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Short Communication

Gadolinium Chloride Suppresses Hepatic Oval Cell Proliferation in Rats with Biliary Obstruction

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Liver injury due to bile duct ligation (BDL) is histologically characterized by cholestasis, bile ductular proliferation, hepatocellular damage, portal fibrosis, and ultimately biliary cirrhosis. Stem cells within the liver may act as progenitor cells for small epithelial cells termed oval cells that can differentiate into bile duct cells or hepatocytes, whereas myofibroblasts are the principal source of collagen production in fibrosis. The aims of this study were to determine 1) whether BDL induces oval cell proliferation and 2) whether blockade of Kupffer cells affects oval cell proliferation, bile duct proliferation, and myofibroblast transformation in experimental biliary obstruction. Male Sprague-Dawley rats were divided into two groups to receive either a single dose of gadolinium chloride (a selective Kupffer cell blocking agent) or vehicle. One day later, the gadolinium- and vehicle-treated groups were further subdivided to receive either BDL or sham operation. The rats were sacrificed on day 7 postoperatively. Serum was collected for measurement of aspartate aminotransferase, γ-glutamyl transpeptidase, and bilirubin levels. Liver tissue was taken for evaluation of fibrosis, bile ductular cells, oval cells, and myofibroblasts. BDL resulted in elevated aspartate aminotransferase, γ-glutamyl transpeptidase, and bilirubin in serum, and gadolinium pretreatment did not modify these effects. BDL induced marked oval cell proliferation, which was completely prevented by gadolinium pretreatment. Gadolinium did not affect the induction of bile duct expansion or myofibroblasts after BDL. We conclude that experimental biliary obstruction induces oval cell proliferation, which can be prevented by gadolinium pretreatment. This suggests that bile ductular proliferation and myofibroblast transformation are not mediated by Kupffer cells and that ductular proliferation can proceed in the absence of oval cells. Alternatively, gadolinium may directly affect oval cell proliferation after BDL. *(Am J Pathol 1998, 152:347–352)*

Liver injury that results from bile duct ligation (BDL) is histologically characterized by cholestasis, bile ductular proliferation, hepatocellular damage, portal fibrosis, and ultimately biliary cirrhosis. Although it is generally accepted that the bile ductular proliferation after BDL results from the proliferation of existing ductules,[1–3] it is possible that stem cells (progenitor cells) within the liver may proliferate, giving rise to small epithelial cells (oval cells) that have the capacity to differentiate into biliary epithelial cells or hepatocytes.[1,4] Oval cells are small cells having scant, lightly basophilic cytoplasm and ovoid nuclei, which express embryonic proteins such as α-fetoprotein, the M_2 isoenzyme of pyruvate kinase (M_2-PK) and π-class glutathione S-transferase.[5] Previous studies of oval cell proliferation after BDL have shown that they are present but not associated with the ductular proliferation occurring in periportal areas.[3,6] Characterization of oval cells in the preceding studies was either based upon utilization of the monoclonal antibody OV-6, which shows strong reactivity against oval cells but which also cross-reacts with other hepatic cells expressing CK14 and CK19,[7] or α-fetoprotein.[6] The use of M_2-PK, which is a more specific marker of oval cells, has not previously been described in the BDL rat model.

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In chronic cholestatic liver injury, one of the earliest events in the development of hepatic fibrosis is the activation of hepatic stellate cells (formerly called Ito cells, lipocytes, or fat-storing cells[8]) and portal periductular fibroblasts to cells with a myofibroblast phenotype.[9–14] These myofibroblasts are largely responsible for the increased production of extracellular matrix components during hepatic fibrogenesis.[9–14] The activation of hepatic stellate cells involves increased cellular proliferation, increased synthesis of extracellular matrix proteins, and the expression of the activation marker α-smooth muscle actin (SMA).[10–14] On the basis of morphological studies of the early events after experimental BDL, it has been proposed that products derived from an expanded population of hepatic macrophages may play an important role in stimulating the subsequent bile ductular proliferation and myofibroblastic transformation leading to fibrosis.[15] However, the specific role of Kupffer cells in the regulation of oval cell and ductular proliferation and the appearance of myofibroblasts has not been determined.

