Biosynthesis and Degradation of Hyaluronan by Nonparenchymal Liver Cells During Liver Regeneration

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Hepatic stellate cells (HSC) and endothelial cells of the liver sinusoids synthesize and degrade hyaluronan, respectively. The roles of these cell types in the biosynthesis and degradation of hyaluronan were studied during regeneration following partial hepatectomy. Pure cultures of HSC and liver endothelial cells (LEC) were obtained from regenerating liver at different stages using a Nycodenz gradient followed by discontinuous Percoll gradient. The HSC that established 3 or 4 days after partial hepatectomy synthesized large amounts of hyaluronan when cultured in the presence of fetal calf serum (FCS) or platelet-derived growth factor B-chain homodimer (PDGF)-BB. These cells, as well as LEC, expressed active PDGF β-receptors. Furthermore, the ability of LEC to degrade hyaluronan was decreased at early stages of liver regeneration. The increased synthesis of hyaluronan by HSC and the failure of LEC to catabolize the polysaccharide resulted in elevated hyaluronan concentrations in the blood. (HEPATOLOGY 1996; 23:1650-1655.)

Hyaluronan is a high-molecular-weight polysaccharide that is ubiquitously distributed in the extracellular matrix and has been assigned many physiological and biological functions, including water and plasma protein homeostasis, and promotion of cell proliferation and migration.[1,2]

Hyaluronan is degraded locally in tissues, and is also secreted into the lymph and taken up in lymph nodes, which are important catabolic sites.[3] The spillover of hyaluronan from lymph nodes enters the circulation and is sequestered by the liver,[4] where liver endothelial cells (LEC) are responsible for the degradation.[5] Normally, low concentrations of hyaluronan circulate in the blood, but, in certain diseases, the hyaluronan levels increase.[6,7]

Several growth factors such as platelet-derived growth factor B-chain homodimer (PDGF)-BB, transforming growth factor *β,* and epidermal growth factor stimulate hyaluronan synthesis.[8,9] The stimulatory effects of PDGF-BB and transforming growth factor *β* in human foreskin fibroblasts were found to be mediated via activation of protein kinase C.[10] Previous studies from our laboratory revealed that the hepatic stellate cells (HSC) isolated from rat liver were the source of hyaluronan production and that PDGF-BB stimulated hyaluronan synthesis in these cells.[11]

It is well known that liver has a remarkable capacity for regeneration.[12-14] Higgins and Anderson[15] established a simple method for partial hepatectomy that made it possible to study the changes in structure, composition, and function of the residual liver. The present study was undertaken to explore the synthesis and degradation of hyaluronan by nonparenchymal cells (NPC), specifically HSC and LEC, during liver regeneration. The expression of PDGF receptors on these cell types was also investigated. For these studies, a simple, rapid, reproducible purification procedure for liver cells from regenerating liver after partial hepatectomy has been developed.

MATERIALS AND METHODS

Materials. Collagenase type I (No. 128F-6815) was obtained from Sigma Chemical Co (St. Louis, MO). Phosphate-buffered saline (PBS) and RPMI 1640 cell culture medium were purchased from Statens Veterinàrmedicinska Anstalt (Uppsala, Sweden). Nycodenz was purchased from Nycomed (Oslo, Norway) and Percoll and Sephadex G-50 from Pharmacia Biotech AB (Uppsala, Sweden). Dulbecco's PBS (10-fold-concentrated) was purchased from Gibco, BRL, Life Technologies AB (Tàby, Sweden). Fibronectin and fluorescein amine-conjugated ovalbumin was kindly provided by Dr. B. Smedsr0d (Troms0, Norway). Formalin-fixed fluorescein-conjugated Staphylococcus aureus was a kind gift from Dr. C. Rydén (Uppsala, Sweden). PDGF A-chain homodimer (AA), PDGF-BB, and ^{125}I-PDGF-BB were generously provided by Dr. C.-H. Heldin (Ludwig Institute, Uppsala, Sweden). Radioactive hyaluronan labeled with ^{3}H in the acetyl groups ([^{3}H]-hyaluronan; 38 μg/mL, 14 x 10^{6} dpm/mL) was a generous gift

Abbreviations: LEC, liver endothelial cell; PDGF-BB, platelet-derived growth factor B-chain homodimer; HSC, hepatic stellate cell; NPC, nonparenchymal cell; PBS, phosphate-buffered saline; PDGF-AA, platelet-derived growth factor A-chain homodimer; BSA, bovine serum albumin; KC, Kupffer cell; FCS, fetal calf serum.

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from Dr. J. R. E. Fraser (Melbourne, Australia). Cefuroxim (Zinacef) was purchased from Glaxo (Greenford, UK).

