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# Cell Proliferation and Oncogene Expression After Bile Duct Ligation in the Rat: Evidence of a Specific Growth Effect on Bile Duct Cells

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**The proliferative response of the rat liver was measured after temporary or permanent total biliary obstruction (BDO) and in different regions after selective ligation of the lobar ducts draining the right 60% of the hepatic mass. The results were compared with those after 70% partial hepatectomy (PH). Cell proliferation was assessed globally by measuring DNA synthesis and stratified to the separate cell populations with cytostaining techniques that allowed distinction of hepatocytes, duct cells, and nonparenchymal cells (NPCs). In selected experimental groups, gene expression was determined of transforming growth factor-$\beta$1 (TGF$\beta$-1), prothrombin, c-*erb*-B2, transforming growth factor alpha (TGF$\alpha$), human Cyclophilin (CyP), and 28S ribosomal RNA. The stimulation of a proliferative response to total BDO required obstruction for longer than 24 hours, but after this deligation did not switch off regeneration. In the first week after permanent BDO, there was progressive infiltration of NPCs, fibrous linkage of some portal areas, and a crescendo of DNA synthesis that was obvious at 24 hours, maximal at 48 hours, and back nearly to baseline at 6 days. At the 2-day mark, the bile duct cells had a 17-fold increase in proliferation, accompanied by a threefold to fourfold increase in hepatocyte renewal. Little or no increase in expression of TGF$\alpha$ or the hepatocyte-specific prothrombin gene was detectable in the first 48 hours, whereas levels of the oncogene c-*erb*-B2 that is associated with cholangiocarcinoma were expressed from 48 to 96 hours. Livers subjected to regional BDO with or without immunosuppressive treatment with FK 506 and cyclosporine had an inflammatory reaction only on the side with ligated ducts. DNA synthesis increased in both the obstructed and freely draining lobes to approximately half the level that occurred after total BDO. The proliferation of the obstructed side was similar to the mixed duct cell/hepatocyte response after total BDO, but this almost exclusively involved duct cells on the freely draining side. In contrast to the findings after BDO, livers after PH regenerated maximally at 24 hours rather than 48 hours, had a predominantly noninflammatory hepatocyte as opposed to duct cell response, and had marked expression of the prothrombin and TGF$\alpha$ genes but only weakly and late of c-*erb*-B2 messenger RNA. The results show that the liver responds as a whole and in a biologically intelligent way to the nature of the injury inflicted on any part of it. It further implies the presence of humoral communications and control networks that assure organ homeostasis and relate this to total body homeostasis. (HEPATOLOGY 1995;21:1070-1078.)**

The kinetic and morphological features of the proliferative response to bile duct ligation in rats[1-5] as well as those to 70% hepatectomy[6-8] have been extensively studied. With the hypothesis that the two response patterns reflect different mechanisms, we have compared the changes including those of oncogene expression in both experimental models. The results have confirmed our hypothesis and have shown the uncanny coordination of the whole liver response to injury to any portion.

## MATERIAL AND METHODS

### *Chemical and Biological Reagents*

Fraction V Bovine Albumin, sodium phosphate, 5 bromo-2′-deoxyuridine (BrdU), ethylenediaminetetra-acetic acid disodium salt, lauryl sulfate sodium salt, and ethidium bromide were purchased from Sigma Chemical Company (St. Louis, MO). Vectastain ABC kit PK 4002, for cell proliferation determination, was purchased from the Vectors Laboratories (Burlingame, CA); [$^3$H]-thymidine (50 to 80 Ci/mmol), from Du Pont-New England Nuclear (Boston, MA); and Aquasol (scintillant solution), from Amersham Corp. (Arlington Heights, IL). Cyclosporine and FK 506 were gifts from Sandoz Pharmaceuticals Inc. (East Hanover, NJ), and the Fujisawa Pharmaceutical Company Ltd. (Osaka, Japan), respectively. RNAzol was purchased from Biotecx, Houston, TX; labeling kits for cDNA probes, from Boehringer Mannheim Co. (India-

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Abbreviations: BrdU, 5 bromo-2′-deoxyuridine; NPC, nonparencymal cells; PH, partial hepatectomy; TGF, transforming growth factor; BDO, bile duct obstruction.

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napolis, IN); and cytokeratin 19 specific monoclonal antibody (CK19) for immunohistochemistry studies and anti-5-bromo-2′-deoxyuridine monoclonal antibody, from Dako Corporation (Carpinteria, CA). Silicone tubes for surgical technique were purchased from Portex Ltd. (Hythe, Kent, UK).

### *Animals*

Male Fischer rats (F-344) weighing 180 to 220 g were purchased from Zivic Miller Laboratories (Zelienople, PA). All the animals were maintained in a temperature- and light-controlled room (light from 6:30 AM to 6:30 PM) for at least 1 week before being used. They received food and water *ad libitum*.