To further elucidate the role of Kupffer cells in the pathogenesis of liver disease, selective Kupffer-cell–suppressing agents such as gadolinium (III) chloride (Gd) have been used. Treatment with Gd has been demonstrated to reduce liver injury and fibrosis in rats receiving chronic ethanol administration or carbon tetracholoride.[16] Gd blocks phagocytosis by rat Kupffer cells and selectively eliminates the large Kupffer cells in zone 1, the periportal region of the acinus.[17] In addition, Gd can reduce superoxide production by isolated Kupffer cells in a dose-dependent manner.[18]

Accordingly, the aims of this study were to determine 1) whether BDL induces oval cell proliferation and 2) whether blockade of Kupffer cells affects oval cell proliferation, bile duct proliferation, and myofibroblast transformation in experimental biliary obstruction.

Materials and Methods

Animals

Male Sprague-Dawley rats (weight 290 to 340 g; Harlan, Indianapolis, IN) were used in this study. All studies conformed to the National Institutes of Health guidelines for the use and care of experimental animals and were approved by the Animal Care Committee, St. Louis University Health Sciences Center. Animals were housed in plastic cages in a controlled environment with standard chow and water *ad libitum*. Rats were quarantined for 2 days before surgery. Food and water were removed on the morning of surgery and returned after the animals had recovered from surgery.

Experimental Biliary Obstruction

Animals were anesthetized using inhalation anesthesia with methoxyflurane (Metofane, Pitman Moore, Washington Crossing, NJ) in a mixture of 95% oxygen and 5% carbon dioxide. Sham laparotomy and bile duct manipulation served as a control (sham group) for the experimental animals that received laparotomy with double ligation and division of the common bile duct (BDL group). Proximal ligatures were placed at the junction of the right and left hepatic ducts. After the surgery the animals were returned to their controlled environment.

Experimental Protocol

The animals were divided into two groups. One group received a single dose of Gd (10 mg/kg intravenously) in sterile saline (Gd, n = 11) according to the protocol of Hardonk et al,[17] whereas the other group received sterile saline only (saline, n = 12). One day later, the Gd and saline groups were further subdivided to receive either BDL or sham operation (Gd + BDL, n = 5; Gd + sham, n = 6; saline + BDL, n = 6; saline + sham, n = 6). The rats were killed on day 7 postoperatively. Blood was collected for measurement of aspartate aminotransferase (AST) and γ-glutamyl transpeptidase (GGT) activities and bilirubin levels. Liver tissue was immediately placed in 10% neutral-buffered formalin (Fischer, Fair Lawn, NJ) for at least 12 hours and then embedded in paraffin. Sections (4 μm) were stained with hematoxylin and eosin and sirius red (sections were placed in 0.02% solution of sirius red in saturated picric acid for 20 minutes and then washed in distilled water, dehydrated, and mounted) to demonstrate collagen fibers.

Immunostaining

Immunohistochemistry was performed using the indirect immunoperoxidase method. Biliary ductules were demonstrated using a specific antibody to cytokeratin 19 (CK19; Dakopatts-MS88, Glostrup, Denmark). The antibody was used at a dilution of 1:100, and the sections were incubated overnight at 4°C. To ensure specificity, positive control sections were included with each batch, and negative sections were included for each case.

For the demonstration of oval cells, specific antibodies against M_2-PK (1:500 dilution; ScheBo Tech, Wettenberg Germany) and the L form of pyruvate kinase (L-PK, 1:200 dilution, a gift from Dr. T. Noguchi, Department of Biochemistry, Fukui Medical School, Japan) were applied for 1 hour at room temperature. The antibody to L-PK was used to demonstrate mature hepatocytes or bile-duct-like cells that have likely developed from oval cell precursors.[19] These antibodies have been shown by Western blotting to be specific to M_2-PK/M_1-PK and L-PK, respectively (raw data not shown). Before immunohistochemistry, enzyme digestion was performed with protease at 37°C for 4 minutes followed by antigen retrieval performed in citrate buffer at 350 W for 2 minutes in a microwave oven and then cooled at room temperature for 15 minutes. Endogenous peroxidase activity was blocked by treating with 3% hydrogen peroxide for 5 minutes. A horseradish-peroxidase-conjugated goat IgG secondary antibody (Diagnostics Pasteur, Marnes-la-Coquette, France) was used for detection. Using a standard graticule, five nonoverlapping fields of view were counted. Cells were scored when they satisfied the mor-