Animals. Male Wistar rats weighing approximately 250 g (ALAB, Sollentuna, Sweden) were used for all experiments. The animals were fed an unrestricted commercial diet (R36, Lab For, Lactamin, Stockholm, Sweden), which was discontinued 12 hours before the operation. The project was approved by the Ethical Committee for laboratory animals in Uppsala.

Partial Hepatectomy. Partial hepatectomy (about 70%) was performed essentially as described by Higgins and Anderson.[15] Briefly, rats were anesthetized with diethyl ether, and the abdomen was cut open with a transverse, subcostal incision. Falciform, gastrohepatic, and left triangular liver ligaments were divided. Left and median liver lobes were ligated and then excised. Cefuroxim solution (15 mg/0.2 mL water) was then applied on the remnant of the liver lobes and the incision was sutured. Postoperatively, the animals were kept under an infrared lamp for 1 hour and had free access to food and tap water.

Isolation and Culturing of NPC. Partially hepatectomized rats at 1, 3, 4, 10, and 20 days after operation were anesthetized with diethyl ether, and the suture of the abdominal incision was divided. Two hundred international units of heparin was then administered via the spleen, and 2 mL of blood was collected from the abdominal aorta. The livers were then perfused with collagenase as previously described.[16] Some rats were partially hepatectomized; the liver stump was then immediately subjected to liver perfusion (0 days). As controls, nonhepatectomized rats were used.

Rat liver NPCs were isolated using the perfusion method originally described by Pertoft and Smedsrod[16] and modified by Heldin et al.[11] Briefly, collagenase-dispersed liver cells were allowed to stay for 15 minutes in the refrigerator (during this time, the majority of hepatocytes sediment at 1 X average g-force). The cell suspension (4 X 50 mL) containing NPC, red blood cells, hepatocytes, and debris was centrifuged at 50 g_{av} for 2 minutes at 4°C to pellet-remaining hepatocytes. The supernatant was then centrifuged at 300 g_{av} for 10 minutes at 4°C to enrich the NPC. The pelleted cells were gently resuspended in 20 mL of Dulbecco's PBS without Ca^{2+} or Mg^{2+} containing 0.3% bovine serum albumin (BSA). The cell suspension was mixed with 16 mL of 27.6% (wt/vol) of Nycodenz, giving a final concentration of 13% (wt/vol), and centrifuged at 900 g_{av} for 30 minutes at 4°C; HSC, LEC, and Kupffer cells (KC) were found above the Nycodenz cushion, whereas the lymphocytes, red blood cells, contaminating hepatocytes, and the debris were pelleted. The NPCs were then removed, diluted up to a volume of 50 mL in PBS-BSA, shaken at 150 rpm for 30 minutes at 4°C in an environmental incubator shaker (New Brunswick Scientific Co., Edison, NJ), and then centrifuged at 300 g_{av} for 10 minutes at 4°C. To separate the HSC from the LEC and the KC, the cell pellet was gently resuspended in 6 mL of PBS-BSA solution and mixed with 6.6 mL of 100% Percoll (9 parts Percoll diluted with 1 part 10-fold-concentrated PBS). Two milliliters of this cell suspension was under-layered a two-step Percoll gradient (4 mL of 50% Percoll, 4 mL of 35% Percoll, and 1 mL of PBS-BSA; 10-mL centrifugation tubes were used) and centrifuged at 900 g_{av} for 30 minutes at 4°C. Three different cell bands were obtained: one at the top of the Percoll gradient enriched in HSC (fraction 1), one at the interface between the two Percoll cushions mainly enriched in LEC (fraction 2), and one near the bottom of the centrifugation tube enriched in KC (fraction 3). After washing fractions 1, 2, and 3 to remove the Percoll solution, the three fractions were resuspended in RPMI 1640 medium containing 4 mmol/L L-glutamine, 100 IU/mL penicillin, 100 μg/mL streptomycin, and 2 μg/mL amphotericin B, to the following final cell concentrations (measured in a Burker counting chamber): fraction 1, 0.1 X 10^6 cells/mL; fraction 2, 0.8 X 10^6 cells/mL; and fraction 3, 0.8 χ 10^6 cells/mL. After addition of fetal calf serum (FCS) to a final concentration of 10%, HSC (fraction 1) were seeded in 12-well Costar culture plates. Isolation of LEC and KC from fractions 2 and 3 was performed by a panning technique described previously[16] with slight modifications. Briefly, cells that adhered on cell culture plastic dishes after 20 minutes of incubation in the absence of FCS yielded pure cultures of KC. To obtain pure cultures of LEC, the nonadherent cells from fraction 2 were removed and reseeded after addition of FCS to a final concentration of 10% to dishes coated with fibronectin (0.5 mg per dish for 1 minute, followed by 3 washes with PBS). Twenty-four hours later, unless otherwise stated, the non-attached cells were removed by gentle washing. Cell number in monolayers was quantified by the insertion of a calibrated reticle between the objective and the ocular lenses. Cells were grown in 5% $C0_2$ at 37°C in a humidified atmosphere.