### *Surgical Procedures*

Surgical procedures were performed between 8 and 10 AM, under 40 mg/kg nembutal anesthesia administered intraperitoneally.

***Temporary Bile Duct Obstruction.*** The common bile duct was isolated and transected 1 cm below the lowest tributary, taking care not to damage the pancreatic ducts. The proximal and distal duct ends were then cannulated and connected through a silicone tube loop (inner diameter 0.28 mm; outer diameter 0.61 mm). The loop was externalized through the abdominal wall and secured with two 6–0 silk threads. The central part of the externalized tube was tied off with a 6–0 silk, causing total obstruction, which was relieved by deligation from 0 to 48 hours later, restoring free bile flow through the tube and into the distal duct. The animals were killed at 48 hours for tissue collections.

***Permanent Total Bile Duct Obstruction.*** After the same dissection, the common duct was ligated and transected, with the identical technique used by Accatino et al.[2]

***Regional Bile Duct Obstruction.*** The bile duct branches of the right lateral, right and left medial, and caudate lobes were doubly ligated and divided, leaving intact the biliary drainage of the left lobe (Fig. 1), which constitutes approximately 40% of the rat liver mass. The absence of ductal cross-communication between the obstructed and nonobstructed lobes was proved with methylene blue injections (Fig. 2). The animals were killed 2 days later for tissue collections.

***Regional Bile Duct Obstruction Plus Immunosuppression.*** Cyclosporine and FK 506 have been shown to augment regeneration after partial hepatectomy[9-11] and portacaval shunt.[11-13] The mechanisms of these hepatotrophic effects are not known. However, they may be by immune modulation of the nonparenchymal cells (NPC). Thus, exactly the same experiment as that just described was performed except that the rats were pretreated for 4 days before operation and the first 2 days afterward with cyclosporine (10 mg/kg/day orally) or FK 506 (1 mg/kg/day intramuscularly).

***Partial Hepatectomy.*** Seventy percent partial hepatectomy (PH) was performed as described by Higgins and Anderson.[14] Thirty animals (three for each group) underwent 70% PH and were killed after 12, 24, 36, 48 hours, and 3, 4, 5, 6, 7, and 8 days.

***Sham Operation.*** Twenty control rats underwent laparotomy only. Ten animals were killed at 0 hours and 10 at 48 hours after the surgery.

## EXPERIMENTAL DESIGN

The groups are summarized in Table 1. In essence, the proliferative and gene expression response to total biliary obstruction (group 3) and the duration of obstruction necessary to evoke these responses (group 2) were defined. In addition, the effect of lobar biliary obstruction on the obstructed versus the drained portion of the liver was determined in untreated (group 4) and immunosuppressed animals (group 5). The results were compared with those in rats after sham operations (group 1 and specific group controls) and with those after 70% hepatectomy (group 6).

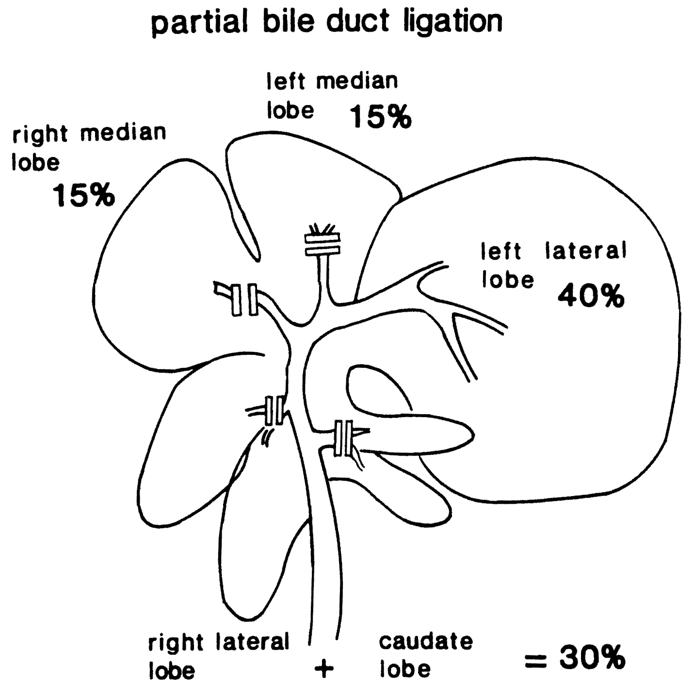


FIG. 1. Regional biliary duct obstruction procedure that permits the study of obstructed and non-obstructed liver in the rat. The drained non-cholestatic portion is approximately 40% of the liver mass.

### *Experimental End Points*

All analyses were of liver fragments obtained at killing from multiple lobes. The specimens for studies of gene expression or DNA synthesis were snap frozen and stored at −80°C.