Table 1. Amount of Portal Tract Collagen and Bile Duct CK19, Numbers of Oval Cells, and SMA Grading in Rats Treated with Gd or Saline before Bile Duct Ligation or Sham Operation

Group	Collagen (pixels)	Bile duct CK19 (pixels)	Oval cells (per 100 hepatocytes)	SMA grading (median)
Saline + sham	2442 ± 285*	61 ± 28	0	0
Gd + sham	2462 ± 471	90 ± 27	0	0
Saline + BDL	2469 ± 330*	181 ± 50	35 ± 6	2+
Gd + BDL	3720 ± 448*	153 ± 56	0	2+

Portal tract collagen and bile duct CK19 were measured by image analysis.
*$P < 0.05$ (Gd + BDL *versus* saline + BDL and saline + sham groups).

phological criteria for oval cells and expressed M_2-PK. Hepatocytes in the same field of view were also scored, and the results are expressed as the number of M_2-PK-positive oval cells per 100 hepatocytes.

Myofibroblasts were demonstrated using a specific monoclonal antibody to the classical marker of the activated phenotype, SMA, as previously described.[20] Immunohistochemistry was performed on sections of formalin-fixed, paraffin-embedded liver using a Dako StreptAB complex/horseradish peroxidase kit (Dako, Santa Barbara, CA), with an anti-SMA primary antibody (1:400, clone 1A4; Sigma Chemical Co., St. Louis, MO). The secondary antibody was a biotinylated rabbit anti-mouse immunoglobulin complex (1:400; Dako), and the chromogenic substrate was diaminobenzidine tetrahydrochloride (DAB; Sigma Chemical Co.). The following histological grading system for SMA immunostaining was used. 0, normal staining pattern showing SMA expression in smooth muscle cells within portal vessel walls only, 1+, mild staining for SMA in a periportal distribution; 2+, moderate staining for SMA in a periportal distribution; 3+, septal and bridging SMA expression between portal tracts; 4+, SMA expression following cirrhotic bands linking portal tracts.

Image Analysis for Sirius Red and CK19 Staining

Tissue sections were scanned using a 20× objective and 10× eyepiece. The images were recorded on video, captured, digitized, and measured using an image analyzer system (VTPRO32 Image Analysis System, Leading Edge, Adelaide, South Australia). An average of 60 randomly selected video fields were measured within each tissue for areas of positive staining of collagen and CK19. The area measured is recorded in pixels with each pixel equating to 0.86 μm^2 of tissue section.

Serum Assay

Serum was prepared from blood samples taken at sacrifice and stored at −20°C until analyzed for AST and GGT activities and bilirubin levels. The AST, GGT, and bilirubin assays were performed using commercially available kits (Sigma Chemical Co.).

Statistical Analysis

Data are presented as the mean ± SE. The data were processed using unpaired *t*-test and analysis of variance (ANOVA). A level of significant difference was considered when $P < 0.05$.

Results

Histopathology

Coded sections were evaluated by a histopathologist (P. Hall). Seven days after surgery, saline + sham animals demonstrated no pathological abnormality, whereas the saline + BDL animals exhibited marked expansion of the portal zone by mild to moderate edema with an increased number of bile ductules and myofibroblasts. The ductules were surrounded by a mild to moderate infiltrate of polymorphonuclear leukocytes. There was a significant increase in the amount of portal tract collagen in the Gd + BDL animals compared with the saline + BDL animals at day 7 ($P < 0.05$, Table 1). Portal tract collagen measurements were similar in the saline + BDL and sham groups. There were no observable histological abnormalities in the Gd + sham animals. Gd pretreatment of BDL rats did not affect the amount of portal tract edema, infiltration with polymorphonuclear cells, or the number of bile ductules or myofibroblasts (Figures 1 and 2).

Oval cells were not detected in saline + sham or Gd + sham animals (Figure 3a). Significant numbers of oval cells were seen in saline + BDL animals (Table 1). Oval cells were observed in periportal regions and throughout the liver lobule (Figure 3b), occasionally forming ductule-like structures (Figure 3c). Gd pretreatment of BDL animals completely suppressed the appearance of oval cells (Figure 3d and Table 1).