Determination of Hyaluronan Levels in Plasma and HSC Cultures. Plasma was immediately prepared from heparinized blood by centrifugation at 2,000 g_{av} for 10 minutes at 4°C. Hyaluronan content was determined using the Pharmacia HA Test (Kabi Pharmacia Diagnostics, Uppsala, Sweden). The amounts of hyaluronan secreted by HSCs (isolated at different times during liver regeneration) into the culture media during the first 24 hours after seeding were determined using the above commercial kit. In some experiments, HSCs isolated immediately after and 4 days after hepatectomy were washed once with RPMI 1640 medium containing 0.5% FCS after the first 24 hours of culture and then incubated for 24 hours in 1 mL of the same medium. Then, the medium was removed and the cells were incubated for a further 24 hours in medium containing 0.5% FCS, 50 ng/mL PDGF-BB, or 10% FCS. The amounts of hyaluronan in the culture media were measured using the same kit.

Degradation of [^{3}H]Hyaluronan by Cultured Endothelial Cells Isolated from Regenerated Liver. LEC purified at different times during liver regeneration were seeded on fibronectin-coated 4-cm^2 dishes (380,000 cells per well) for 1 hour, followed by extensive washing to remove nonattached cells. Then, plates were incubated at 37°C with [^{3}H]hyaluronan (0.5 μg/mL) in RPMI 1640 medium without FCS in a total volume of 0.5 mL. After incubation periods of 1, 2, 3, and 4 hours, the medium from each sample was removed and the cell layers were washed separately three times with 0.2 mL of the culture medium. The media and the corresponding washing solutions were combined. The cell layers were then treated with 0.6 mL of 0.3 mol/L NaOH/1% sodium dodecyl sulfate for 30 minutes. Then, 0.1 mL of 2 mol/L HCl was added to the suspension and the plates were washed twice with 0.2 mL of 0.5 mol/L NaCl/0.02% NaN_3/l% Tween 20. The cell lysates and the corresponding washes were also combined. Media and the corresponding cell fractions were chromatographed on a column of Sephadex G-50 (1 x 10 cm) equilibrated with 0.5 mol/L NaCl/0.02% NaN_3/l% Tween 20. Fractions of 0.4 ml were collected, mixed with 2 mL scintillation cocktail (Ready Safe; Beckman, Sweden), and subjected to scintillation counting in a Pharmacia LKB Wallac Scintillation Counter. Unspecific binding of [^{3}H]hyaluronan on the walls of the dishes was determined by processing the same amount of [^{3}H]hyaluronan in the absence of cells. The radioactive material that moved from the void volume position

(undegraded hyaluronan) to an included position was regarded as degraded hyaluronan.

Determination of PDGF a- and β-Receptors on Regenerating HSC and LEC. HSC at a cell density of 60 X 10^3 cells/4 cm^2 (24 hours after seeding) and LEC cultures at a cell density of 380 x 10^3 cells/4 cm^2 (24 hours after seeding) in 12-well dishes were washed with 1 mL of PBS containing 0.1 mg/mL Ca^{2+}, 0.1 mg/mL Mg^{2+}, and 1 mg/mL BSA. Then, 0.5 mL of the above PBS solution was added to each well, together with ^{125}I-PDGF-BB (1.28 ng/mL; 100,000 cpm/mL), in the absence or presence of unlabeled PDGF-AA or PDGF-BB (500 ng/mL). The plates were incubated with gentle agitation at 4°C for 2 hours; the binding buffer was then removed and the cell cultures washed 5 times with PBS/0.1% BSA. Thereafter, the cells were solubilized in 0.5 mL of 20 mmol/L HEPES buffer, pH 7.4, containing 1% Triton X-100 and 10% glycerol, at room temperature for 1 hour. The cell-bound radioactivity was counted in a gamma counter.

Statistics. Comparisons among more than two data groups were performed with an ANOVA corrected with Bonferroni's *post hoc.* Comparisons between two data groups were performed with a paired Student's *t* test, after checking the variances with Fisher's exact test. Statistical significance error was set to 5%. All statistical calculations were performed with Stat View 4.01 (Abacus Concepts, Inc., Berkeley, CA).