***Assessment of Cell Proliferation.*** The animals whose DNA synthesis was measured chemically by thymidine incorporation were given an intraperitoneal injection of 200 $\mu$Ci/kg $^3$H-thymidine 2 hours before killing. Frozen samples were analyzed as previously reported.[15]

Animals studied histopathologically for localization of the proliferating cells were injected intraperitoneally with 120 mg/kg BrdU 2 hours before killing. Liver samples, 5 mm thick, from the different lobes were fixed in buffered formalin, imbedded in paraffin, sectioned at 4 $\mu$m, and stained with hematoxylin-eosin and trichrome.[16] BrdU incorporation was detected with a monoclonal anti-BrdU antibody at a 1:20 dilution.[17-19] The reaction product was developed by 3-amino-9-ethylcarbazole (AEC).[20] Bile duct epithelial cell proliferation was determined in 10 randomly selected portal areas in which the number of nuclear labeled and unlabeled epithelial cells was counted. At least 500 cell counts were obtained from each liver lobe. To measure hepatocyte proliferation, 25 parenchymal fields of 1,000 hepatocytes were counted, from which the percent of BrdU-positive cells was determined.


FIG. 2. Evidence of lack of ductal cross-communication between obstructed and non-obstructed lobes proved with methylene-blue injection into the common bile duct. Right lobe obstructed and left lobe nonobstructed.

The number of inflammatory cells was counted in the regional bile duct obstruction experiment 7 days after surgery. In addition, cytokeratin 19 expression (C-K 19; mol wt 40.000 in the catalogue of Moll et al[21]) was used as a specific marker of bile duct cells.[22-24]

***Gene Expression Determination.*** These studies were performed with previously described methods.[25] Briefly, total cellular RNA from frozen hepatic tissue samples was extracted by RNAzol using the manufacturer's procedure. Total RNA (20 $\mu$g) was subjected to electrophoresis in a 1% agarose gel and then transferred to Zitabind nylon membrane (Whatman Co., Maidstone, UK) overnight in 20× standard saline citrate. After transfer, the blot was fixed by ultraviolet light (short wave, 254 nm). Complementary DNA probes were labeled with $^{32}$P with a random primed labeling kit. Prehybridization and hybridization were performed at 70°C with Church buffer (1% bovine serum albumin 7% sulfate sodium salt, 0.5 mol/L sodium phosphate, 1 mmol/L ethylenediaminetetra-acetic acid). The membranes then were washed, air-dried, and autoradiographed at −70°C. To check for equivalent transfer of RNAs from agarose gel to membrane, the gel was stained with ethidium bromide after capillary transfer and examined; little RNA was found to remain in any lane. The probes used were c-*raf* (purchased from American Type Culture Collection, Rockville, MD); transforming growth factor-$\beta$1 (TGF-$\beta$1) (gift from R. Derynk; Genetech, San Francisco, CA); prothrombin (gift from S. J. F. Degen, University of Pittsburgh, PA); c-*erb*-B2 (gift from Wataru Yasui, Hiroshima University, Japan), rodent TGF$\alpha$ (gift from G. Lee, University of North Carolina). The TGF-alpha and $\beta$ and c-*raf* probes were selected because they have been frequently used to study posthepatectomy regeneration.[6,26] The c-*erb*-B2 probe was selected because this gene, like c-*raf*, has been specifically associated with cholangiocarcinoma.[27] For internal controls, we used 28S ribosomal RNA (Oncogene Science, Inc., Uniondale, NY), and human cyclophilin G (gift from C. T. Walsh, Harvard University, Cambridge, MA).

## STATISTICAL ANALYSIS

Statistical analysis was performed using a two-way ANOVA (Epistat software) available on IBM (Danbury, CT) PC and assuming statistical significance only with a $P < .05$. Data were expressed as (M ± SD).

## RESULTS

### *Total Bile Duct Obstruction*

***Standard Histopathology.*** The livers of sham-operated rats were normal. Livers with biliary obstruction had a light infiltration of inflammatory cells in the portal areas by 2 days. By day 7, there were signs of intensive proliferation and formation of new bile ducts (Fig. 3), which were surrounded by thin fibrous connective tissues linking some portal areas, as has been reported.[1-5,28,29] The hyperplastic ducts as well as the normal ducts and ductules in the sham-operated livers were positive for cytokeratin 19, whereas hepatocytes were always negative (data not shown) as reported by Alpini et al.[23,24]

