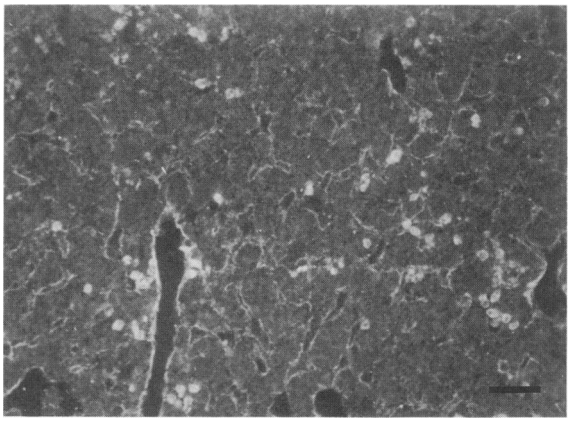

Figure 4. *Immunofluorescent localization of perlecan in a 18-day-old fetal liver. Bar = 100 μ (×110).*

perlecan in the space of Disse and intracellular staining in sinusoidal endothelial cells (Figure 5A). Both Ito cells and hepatocytes lacked intracellular staining for perlecan (Figure 5B).

Perlecan Gene Expression

Northern-blot analysis confirmed immunoelectron findings. Freshly isolated hepatocytes from both adult and an 18-day-old fetus did not contain detectable perlecan mRNAs (Figure 6A, lanes 2 and 3), whereas rat parietal yolk sac cells used as a positive control contained high levels of 12 kb perlecan mRNA species (Figure 6A, lane 1). Perlecan mRNAs were detectable in malignant nodules from diethylnitrosamine-treated rat livers (Figure 6A, lane 4) and absent in hepatocytes from nontumorous areas (Figure 6A, lane 5).

When normal adult hepatocytes were cultured in standard conditions, they began to produce perlecan mRNAs. In the absence of hydrocortisone hemisuccinate, perlecan mRNAs were detectable as early as 24 hours after cell seeding and became abundant after 48 hours (Figure 6B, lane 2). The steady-state perlecan mRNA level was not significantly changed after 4 days (Figure 6B, lane 6). In the presence of 7×10^{-6} M hydrocortisone hemisuccinate, the steady-state perlecan mRNA level was lower in 2-day-old culture compared with that in untreated cells (Figure 6B, lane 3). However, the differences were only transient. No significant change was found between untreated and hydrocortisone-treated cultures after 4 days (Figure 6B, lane 7). When media were supplemented with 2% DMSO perlecan mRNAs were only slightly detectable, even in 4-day-old cultures (Figure 6B, lanes 4 and 8). In the presence of 25 mM nicotinamide, perlecan mRNAs were almost undetectable (Figure 6B, lanes 5 and 9). Only a 10-day exposure of the filter allowed a slight detection of perlecan mRNA species (data not shown). In 4-day-old co-cultures, both hepatocytes and RLECs were found to contain perlecan mRNAs, the steady-state perlecan mRNA level being higher in RLECs (Figure 6B, lanes 10 and 11). Filters were subsequently washed and hybridized with a ^{32}P-labeled albumin cDNA probe (Figure 6B). In conventional conditions, the steady-state albumin mRNA level was found to dramatically decrease in 2- and 4-day-old primary hepatocyte cultures compared with freshly isolated cells. The decrease was slightly prevented in the presence of hydrocortisone. When media were supplemented with either 2% DMSO or 25 mM nicotinamide, the steady-state albumin mRNA levels in 2- and 4-day-old hepatocyte cultures were similar to that in freshly isolated cells.