RESULTS

Isolation and Characterization of Liver NPC. The separation technique used in this study to isolate NPC from normal, as well as regenerating livers (Fig. 1), yielded essentially pure fractions of HSC, LEC, and KC. Fraction 1, which banded at a density ≤ 1.05 g/mL, was composed of HSC to almost 100%, as determined by their typical light microscopic appearance and the rapidly fading autofluorescence of vitamin A when irradiated at a wavelength of 328 nm.[17] The yield of HSC from normal, as well as from livers 10 to 20 days' posthepatectomy, which had regenerated to approximately normal size, was approximately 2 X 10^6 cells/g liver. During the exponential course of liver regeneration, about 1.3 X 10^6 cells and 1.7 X 10^6 cells/g regenerated liver were isolated at the first and the third or fourth day after hepatectomy, respectively

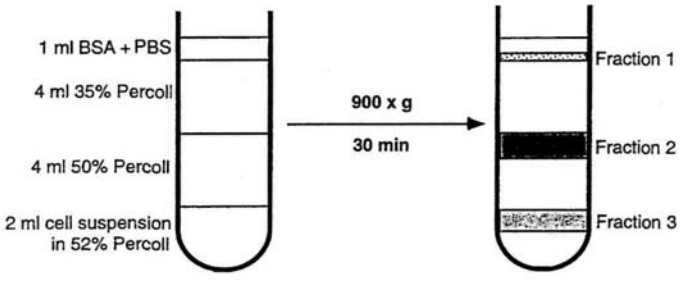


FIG. 1. Schematic depiction of separated liver NPC bands after Percoll centrifugation. One milliliter of cells was mixed with 1.1 mL of 100% Percoll underlayered a two-step Percoll gradient and centrifuged at 900 g_{av} at 4°C for 30 minutes. Three distinct boundaries containing cells were obtained: one at the top of the Percoll gradient enriched in HSC, one at the interface between the two Percoll cushions enriched in LEC, and one at the boundary between cell suspension in 52% Percoll and 50% Percoll enriched in KC.

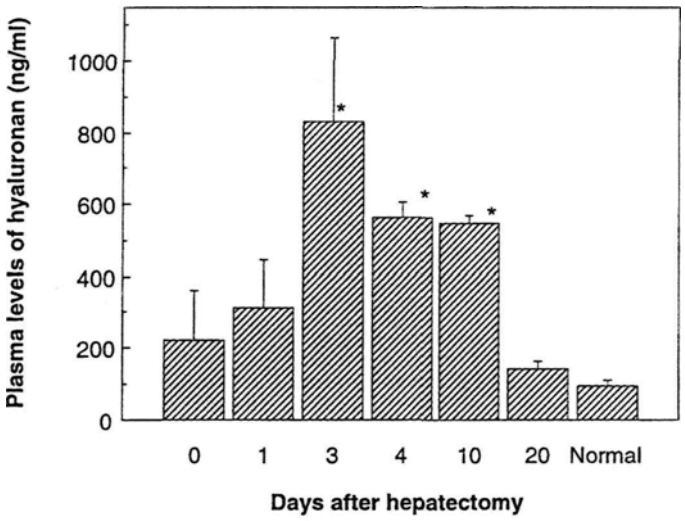


FIG. 2. Blood levels of hyaluronan during liver regeneration. Blood collected from the abdominal aorta (2 mL) was centrifuged at 2,000 g_{av} for 10 minutes at 4°C, and the hyaluronan content in the supernatant (plasma) was determined using a commercial kit for hyaluronan. Data represent the mean ± SD of three different experiments. *$P < .05$ compared with the group of normal rats.

(data not shown). By modifying the discontinuous two-step Percoll gradient composition previously used in our laboratory from 3 mL of 50% Percoll + 4 mL of 30% Percoll[11] to 4 mL of 50% Percoll + 4 mL of 35% Percoll, LECs were more efficiently separated from KCs (Fig. 1). Fraction 2, which appeared at the interface between the two Percoll cushions, was composed of 70% LEC, as determined by their ability to take up fluoresceinamine-labeled ovalbumin, and 30% KC, as determined by their ability to ingest formalin-fixed fluorescein-conjugated S. aureus; these two cell types were separated from each other by panning, making use of the observation that only KC attach and rapidly spread on tissue-culture plastic.[16] Approximately 6 X 10^6 LEC/g were obtained from regenerating livers. Fraction 3, which banded at the interface between cell suspension in 52% Percoll and 50% Percoll, contained mainly KC and was contaminated up to 30% by LEC. This fraction was not studied further.

Determination of Hyaluronan Levels in Plasma. Analysis of plasma levels of hyaluronan in normal and hepatectomized rats at different periods after operation is shown in Fig. 2. Significantly elevated plasma hyaluronan levels were observed at 3, 4, and 10 days after hepatectomy. The return to normal plasma hyaluronan levels observed at day 20 after operation coincides with the time when the liver returns to approximately normal size.[12] No statistically significant differences between hyaluronan values in plasma were apparent immediately after removal of about 70% of the liver or 1 day posthepatectomy compared with nonhepatectomized rats.