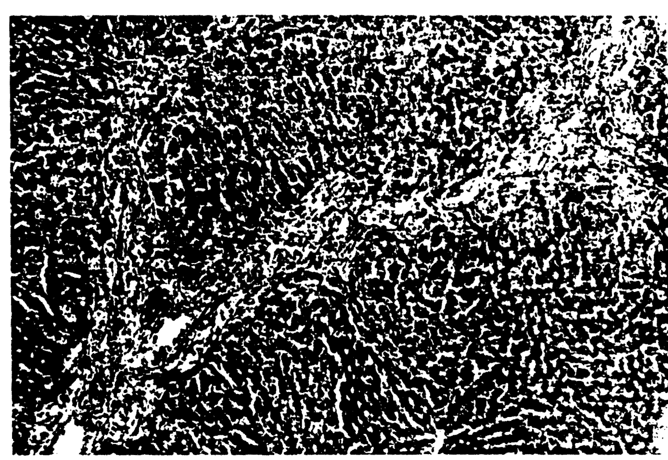

FIG. 3. Rat liver 7 days after common bile duct ligation. Proliferation of bile ducts and nonparenchimal liver cells, that form bridging connection between portal areas. (Trichrome stain; original magnification ×100.)

TABLE 1. Study Groups

| Group | N | Day Killed | Liver Tissue Studies |
|---|---|---|---|
| 1. Sham-operated rats | 10 | 0 | BrdU (n = 5 each time) |
| | 10 | 2 | $^3$H-Thymidine (n = 5 each time) |
| | | | Gene expression (n = 5 each time) |
| 2. T-BDO (for 0, $\frac{1}{2}$, 1, $1\frac{1}{2}$, or 2 days) | 25 | 2 | BrdU (n = 5 each subgroup) |
| 3. BDO | 80 | $\frac{1}{2}$, 1, $1\frac{1}{2}$, 2 | BrdU (n = 3 each group) |
| | | 3, 4, 5, 6, 7, 8 | $^3$H-Thymidine (n = 5 each group) |
| | | | Gene expression (n = 3 each group) |
| 4. R-BDO | 20 | 2 | BrdU (n = 10) |
| | 5 | 7 | $^3$H-Thymidine (n = 10) |
| | | | Inflammatory cells (n = 5) |
| 5. R-BDO* | 20 | 2 | BrdU (n = 10) |
| CyA | | | $^3$H-Thymidine (n = 10) |
| FK506 | | | |
| 6. 70% hepatectomy | 30 | $\frac{1}{2}$, 1, $1\frac{1}{2}$, 2 | BrdU (n = 3 each group) |
| | | 3, 4, 5, 6, 7, 8 | Gene expression (n = 3 each group) |

Abbreviations: T-BDO, temporary bile duct obstruction (total); BDO, bile duct obstruction (total); R-BDO, regional bile duct obstruction; BrdU, 5-bromodeoxyuridine.

* See text for drug dose schedule.

***DNA Synthesis.*** A crescendo of DNA synthesis began at 24 hours, was maximal at 2 days, and receded almost to baseline by day 6 (Fig. 4).

***BrdU Incorporation.*** The increased DNA synthesis could be seen in the nuclear labeled BrdU-positive cells to be mainly a reflection of duct cell proliferation rather than of hepatocytes (Fig. 5). In the animals of group 3, 17% of bile duct cells were labeled at the peak of the response at 2 days compared with 1% at the outset. A much smaller response, reaching 4.8% at 2 days, was seen in the hepatocytes of these livers (Fig. 6, top panels).

These results were strikingly different than those following 70% PH (Fig. 6, lower panels). With PH, a bile duct response also promptly occurred, but it was short-lived compared with bile duct obstruction (BDO). In addition, the percentage of BrdU-positive hepatocytes was more than 5 times greater than after BDO (Fig. 6).

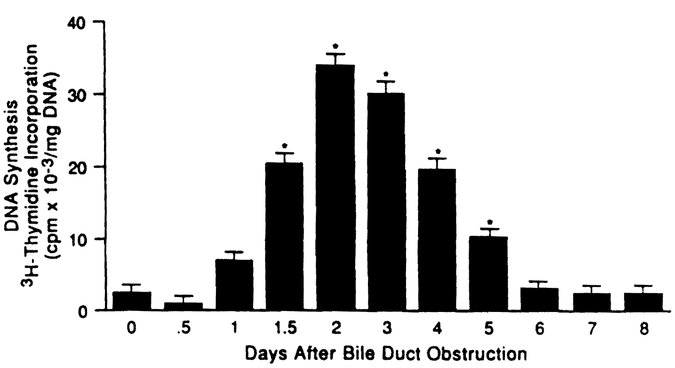


FIG. 4. DNA synthesis (mean ± SD) on successive days after total bile duct obstruction (n = 5 rats at each time point). *$P < .005$ vs. 0 time control.

***Required Time of Obstruction.*** In the animals of group 2, biliary obstruction for 12 hours or even 24 hours did not cause significant increases in BrdU-positive cells in the livers obtained at killing at 2 days. However, with obstruction for 36 hours that was then relieved or with continuous obstruction throughout the 48-hour duration of the experiment, the duct cell proliferation (>15% of total) was similar to that found in the animals of group 3 (Fig. 7).