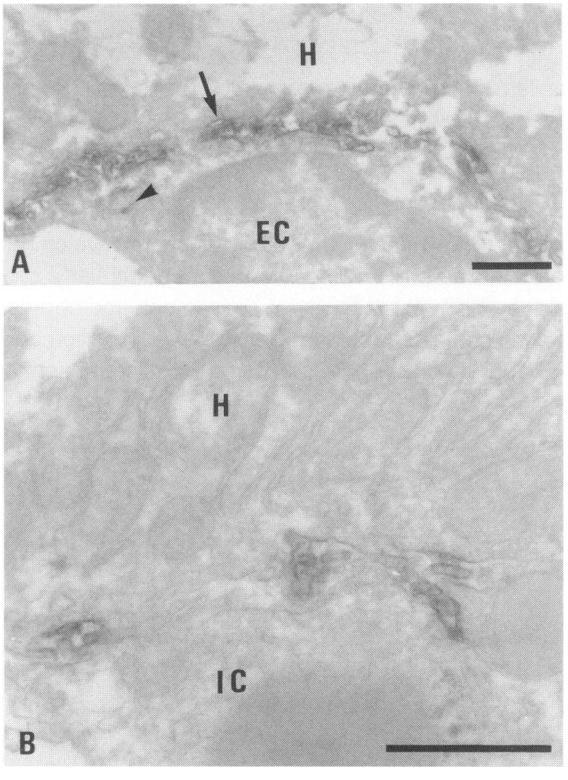

Figure 5. *Electron microscopic immunolocalization of perlecan in a 18-month-diethylnitrosamine–treated rat liver. In the sinusoid, perlecan is abundant in the space of Disse (arrow). Intracellularly perlecan is detectable in the rough endoplasmic reticulum (arrowhead) of endothelial cells (EC), but not in hepatocytes (H) and Ito cells (IC). Bars = 1 μ* (**A,** ×10,200; **B,** ×21,800).

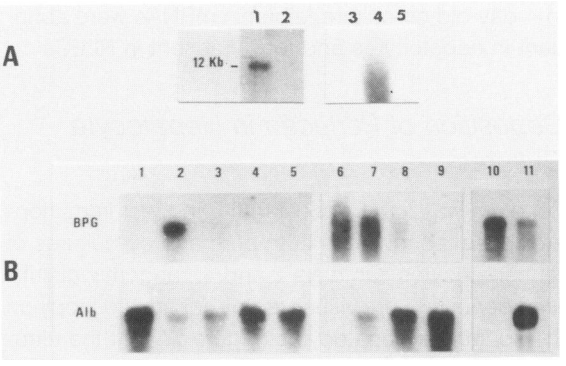

Figure 6. *Northern-blot analysis.* (**A**) *The steady-state level of perlecan mRNAs was studied in hepatocytes from 18-day-old fetal (lane 2 and normal adult livers (lane 3), and in nodular formations (lane 4) and hepatocytes from nontumorous areas (lane 5) in 18-month-diethylnitrosamine–treated rat livers. The parietal yolk sac tumor (lane 1) was used as a positive control.* (**B**) *The steady-state level of perlecan mRNAs (BPG) was studied in adult hepatocyte primary cultures maintained in serum-free medium without (lanes 2 and 6) or with hydrocortisone hemisuccinate (lanes 3 and 7) and supplemented with 2% DMSO (lanes 4 and 8) or 25 mM nicotinamide (lanes 5 and 9). Lane 1, freshly isolated hepatocytes; lanes 2 to 5, 2-day-old pure cultures; lanes 6 to 9, 4-day-old pure cultures; lanes 10 and 11, 4-day-old co-cultures (lane 10, RLECs; lane 11, hepatocytes). The same blots were used for hybridization with an albumin cDNA probe (Alb).*

In 4-day-old co-culture, albumin mRNAs were abundant in hepatocytes and totally absent in RLECs.

Deposition of Perlecan in Hepatocyte Cultures

Perlecan was localized using the indirect immunoperoxidase technique in both pure and co-cultures of rat hepatocytes. On days 2 and 4, randomly distributed deposits were visualized by light microscopy on the surface of hepatocytes (Figure 7A). At the ultrastructural level, electron-dense deposits were found extracellularly sparsely located on the surface of hepatocytes and intracellularly in the endoplasmic reticulum of all cultured hepatocytes (data not shown).

Unlike its distribution in pure culture, intense staining for perlecan was found in 4-day-old co-culture of hepatocytes and RLECs, specifically around hepatocyte cords (Figure 7B). At the ultrastructural level, perlecan was detected within a basement membranelike structure located close to the plasma membrane of hepatocytes at the level of intima contacts with RLECs (Figure 8A). The endoplasmic reticulum of RLECs was strongly labeled, whereas small quantities of perlecan were found in that of hepatocytes (Figure 8B). In pure RLEC cultures used as control, perlecan was detected intracellularly in the rough endoplasmic reticulum of the cells, but not extracellularly (data not shown).