Determination of Hyaluronan Synthesis in HSC Cultures. Previous studies showed that HSC, in response

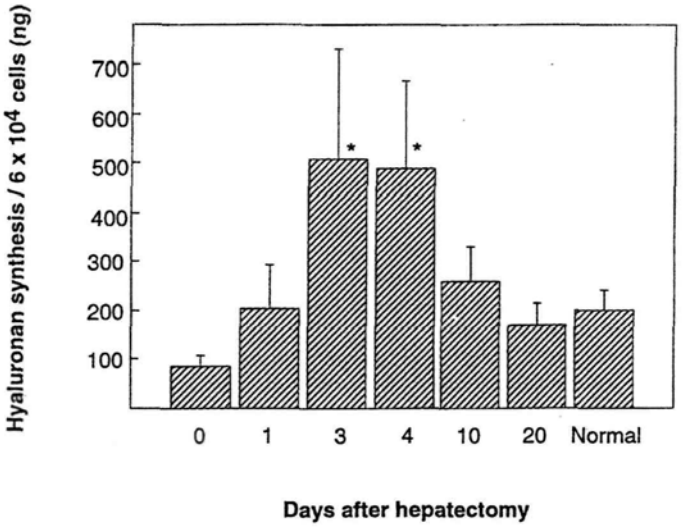
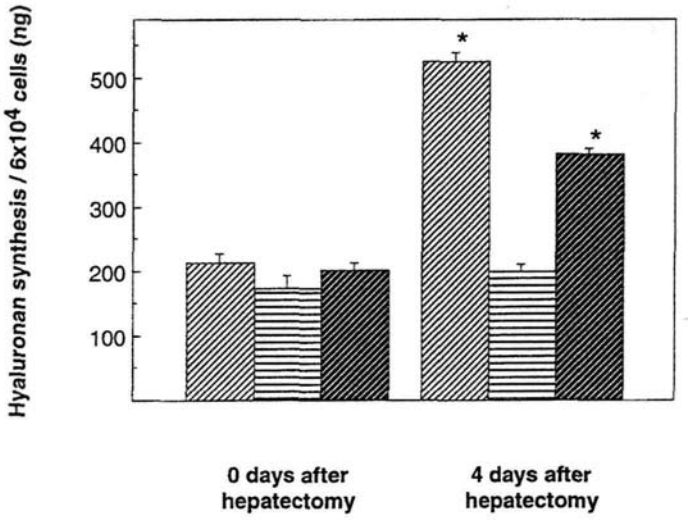


FIG. 3. Effect of FCS on hyaluronan synthesis in cultures of HSCs. HSCs were cultured in the presence of 10% FCS. The amount of hyaluronan secreted into the culture medium by HSCs was determined during the first 24 hours after seeding. Data are the means ± SD of three different experiments. *P < .05 compared with the group of normal rats.

to PDGF-BB and other growth factors, produce hyaluronan and other matrix components in normal and inflamed liver.[1118] To investigate whether HSCs from regenerating livers are the source of hyaluronan production, the effects of 10% FCS or PDGF-BB (50 ng/mL) on hyaluronan synthesis in HSC cultures were studied at different periods after partial hepatectomy (Figs. 3 and 4, respectively). More than a twofold in-

FIG. 4. Effect of PDGF-BB on hyaluronan synthesis in cultures



ofHSCs. Quiescent HSCs cultured in RPMI 1640 medium were given 10% FCS (B) or 50 ng/mL PDGF-BB (■). After 24 hours, the amounts of hyaluronan secreted into the culture media were determined. As a control, the cells were cultured in 0.5% FCS serum (■). Data are the means ± SD of three different experiments. *P < 05 compared with the group of normal rats.