***Gene Expression.*** Hepatic gene expression was different after BDO than after 70% PH, and the differences had a physiologic correlation. The features that appeared to be most typical of BDO during the first 48 hours were lack of expression of TGF$\alpha$, weak and delayed prothrombin gene expression, but with prompt

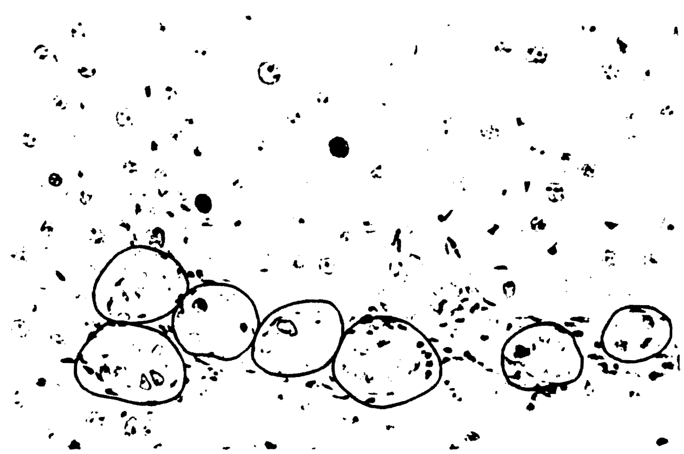

FIG. 5. Rat liver 6 days after common bile duct ligation. BrdU incorporation in proliferating bile duct epithelial cells (circled) and hepatocytes. (Immunostain with BrdU-antibodies and hematoxylin; original magnification ×400.)

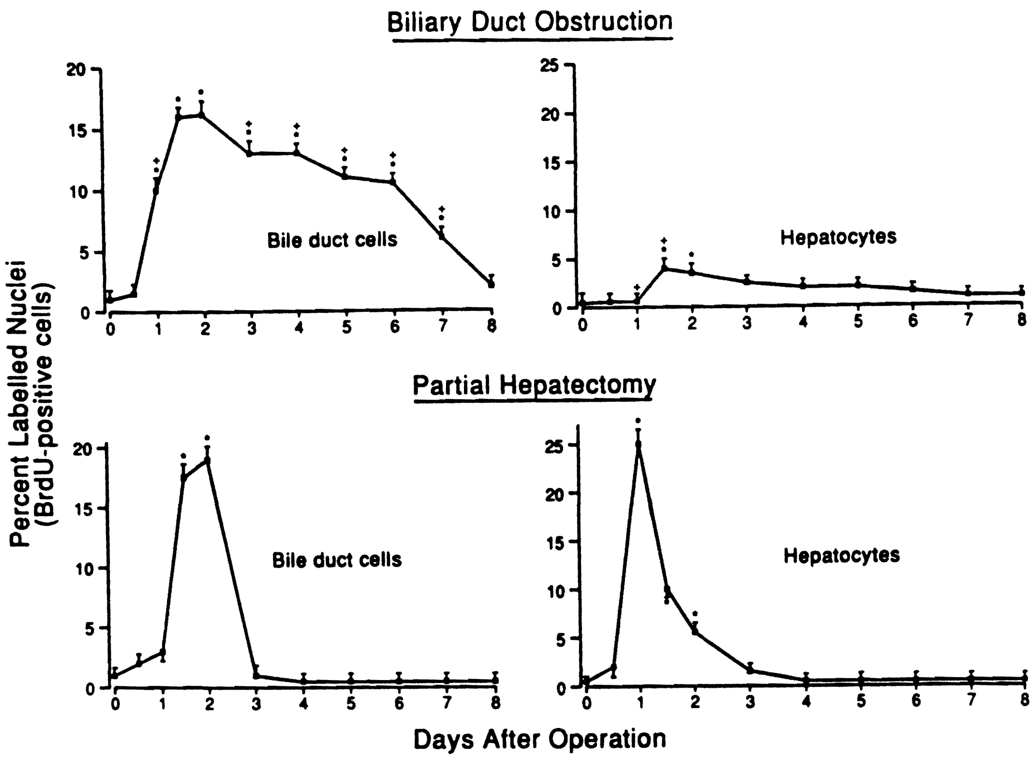


FIG. 6. Percent (mean ± SD) of hepatocytes and bile duct cells with nuclear labeling with BrdU at successive times after total bile duct obstruction (upper) and 70% hepatectomy (lower) (n = 3 rats each time point). The control value (0 time) is the mean average of five sham-operated rats. *$P < .005$ vs. sham controls. †$P < .005$ vs. value of PH rats at same time point.

appearance of c-*erb*-B2 messenger RNA. In contrast, the typical response to PH was strong expression of TGF$\alpha$ and prothrombin by 12 hours but without the appearance of c-*erb*-B2 messenger RNA.