Immunohistochemistry for L-PK demonstrated diffuse and uniform staining of hepatocytes in saline + sham and Gd + sham animals (Figure 4a). There was no detectable staining for L-PK in the bile ducts of these animals. After BDL, hepatocytes remained positive for L-PK whereas proliferating bile-duct-like structures demonstrated either positive staining for L-PK (Figure 4b) or a mixed pattern of staining with positive- and negative-staining epithelial cells in the same ductule (Figure 4c). Mature bile ducts exhibited negative staining for L-PK (Figure 4d). Gd pretreatment of BDL animals did not affect L-PK staining.

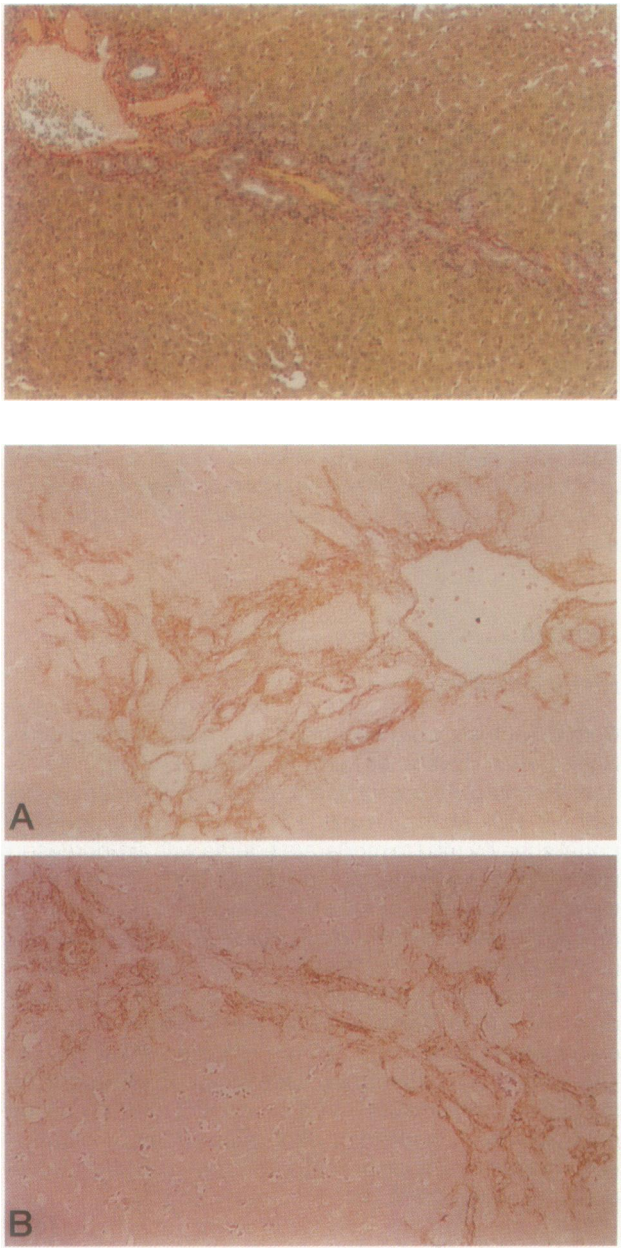

Figure 1. Hepatic histology in rats 7 days after BDL. BDL resulted in cholestatic changes within hepatocytes, moderate edema within the portal zone, and an increased number of bile ductules surrounded by an infiltrate of polymorphonuclear leukocytes. Pretreatment with Gd did not change the number of bile ductules induced by BDL. Sirius red; magnification, ×50.

Figure 2. Gd pretreatment does not change the number of hepatic myofibroblasts induced by BDL. Immunohistochemical detection of SMA in liver tissue 7 days after BDL in rats pretreated with saline (**A**) or Gd (**B**). SMA is expressed by myofibroblasts and smooth muscle cells of blood vessels. BDL caused an increase in the number of myofibroblasts in the periportal region, and these cells often surrounded areas of hyperplastic biliary epithelial cells (**A**). Pretreatment with Gd did not change the number of myofibroblasts induced by BDL (**B**). Magnification, ×50.