Inhibition of Hepatocyte Attachment to Perlecan by Anti-β_1–Integrin Antibodies

Human hepatocytes were incubated with monoclonal anti-β_1–subunit of integrin antibodies, then assayed for their attachment to perlecan substrate (Figure 9). Attachment of hepatocytes was inhibited in a dose-dependent manner by monoclonal antibodies. At a concentration of 400 µg/ml, the antibodies inhibited adhesion of hepatocytes by 75% when compared with controls.

Discussion

Proteoglycans are a diverse class of macromolecules, some of which anchored in cell membrane and others associated with extracellular matrices. We have investigated the expression of perlecan, the major basement membrane proteoglycan in the liver, because this organ exhibits unique patterns of basement membrane distribution depending on its functional stage. Indeed, unlike most epithelia, normal adult hepatocytes lack a continuous basement membrane. Our study confirms previous immunogold localization of heparan sulfate proteoglycan in the space of Disse[33] and extends the particular feature of laminin and collagen IV distribution in the liver to perlecan. Indeed, although not forming a complete meshwork basement membrane components interact with hepatocyte microvilli.[18,31,34,35] Lack of basement membrane formation could be related to uncoordinated expression of corresponding genes.[21,36] Another explanation is the synthesis of incomplete basement membrane proteins, such as laminin, that could modify their biological properties, including self-assembly and interactions with specific receptors.

Basement membranelike structures containing laminin and collagen IV[20] and perlecan (this study) interact with immature hepatocytes in the developing liver. As shown in other tissues,[37] particularly the kidney,[38,39] expression of basement membrane genes is highly regulated both spatially and temporally during development. It is likely that interaction of

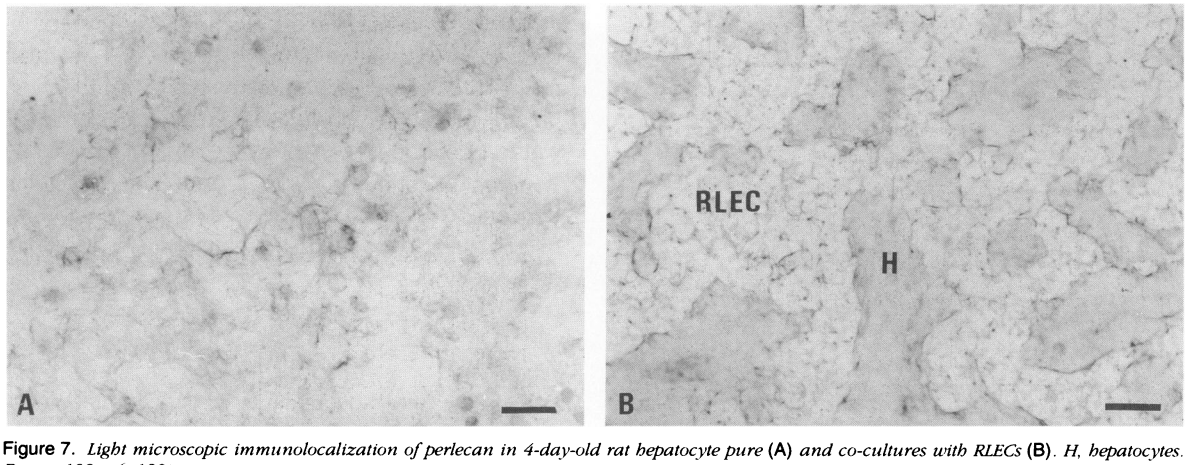

Figure 7. *Light microscopic immunolocalization of perlecan in 4-day-old rat hepatocyte pure* **(A)** *and co-cultures with RLECs* **(B)**. *H, hepatocytes. Bars = 100 µ (×120).*