Essentially no increase of TGF$\alpha$, a potent hepatocyte mitogen, was detectable during the first 48 hours after BDO when DNA synthesis was maximum, whereas TGF$\alpha$ was markedly increased after PH (Fig. 8). Elevated levels of c-*erb*-B2, an oncogene associated with cholangiocarcinoma,[27] were clearly expressed from 48 to 96 hours after BDO, but appeared only at 72 hours after PH (Fig. 9). c-*raf*, a proto-oncogene associated with hepatocytes proliferation after PH,[26] and also with cholangiocarcinoma,[27] increased significantly 48 to 96 hours after BDO, whereas it was slightly detectable after PH (Fig. 9). Interestingly, the expression of the hepatocyte-specific prothrombin gene was enhanced during the increased DNA synthesis and mitoses after PH, but not after BDO (Fig. 10). TGF-$\beta$1 messenger RNA expression was similar both after PH and BDO (Fig. 11) and was similar to that previously reported for PH.[6]

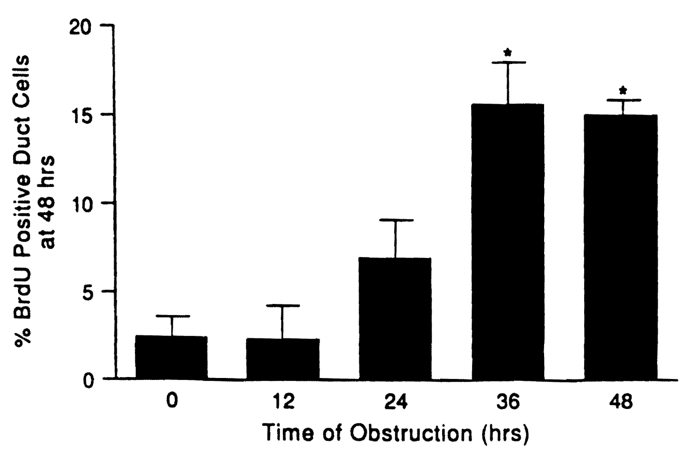


FIG. 7. Effect of total biliary obstructions for different durations on the percent of BrdU nuclear labeling of duct cells (mean ± SD) in animals killed at 2 days (n = 5 rats at each time point). *$P < .005$ vs. value at time 0.

### *Regional Bile Duct Obstruction*

***Standard Histopathology.*** The obstructed liver lobes in the rats of group 4 at 7 days had early changes similar to those in the totally obstructed livers of group

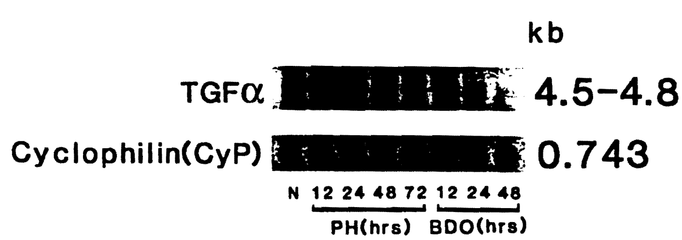


FIG. 8. Northern blot analysis of RNA from rat liver at different times after PH and BDO, and hybridized to probes for TGF$\alpha$ or cyclophilin (n = 3 rats for each time point).

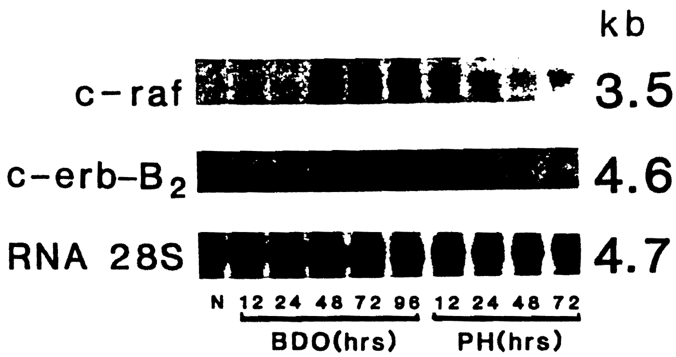


FIG. 9. Northern blot analysis of RNA from rat liver at different times after PH or BDO, and hybridized to probes for *c-raf* or *c-erb-*B2.

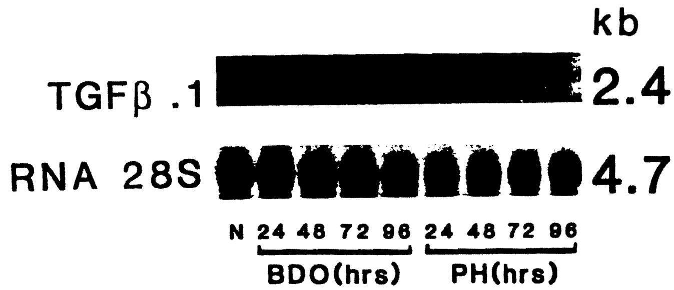


FIG. 11. Northern blot analysis of RNA from rat liver at different times after PH and BDO, and hybridized to the probe of TGF-$\beta$1.