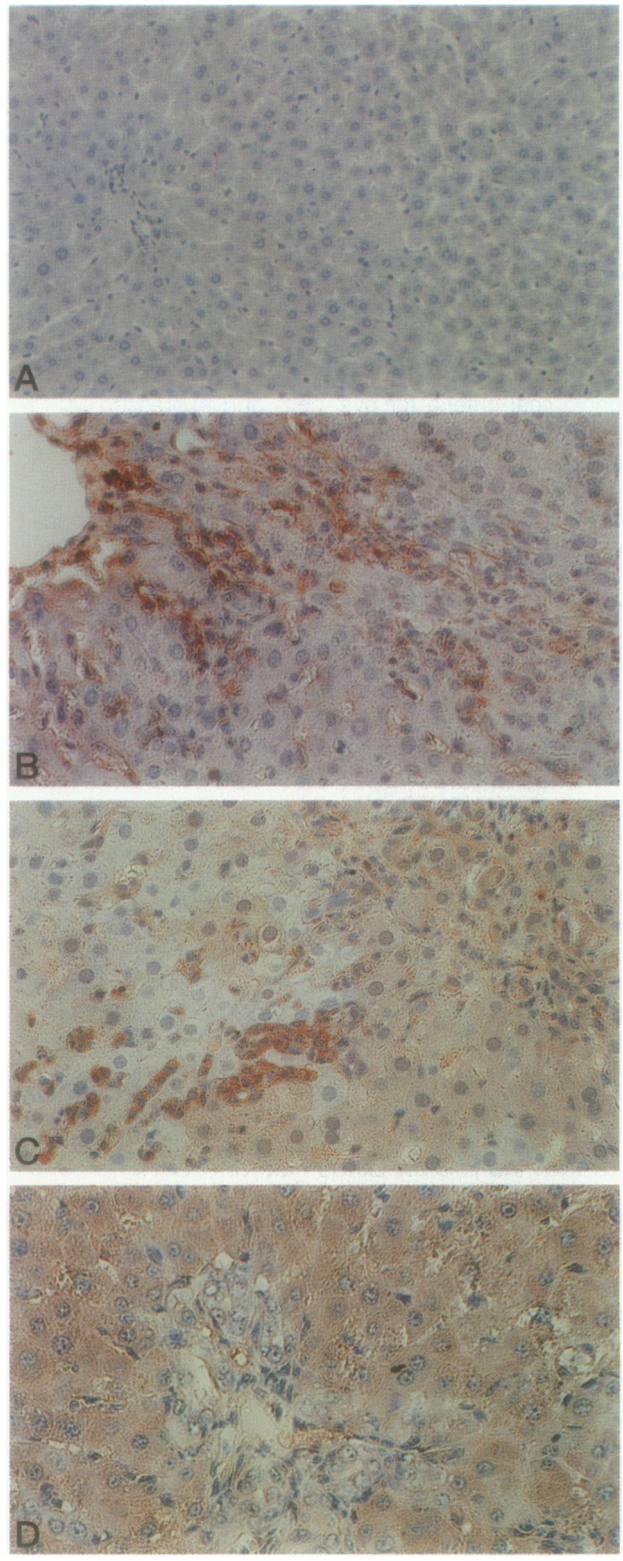

Figure 3. Gd pretreatment suppresses the appearance of oval cells induced by BDL. Oval cells were detected in liver tissue using immunohistochemical staining for M_2-PK, an oval cell marker. Oval cells were not detected in saline + sham or Gd + sham rats (**A**). Rats were pretreated with saline (**B** and **C**) or Gd (**D**) before BDL and sacrificed after 7 days. BDL resulted in the appearance of oval cells in both the portal zone and the liver lobule (**B**), occasionally forming ductular-like structures (**C**). Gd completely suppressed the emergence of oval cells in BDL rats (**D**). Magnification, ×100.