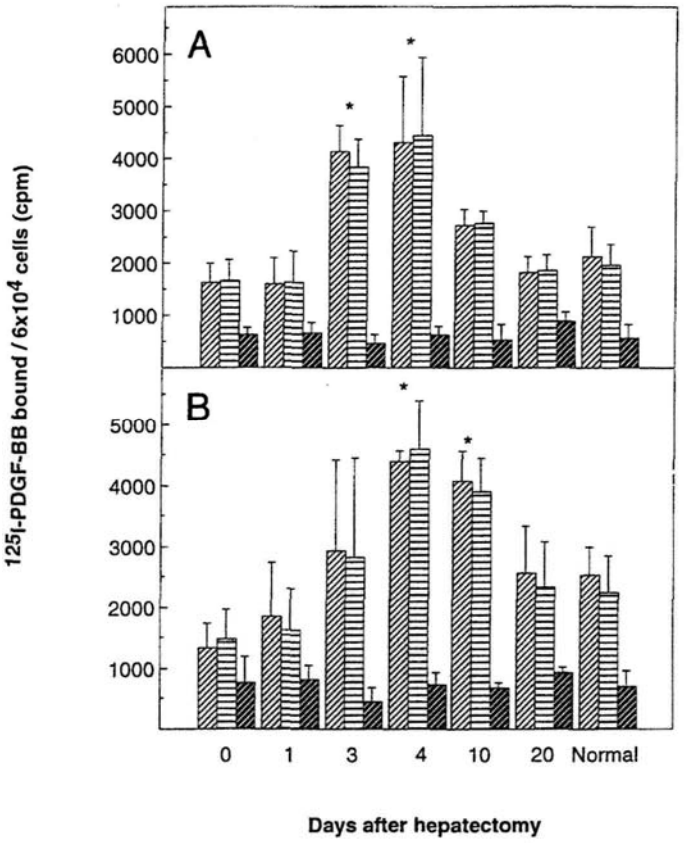


FIG. 5. Determination of PDGF receptors on HSC during Liver Regeneration. PDGF receptors expressed on the surface of (a) HSC and (b) LEC were determined by binding of ^{125}I-PDGF-BB in the (▒) absence or (■) presence of PDGF-AA or (■) PDGF-BB at the indicated times after hepatectomy. Data are the means ± SD of three different experiments. *P < .05 compared with the group of normal rats.

crease in the hyaluronan synthesizing activity was observed in the HSC cultured in medium containing 10% FCS at 3 and 4 days after hepatectomy, compared with that of the normal rats (Fig. 3). Exogenously added PDGF-BB did not significantly affect hyaluronan biosynthesis in HSC cultures isolated from the liver stump immediately after hepatectomy, whereas it powerfully stimulated hyaluronan synthesis in cultures obtained at 4 days after operation (Fig. 4). Thus, PDGF-BB induces stimulation of hyaluronan biosynthesis in stellate cells taken from the early phase of liver regeneration.

Detection of PDGF β-Receptors on Cultured HSC and LEC. To investigate why PDGF-BB stimulated hyaluronan synthesis in HSC cultures isolated at 4 days' post-hepatectomy but not in cells taken immediately after the operation, we determined the expression of PDGF receptors on the HSC at different times during liver regeneration. As shown in Fig. 5A, all cells were found to express binding sites for I-PDGF-BB, which were competed for by PDGF-BB but not by PDGF-AA indicating that they represented PDGF β-receptors which

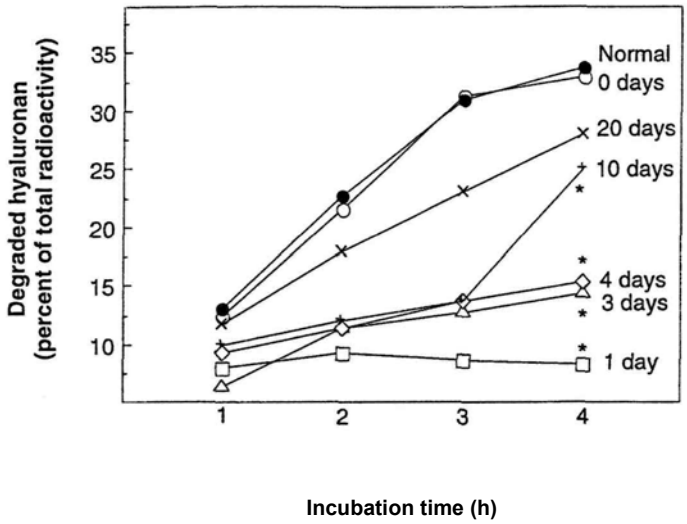


FIG. 6. Hyaluronan degradation by LEC During Liver Regeneration. [^{3}H]Hyaluronan (0.25 μg) was added to LEC cultures (380,000 cells/4 cm^2) and incubated at 37°C for the indicated times. The corresponding media were then collected and the low-molecular-weight [^{3}H]hyaluronan degradation products were evaluated as described in Materials and Methods. Each point represents the mean ± SD of three different experiments. *P < .05 compared with the group of normal rats.

bind only PDGF-BB, rather than α-receptors, which bind both PDGF-AA and PDGF-BB. Interestingly, an upregulation of the receptors was observed at 3 and 4 days after partial hepatectomy (Fig. 5A).

We also investigated whether cultures of LEC expressed PDGF receptors. ^{125}I-PDGF-BB bound to LEC, and the binding was specifically inhibited by unlabeled PDGF-BB but not PDGF-AA (Fig. 5B); furthermore, on these cells, an increase in the amount of PDGF β-receptors was observed at days 3, 4, and 10 after operation. Thus, different levels of PDGF β-receptors are expressed on both HSC and LEC at different times after hepatectomy, with the more pronounced expression at day 4 after operation.

Ability of LEC from Regenerating Liver to Degrade Hyaluronan. To explore whether the increased hyaluronan plasma levels observed 3 to 10 days after operation (Fig. 3) were due to a failure of LEC to degrade hyaluronan, the ability of LEC isolated from regenerating livers at different times after operation to degrade exogenously added [^{3}H]hyaluronan was tested. As shown in Fig. 6, LEC isolated from the liver stump immediately after the operation exhibited the same capacity to degrade exogenously added [^{3}H]hyaluronan as the LEC from normal rats. However, LEC isolated at 1, 3, and 4 days after partial hepatectomy degraded exogenously added [^{3}H]hyaluronan very inefficiently. An increase in the capacity of LEC to degrade hyaluronan at days 10 and 20 posthepatectomy was observed. Interestingly, the decrease in capability of LEC to degrade hyaluronan after partial hepatectomy coincides with the time when the hyaluronan concentration in the plasma is high. Exogenously added PDGF-BB did not alter the ability of cultured LEC to degrade hyaluronan (data not shown).