3, including the appearance of inflammatory cells (Table 2). The freely draining left lobes had a normal number of inflammatory cells, but there was striking proliferation and formation of new ducts.

***DNA Synthesis.*** At 48 hours, which was the time of the maximal proliferative response in the total biliary obstruction experiments previously described, DNA synthesis was significantly and almost equally elevated, in the obstructed as opposed to the freely draining lobes (Fig. 12). The magnitude of DNA response in both locations was less than half that caused by total biliary obstruction.

***BrdU Incorporation.*** As in the total biliary obstruction experiments of group 3, the predominant proliferative response was that of the duct cells. This was true both in the obstructed and freely draining lobes (Fig. 13). Interestingly, increased hepatocyte proliferation occurred only in the obstructed lobes. In the drained lobes, hepatocyte proliferation was negligible although significantly more than in the sham-operated controls (Fig. 13).

***Addition of Immunosuppression.*** Preoperative and postoperative treatment with cyclosporine and FK 506 did not significantly affect the results (data not shown).

## DISCUSSION

The literature on an increasing number of hepatic growth factors has accumulated rapidly in the last two decades.[6-8,30] However, this information has not been synthesized into a generally accepted explanation of hepatic regeneration. Because hepatocytes, biliary duct cells, and NPCs have long been known to have different kinetic orders of proliferation,[31] a reasonable implication is that they are variably cross-regulated. NPCs have been particularly attractive candidates for such a role.[32-36]

The biliary obstruction models have lent themselves well to proliferation studies of the duct cell component of the liver. These cells constitute approximately 30% of the total liver mass. Previous investigations have defined the morphologic consequences of duct obstruction, which include pronounced ductular hyperplasia, a modest hepatocyte proliferative response, and infiltration of inflammatory cells.[1-5,23,24] These findings were confirmed in rat livers after common duct ligation and in liver lobes that were selectively obstructed. The duct cell and hepatocyte proliferative responses were not simultaneous but they were closely related temporally. These were compared with the changes after 70% partial hepatectomy.

With PH, the duct cells and hepatocytes both participated vigorously in the regeneration, the peak hepatocyte response being at 24 hours, with the duct cells only slightly behind. The global proliferative response to BDO or regional BDO as defined by DNA synthesis was similar to PH except that its peak was at 2 days instead of 1, with a slower return to baseline. However, the differences between the PH and BDO models were


FIG. 10. Northern blot analysis of RNA from rat liver at different times after PH and BDO, and hybridized to the probe of prothrombin.

**TABLE 2. Inflammatory Cells Infiltrating Portal Tracts in Sham-Operated and Drained Versus Nondrained Lobes 7 Days After Regional Bile Duct Ligation Operated Rats**

| | Sham Rat Lobes | Cholestatic Lobes | Noncholestatic Lobes |
|---|---|---|---|
| Neutrophils | 0.17 ± 0.03 | 3.98 ± 2.5* | 0.28 ± 0.1 |
| Macrophages | 7.2 ± 3.0 | 22.7 ± 6.8* | 8.9 ± 3.5 |
| Lymphocytes | 0.6 ± 0.4 | 1.4 ± 0.8 | 0.9 ± 0.5 |

NOTE. The values, M ± SD, are the inflammatory cell numbers observed in 0.02 $mm^2$ of the portal areas.

* Significantly different from the sham and noncholestatic lobes, $P < .05$.

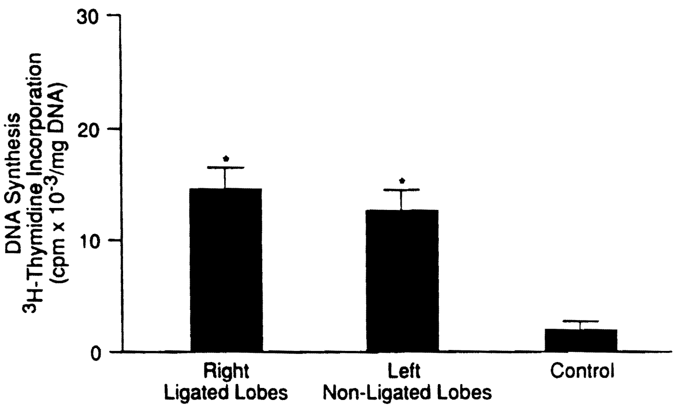


FIG. 12. DNA synthesis (mean ± SD) in ligated and nonligated liver lobes of rats with regional biliary tree obstruction (n = 10 rats for the experimental bars and 5 for controls). *$P < .005$ vs. controls.

more than temporal. Cell labeling studies demonstrated that most of the heightened DNA synthesis after biliary obstruction was occurring in the duct cells, with only a minor contribution from the hepatocytes. The required stimulus in the BDO model was continuation of the obstruction for more than a day. Blockage for 24 hours or less was without significant effect at the time of killing at 2 days.