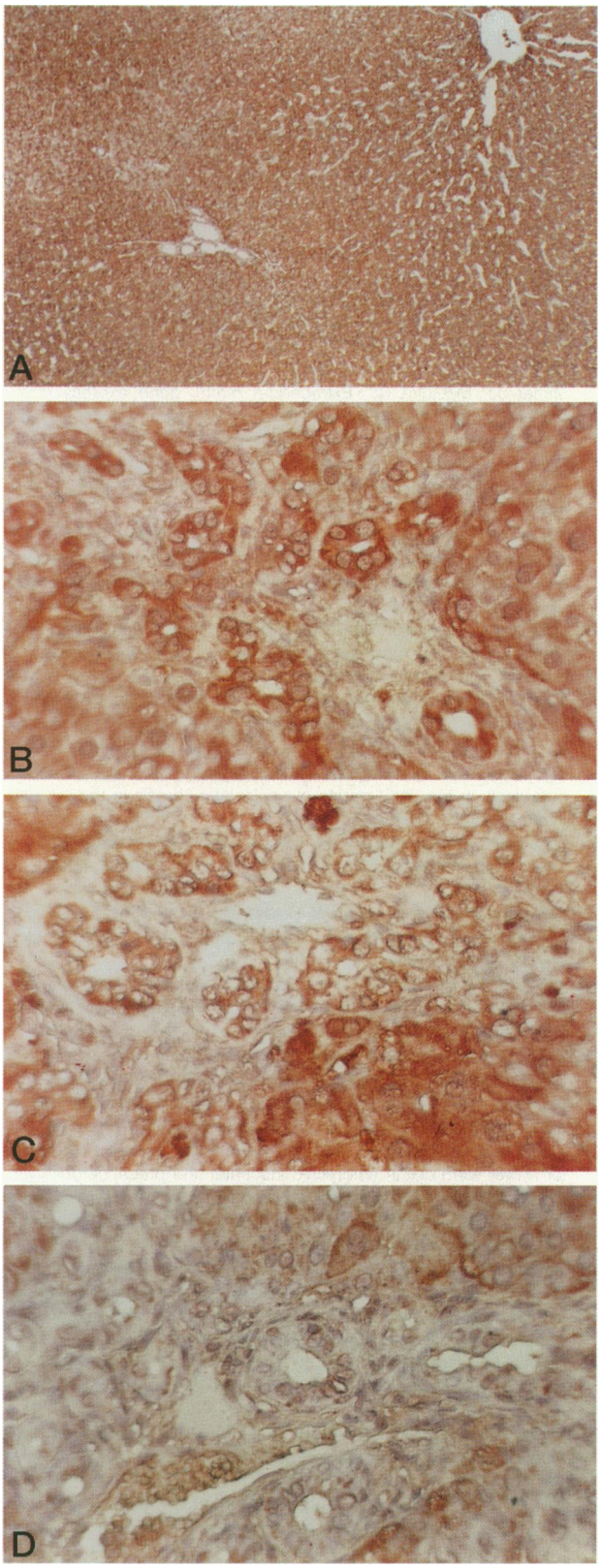

Figure 4. BDL induces L-PK staining in proliferating bile ductules. Diffuse, uniform staining for L-PK is observed in hepatocytes but not in bile ducts of saline + sham and Gd + sham animals (**A**). After BDL, proliferating ductules demonstrate either positive (**B**), mixed (**C**), or negative (**D**) staining for L-PK. Magnification, ×50 (**A**) and ×100 (**B** to **D**).

Serum AST, GGT, and Bilirubin Levels in BDL and Sham-Operated Animals

The saline + BDL and Gd + BDL rats when compared with the saline + sham and Gd + sham groups had significantly higher AST (81 ± 10 and 59 ± 12 compared with 22 ± 3 and 31 ± 3 U/L, respectively), GGT (22 ± 1 and 36 ± 6 compared with 1 ± 1 and 3 ± 0.5 U/ml, respectively), and bilirubin (7 ± 1 and 10 ± 1 compared with 0.06 ± 0.02 and 0.06 ± 0.02 mg/dl, respectively; $P < 0.01$). The Gd + BDL group had significantly elevated GGT and bilirubin levels compared with the saline + BDL group ($P < 0.05$). Serum GGT levels were also elevated in the Gd + sham animals compared with the saline + sham group ($P < 0.05$).