DISCUSSION

Recent research, which aimed at understanding the molecular mechanisms behind liver regeneration, has focused on studies of growth control in cultures of isolated hepatocytes. However, very little is known about changes in NPC.[12-14] To understand the role of the NPC during liver regeneration, *in vitro* culturing of pure individual cell types is necessary. In this study, we further developed our previously described purification method for the preparation of pure cultures of NPC derived from normal rat livers.[11] Following perfusion of regenerated livers from rats weighing 250 g, use of Nycodenz gradient, followed by a Percoll density gradient and a panning technique, reproducibly revealed pure and viable cultures of HSC (17 X 10^6 cells), LEC (60 X 10^6 cells), and KC (23 X 10^6 cells) from fully regenerated livers.

In liver regeneration following partial hepatectomy, cells in the entire remaining liver undergo proliferation; hepatocyte proliferation the first day after operation precedes proliferation of the NPCs, which is maximally 48 to 72 hours posthepatectomy. As regeneration continues, vascularization and deposit of extracellular matrix within hepatocyte clusters occur.[13] It has been demonstrated that HSCs synthesize and secrete into the culture medium hyaluronan and other glycosaminoglycans and extracellular proteins.[1119] We found that HSCs obtained at the third and fourth days posthepatectomy produced significant quantities of hyaluronan (Fig. 3). The functional significance of the increased production of hyaluronan during liver regeneration is not known. Hyaluronan has been shown to influence the proliferation and migration of certain cell types[1] and may have a similar role in the regenerating liver. It is also possible that the expanding properties of hyaluronan contribute to the restoration of the extracellular space that becomes encroached the first day after hepatectomy due to swelling of hepatocytes.[112]

The circulating hyaluronan concentrations also increased the third and fourth days after hepatectomy (Fig. 2). The increase of hyaluronan in the blood may be due to the overproduction of hyaluronan by HSC and/or the inability of LEC to degrade the polysaccharide. The fact that serum hyaluronan concentration is still low the first day after operation - although LECs have already lost a large part of their ability to catabolize hyaluronan (Fig. 6)—suggests that LEC has an overcapacity to deal with the polysaccharide. When the HSCs overproduce hyaluronan, elevation of the polysaccharide first is observed in blood. Therefore, the major reason for increased serum levels seems to be increased production of hyaluronan, rather than decreased clearance. This is presumably facilitated by the direct input of HSC hyaluronan in the circulation. A rough calculation, assuming that the stellate cells synthesize the same amount of hyaluronan *in vivo* and *in vitro,* shows that it is quite feasible that the HSCs

should contribute the serum hyaluronan level. If the rat lymph contributes to circulating hyaluronan proportionally by weight to a human, one should expect 0.1 mg hyaluronan from the peripheral tissues per day.[20] The HSC in the regenerating liver should contribute up to 0.65 mg/d according to the observations in the present investigations. It is likely that the HSCs are the major source of hyaluronan produced in the liver, because, in previous studies, it has been shown that these cells synthesize significant amounts of hyaluronan, whereas hepatocytes did not produce hyaluronan and only low amounts were found in cultures of KC from normal livers.[21] However, it cannot be excluded that during liver regeneration, cells other than HSC contribute to the production of hyaluronan.

Previous studies on hepatocyte cultures revealed the identification of liver-specific factors such as hepatocyte growth factor, transforming growth factor a, and epidermal growth factor, which are implicated in control of liver growth after partial hepatectomy.[14] These factors act in autocrine, as well as paracrine, ways. In addition, serum factors control the growth in regenerating liver.[14] Our finding that HSC expressed PDGF β-receptors and the PDGF-BB is able to induce stimulation of hyaluronan synthesis (Fig. 4) supports the possibility that PDGF-BB may be of importance in the restoration of the residual liver. The fact that LEC expressed significant PDGF β-receptors led to the question of whether the receptors influence the binding and degradation of hyaluronan by LEC. However, no such effect of PDGF-BB was seen (data not shown). It is of interest to point out that LEC express only PDGF β-receptors when cultured on fibronectin-coated dishes (Fig. 5B), whereas these cells have been found to have only PDGF α-receptors when cultured on uncoated plastic dishes.[11] Thus, extracellular matrix proteins appears to be important for the expression of receptors on LEC.

The method described in this communication to prepare pure cultures of various NPC of rat liver will allow a detailed characterization of the properties of these cells under different conditions, such as regeneration, inflammation, or cirrhosis.