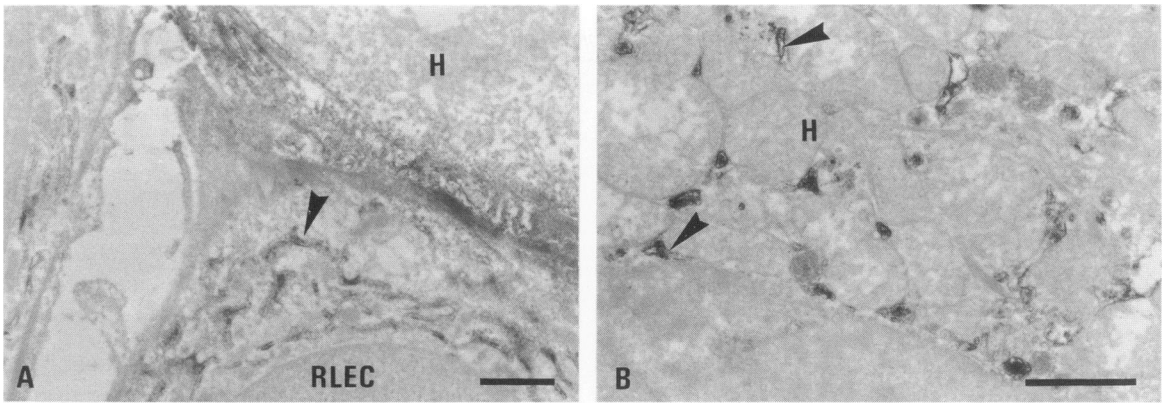

Figure 8. *Electron microscopic immunolocalization of perlecan in 4-day-old hepatocyte co-cultures. Perlecan is present extracellularly between an hepatocyte (H) and RLECs, and intracellularly in the rough endoplasmic reticulum (arrows) of both cells. Bars = 1 μ* (A, ×9,500; B, ×14,700).

basement membrane components with immature hepatocytes plays a major role during liver differentiation. *In vitro* studies have shown that liver-specific functions and transcription of liver-specific genes are maintained several days when hepatocytes are cultured on a reconstituted basement membrane.[40] However, the influence of perlecan on hepatocyte functions is unclear. Proteoglycans (eg chondroitin sulfate and dermatan sulfate proteoglycans, and liver-derived heparin) have been shown to improve electrical and dye coupling slightly, as well as the level of gap junction protein mRNAs in cultured hepatocytes.[41] In contrast, Caron[42] failed to detect a significant effect of perlecan on the steady-state albumin mRNA level in cultured adult hepatocytes.

Diethylnitrosamine treatment results in an increase in perlecan deposition in altered areas. Other basement membrane components, including collagen IV and laminin, are also abundant in the space of Disse during hepatocarcinogenesis.[21,43] It has been shown that basement membranes play a role in the transformation process.[37] Further studies should address whether this is relevant to liver cells. Interestingly, it has been shown *in vitro* that purified basement membrane components modulate proliferation of both transformed epithelial liver cells[44] and rat and human hepatoma cells (Levavasseur et al, unpublished data).

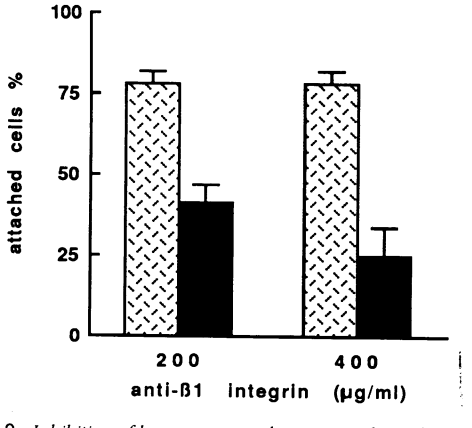

Figure 9. *Inhibition of hepatocyte attachment to perlecan by anti-β_1-integrin monoclonal antibodies. Tissue-culture plates (0.32 cm² well) were coated with 2 μg of perlecan purified from the EHS tumor. Anti-β_1-integrin antibodies were mixed with hepatocytes for 10 minutes before seeding. Control cells were first incubated with Ca^{2+}-Mg^{2+}-free PBS then assayed for their adhesion on perlecan. Each assay was conducted in duplicate in two independent experiments.* ■, *assay;* ⊠, *control.*

Whatever the situation, immunoelectron studies indicate that the major sources of perlecan are bile duct cells and vascular endothelial cells in portal spaces, and endothelial cells in the sinusoids. Other cells (eg Ito cells and hepatocytes) lacked precursors of perlecan. It cannot be ruled out that these cells could express perlecan below the threshold of detection by immunohistochemistry. However, perlecan mRNAs were not detectable by Northern-blot in hepatocyte and Ito cell populations purified from normal liver, thus suggesting that its expression, if any, might be biologically not appreciable. Increased steady-state perlecan mRNA levels in nodules from diethylnitrosamine-treated rat livers were likely related to endothelial cells, which were positive by immunoelectron microscopy. Endothelial cells could play a central role in basement membrane formation during liver injury. This particular cell type produces laminin and collagen IV[18,45] and undergoes striking morphological alterations during the early steps of sinusoidal fibrogenesis, particularly a dramatic decrease in both the size and number of fenestrae.[46] That Ito cells appeared negative for perlecan is unexpected. Indeed, besides their role in the storage of vitamin A, extracellular matrix protein synthesis is likely a major function of Ito cells. Because these