Discussion

This study was designed to examine the effect of BDL on oval cell proliferation and whether Kupffer cell blockade inhibits oval cell proliferation, bile ductular proliferation, and the appearance of myofibroblasts (as evidenced by SMA expression) in experimental biliary obstruction. We have shown that oval cell proliferation is induced 7 days after BDL using M_2-PK as a marker for oval cells. Kupffer cell blockade with Gd prevented the appearance of oval cells but did not affect bile ductular proliferation or the number of myofibroblasts in BDL animals. These data are consistent with the hypothesis that bile ductular proliferation after BDL occurs predominantly by replication of existing ducts rather than through proliferation and differentiation of oval cells into biliary ductules. Furthermore, these results suggest that Kupffer cells may not be essential for the occurrence of bile ductular proliferation or myofibroblastic activation after BDL as selective elimination of Kupffer cells before BDL had no effect on either of these parameters. Alternatively, a direct effect of Gd on oval cells has not been excluded in our study and could equally explain the inhibition of oval cell proliferation observed after the administration of Gd in this animal model.

In the current study, immunohistochemistry for L-PK demonstrated diffuse uniform staining of hepatocytes in the BDL and non-BDL groups. Mature bile ducts in non-BDL animals did not demonstrate staining for L-PK, consistent with the differentiated biliary epithelial phenotype. However, proliferating bile-duct-like structures, which may be derived from oval cells,[19] rather than extant bile ducts in BDL animals demonstrated mixed patterns of staining for L-PK, suggesting that biliary epithelial cells in these conditions can progress along the hepatocyte lineage.

Several studies have demonstrated evidence of hepatic fibrosis occurring within the first 2 weeks after BDL.[21,22] We observed marked increases in the numbers of bile ductules and myofibroblasts in periportal regions. The total amount of portal tract collagen was measured in sirius-red-stained sections and was not found to be increased in the saline + BDL animals. This may be due to the short time course (7 days) of our study. Our results are consistent with the previous observation of Hines et

al,[15] who demonstrated an increased number of myofibroblasts 7 days after bile duct ligation. Gd pretreatment of BDL animals resulted in significantly increased portal tract collagen and elevated GGT and bilirubin levels compared with the saline + BDL animals. These data suggest that blockade of Kupffer cells increases liver injury, which may indicate that Kupffer cells have a protective role after BDL. The exaggerated response of the liver to injury after administration of Gd has also been observed after partial hepatectomy where animals treated with Gd followed by partial hepatectomy demonstrated an increased proliferative response of hepatocytes compared with those treated with partial hepatectomy alone.[23] Taken together, these data suggest that Gd interferes with Kupffer cell mechanisms that normally restrict collagen deposition, cholestasis, and hepatocyte regeneration.

In conclusion, we have studied the effect of BDL on oval cell proliferation and whether Kupffer cell blockade inhibits oval cell proliferation, bile ductular proliferation, and myofibroblastic transformation. We have shown that oval cell proliferation is seen by day 7 after BDL. Kupffer cell blockade with Gd significantly reduced oval cell proliferation but did not affect bile ductule proliferation or myofibroblast transformation in BDL animals, supporting the hypothesis that bile ductular proliferation after BDL occurs predominantly by replication of existing ducts rather than through proliferation and differentiation of oval cells into bile-duct-like structures. Furthermore, Kupffer cells may not be essential for the occurrence of bile ductular proliferation or myofibroblast transformation after BDL. The ability of Gd pretreatment to prevent the appearance of oval cells suggests that either Kupffer cells or their products are required to facilitate oval cell proliferation or oval cells are directly affected by Gd.