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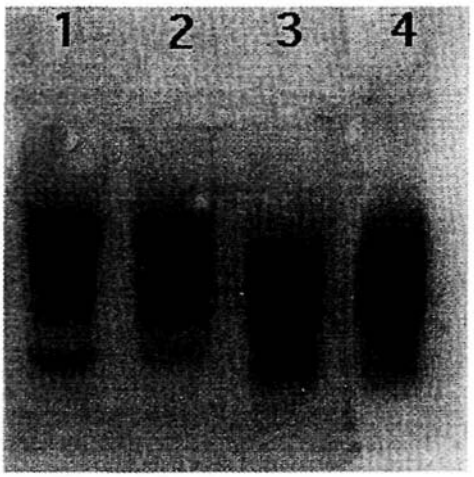

Figure 1. Proteoglycans from arthrotic cartilage (1), normal cartilage (2), reactive arthritis synovial fluid (3) and arthrosis synovial fluid (4), blotted to PVDF-P at high ionic strength and stained with Toluidine Blue.

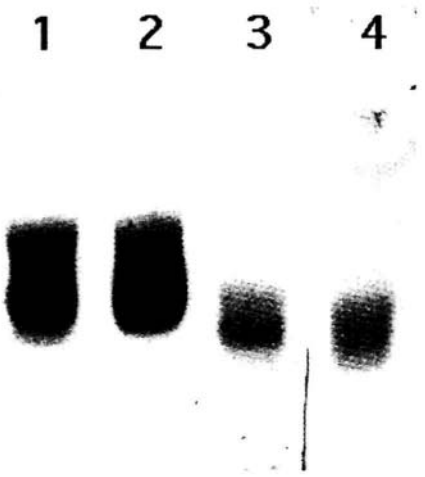

Figure 2. Mab 1C6, same samples as Figure 1. N-terminal globular domains G1 and G2 were detected after reduction and alkylation and digestion of the immobilized proteoglycans with chondroitinase ABC and Keratanase II. Equal amounts (10µg) of glycosaminoglycan was applied in each lane. Cartilage PGs contain both G1 and G2, while the synovial fluid PGs possibly contains only G2.

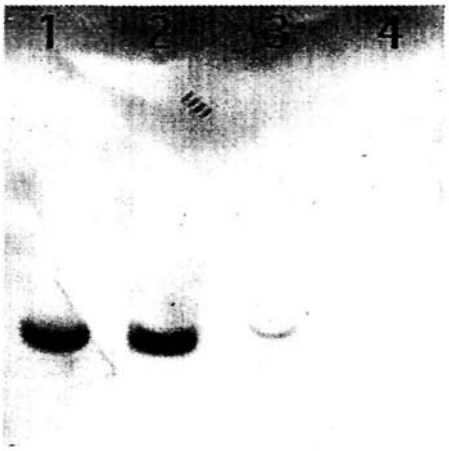

Figure 3. Mab 2B6, same samples as in Figure 1. Detection of unsaturated disaccharides of chondroitin-4-sulfate in native proteoglycans. Only the small proteoglycans, and not aggrecan, of the cartilage extracts contain the exposed epitope. Neither synovial fluid contains the epitope.

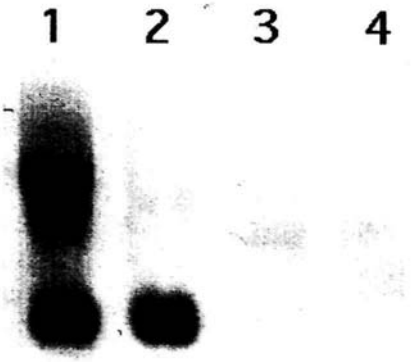

Figure 4. Mab 2B6, same samples as in Figure 1. Detection of unsaturated disaccharides of chondroitin-4-sulfate after digestion with Chondroitinase ABC of the immobilized proteoglycans The aggrecan from arthrotic cartilage, but not from normal cartilage exhibits this epitope. Neither synovial fluid exhibits the epitope after chondroitinase.

perse with an extrapolated Mw of 360 kD. An intermediate polydisperse population had a Mw of 270 kD. The third, most abundant population, had a Mw of 170kD. Deglycosylation was more efficient with released PGs than with immobilized PGs (not shown).

Conclusion

A 10µg sample is sufficient for separating PGs from knee joint cartilage or synovial fluid into three different populations with subsequent characterization of their antigenic epitopes or core protein sizes. Both synovial fluid populations contained lC6-epitope and are thus C-terminal fragments of aggrecan cleaved in the interglobular domain. The most abundant population in normal human knee cartilage had a core protein M_w of 180kDa, that was considerably lower than the most abundant proteoglycan population in synovial fluid which had a core protein M_w of 260kDa. The large size fragments in synovial fluid may be derived from a separate, newly synthesized pool of large, untruncated molecules.

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