Table 1.
Immunoelectron Localization of Basement Membrane Components in Rat Livers

	Space of Disse	Ito cells	Endothelial cells	Hepatocytes			
				Adult	Fetal	DN	In vitro
Collagen IV	±	+	+	−	−	−	+
Laminin	±	+	+	−	+	+	+
Entactin/nidogen	±	+	+	−	ND	ND	+
Perlecan	+	−	+	−	−	−	+

Antigens were either absent (−) or present (±, weak or +, strong staining) extracellularly in the space of Disse and intracellularly in the rough endoplasmic reticulum of the cells. DN, 18-month-diethylnitrosamine–treated rat livers. ND, not determined. Taken from refs. 18, 20–22, and 31, and Loréal et al. (unpublished data).

cells express collagen IV and laminin, this suggests that basement membrane genes are not coordinately regulated. Recent Northern-blot analyses support this hypothesis. Freshly isolated Ito cells were found to contain high levels of procollagen α_1(IV) and laminin B2 mRNA, whereas perlecan and laminin A and B1 mRNAs were almost undetectable[47,48] (Loréal et al., unpublished data).

Although not producing appreciable amounts of perlecan *in vivo*, hepatocytes express this proteoglycan after a few hours in culture. Similar observations have been reported for other basement membrane components, including α_1(IV) procollagen,[22] laminin B1 and B2 chains,[21] and entactin genes (Loréal et al., unpublished data). The mechanisms responsible for this induction during their isolation and culture may represent an attempt for hepatocytes to adapt to an artificial environment. Another explanation is the relationship of basement membrane component expression with the cell cycle. Sequential activation of the proto-oncogenes (*c-fos*, *c-myc*, *jun* family, and *c-Ki-ras*), which are correlated with progression into the cell cycle, were described in hepatocytes following liver disruption and after a few hours in culture.[49] Whether expression of basement membrane genes is related to this activation and the entry of hepatocytes into the cell cycle is currently unknown. It is noteworthy that perlecan expression appears to be dramatically reduced in primary hepatocyte culture containing various factors (including corticoids, DMSO, and nicotinamide) that result in improvement of hepatocyte survival and function,[26,27] in the absence of cell proliferation.[50] That sinusoidal laminin increases in regenerating liver[51] and affinity of hepatocytes to this glycoprotein is enhanced in response to epidermal growth factor[52] sustain the hypothesis of a relationship between hepatocyte growth and basement membrane gene expression. Studies on perlecan expression during liver regeneration and in hepatocytes cultured in the presence of epidermal growth factor, pyruvate, and insulin, which allow hepatocytes to divide, should address this issue.

Deposition of perlecan within a basement membranelike structure around hepatocytes occurs in co-culture only (ie following reestablishment of specific cell–cell interactions). This suggests that perlecan deposition requires cellular cooperation. The main source of perlecan in co-culture appears to be RLECs, hepatocytes being a lower producer of this component and rather a potent regulator of its aggregation within a basement membrane. Stabilization of both functions and specific membrane domains in co-culture could modulate perlecan deposition through specific receptors. We have previously shown that the core protein of perlecan interacted with hepatocytes through specific membrane-associated proteins.[15] Here we demonstrate that hepatocytes may also bind to perlecan using β_1-subunit of integrin. That multiple receptors are implicated in hepatocyte-perlecan interaction is not unique. It has been shown that this cell type binds to laminin through both $\alpha_1\beta_1$-integrin and the 32/67 kd laminin receptor.[53,54] Also an M_r = 80,000 protein was found on the cell surface of hepatocytes interacting with collagen IV, laminin, and perlecan.[16] It is likely that these multiple mechanisms of hepatocyte-basement membrane interactions provide specific signals for tissue development and differentiation.

This study, together with those on collagen IV, laminin, and entactin/nidogen in the liver,[18,20–22] brings new insights into the role of liver cells in the formation of basement membrane components in the space of Disse in various physiopathological situations (Table 1). Both Ito and endothelial cells are the main sources of basement membrane components in normal and fibrotic livers, with hepatocytes being potent modulators of their deposition in the extracellular spaces. Interestingly, hepatocytes express the whole set of basement membrane components when put in culture and only laminin in fetal livers.

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