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References

1. Van Eyken P, Desmet V: Ductular metaplasia of hepatocytes. Biliary and Pancreatic Ductal Epithelia: Pathobiology and Pathophysiology. Edited by Sirica AE, Longnecker DS. New York, Marcel Dekker, 1997, pp 201–228
2. Slott PA, Liu NM, Tavoloni N: Origin, pattern, and mechanism of bile duct proliferation following biliary obstruction in the rat. Gastroenterology 1990, 99:466–477
3. Factor VM, Radaeva SA, Thorgeirsson SS: Origin and fate of oval cells in dipin-induced hepatocarcinogenesis in the mouse. Am J Pathol 1994, 145:409–422
4. Sirica AE, Gainey TW, Harrell MB, Caran N: Cholangiocarcinogenesis and biliary adaptation responses in hepatic injury. Biliary and Pancreatic Ductal Epithelia: Pathobiology and Pathophysiology. Edited by Sirica AE, Longnecker DS. New York, Marcel Dekker, 1997, pp 229–290
5. Smith P, Yeoh G: Chronic iron overload in rats induces oval cells in the liver. Am J Pathol 1996, 149:389–398
6. Omori Masalo O, Evarts RP, Omori N, Hu Z, Marsden ER, Thorgeirsson SS: Expression of α-fetoprotein and stem cells factor/c-kit system in bile duct ligated young rats. Hepatology 1997, 25:1115–1122
7. Hixson DC, Fowler LC: Development and phenotypic heterogeneity of intrahepatic biliary epithelial cells. Biliary and Pancreatic Ductal Epithelia: Pathobiology and Pathophysiology. Edited by Sirica AE, Longnecker DS. New York, Marcel Dekker, 1997, pp 1–40
8. Ahern M, Hall P, Halliday J, Liddle C, Olynyk J, Ramm G, et al: Hepatic stellate cell nomenclature. Hepatology 1996, 23:193
9. Maher JJ, McGuire RF: Extracellular matrix gene expression increases preferentially in rat lipocytes and sinusoidal endothelial cells during hepatic fibrosis in vivo. J Clin Invest 1990, 86:1641–1648
10. Hines JE, Johnson SJ, Burt AD: *In vivo* responses of macrophages and perisinusoidal cells to cholestatic liver injury. Am J Pathol 1993, 142:511–518
11. Miyazaki H, van Eyken P, Roskams T, de Vos R, Desmet VJ: Transient expression of tenascin in experimentally induced cholestatic fibrosis in rat liver: an immunohistochemical study. J Hepatol 1993, 19:353–366
12. Rockey DC, Boyles JK, Gabbiani G, Friedman SL: Rat hepatic lipocytes express smooth muscle actin upon activation in vivo and in culture. J Submicrosc Cytol Pathol 1992, 34:193–203
13. Tang L, Tanaka Y, Marumo F, Sato C: Phenotypic change in portal fibroblasts in biliary fibrosis. Liver 1994, 14:76–82
14. Tuchweber B, Desmouliere A, Bochaton-Piallat M-L, Rubbia-Brandt L, Gabbiani G: Proliferation and phenotypic modulation of portal fibroblasts in the early stages of cholestatic fibrosis in the rat. Lab Invest 1996, 74:265–278
15. Hines JE, Johnson SJ, Burt AD: *In vivo* responses of macrophages and perisinusoidal cells to cholestatic liver injury. Am J Pathol 1993, 142:511–518
16. Adachi Y, Bradford BU, Gao W, Bojes HK, Thurman RD: Inactivation of Kupffer cells prevents early alcohol-induced liver injury. Hepatology 1994, 20:453–460
17. Hardonk MJ, Dijkhuis FWJ, Hulstaert CE, Koudstaal J: Heterogeneity of rat liver and spleen macrophages in gadolinium chloride-induced elimination and repopulation. J Leukocyte Biol 1992, 52:296–302
18. Iimuro Y, Yamamoto M, Kohno H, Itakura J, Fuji H, Matsumoto Y: Blockade of liver macrophages by gadolinium chloride reduces lethality in endotoxaemic rats: analysis of mechanism of lethality in endotoxaemia. J Leukocyte Biol 1994, 55:723–728
19. Tian YW, Smith PGJ, Yeoh GCT: The oval shaped cell as a candidate for a liver stem cell in embryonic, neonatal and precancerous liver: identification based on morphology and immunohistochemical staining for albumin and pyruvate kinase isoenzyme expression. Histochem Cell Biol 1997, 107:243–250
20. Ramm GA, Crawford DHG, Powell LW, Walker NI, Fletcher LM, Halliday JW: Hepatic stellate cell activation in genetic hemochromatosis: lobular distribution, effect of increasing hepatic iron and response to phlebotomy. J Hepatol 1997, 26:584–592
21. Aldana PR, Goerke ME, Carr SC, Tracy TF Jr: The expression of regenerative growth factors in chronic liver injury and repair. J Surg Res 1994, 57:711–717
22. Tracy TF Jr, Goerke ME, Bailey PV, Sotelo-Avila C, Weber TR: Growth-related gene expression in early cholestatic injury. Surgery 1993, 114:532–537
23. Rai R, Yang SQ, McClain C, Karp CL, Klein AS, Diehl AM: Kupffer cell depletion by gadolinium chloride enhances liver regeneration after partial hepatectomy in rats. Am J Physiol 1996, 270:G909–G918