Hypotheses have been proposed to explain the hyperplastic duct cell response to biliary obstruction in humans and animals. Excluding the possibility that there are pluripotent (oval) stem cells,[37] these fall into two general categories. In one of these generic alternatives, Slott et al[4] suggested that proliferating hepatocytes were able to invade the basal membrane of the obstructed duct, taking up residence in the epithelium, where they differentiated to the phenotypic characteristics of biliary duct cells. Our experiments provided no support for this concept. Instead, the primary proliferative response of the totally obstructed liver was of the duct cells, the hepatocytes being relatively minor participants. The observations in the regional biliary obstruction experiments were considerably more damaging to the ectopic hepatocyte hypothesis. Here, the duct cell proliferation in the ostensibly healthy freely draining lobes was of the same magnitude as in the lobes with ligated ducts. In both the draining and cholestatic lobes, the muted hepatocyte response (which was slightly greater in the obstructed lobes) presumably was in response to partial obstruction to the injury caused unilaterally by the duct ligation. This response to partial obstruction was only about half of that caused when the entire biliary system was occluded, as would be expected with the proportionately less complete damage to the parenchyma.

In an alternative general hypothesis explaining the ductular hyperplasia after biliary obstruction, Buyssens[33] has proposed a contributory or regulatory role of NPC by-products of the lipooxygenase pathway,[34] which are chemotactic for and activate polymorphonuclear leukocytes[30,31] as well as multiple cytokines. A histopathologic basis for such a mechanism was evident in that there was a striking leukocyte inflammatory reaction that appeared to include multiple NPC lineages in the obstructed liver tissue, whether this obstruction was of the whole organ or only part of it. However, in the partial obstruction experiments the vigorous duct cell proliferation in the freely draining lobes that did not have a significant inflammatory reaction showed that the local presence of NPCs was not a direct paracrine requirement for stimulation of duct cell hyperplasia, while not ruling out a humorally transmitted effect of cytokines from leukocytes on the contralateral side. The role of these immunologically capable NPCs in the events after duct ligation remains to be determined. Although the T-cell–directed drugs, cyclosporine and FK 506, both augment the proliferation associated with hepatectomy[9-11] and Eck's fistula,[11-13] these immunosuppressive agents did not affect the outcome of the regeneration after regional biliary obstruction. If a stem cell actually exists,[37] it could be accommodated by this paradigm.

Whether or not an immune component was involved,

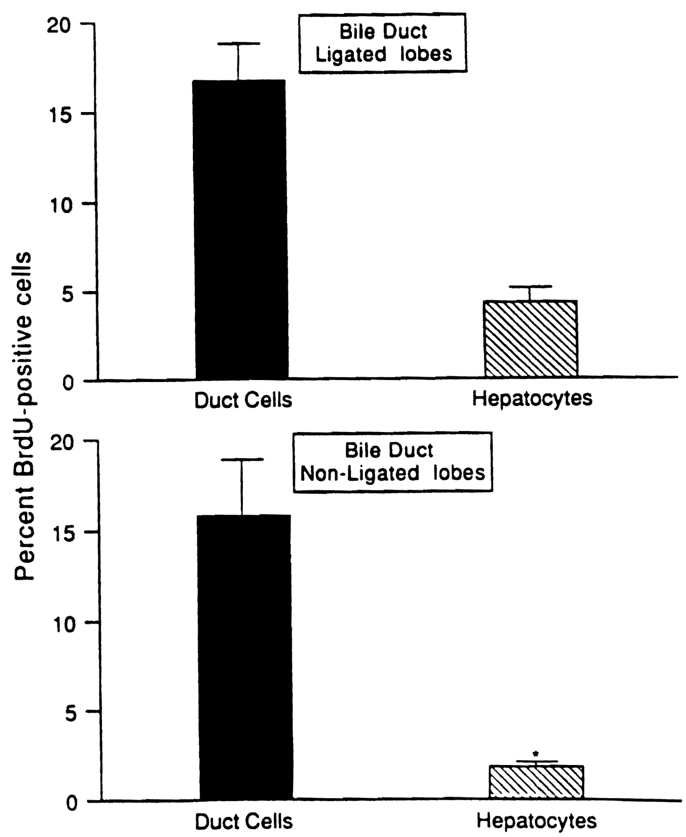


FIG. 13. Ligated lobe of rat liver 48 hours after regional bile duct ligation. BrdU incorporation in proliferating bile duct epithelial cells and hepatocytes. *$P < .05$ vs. hepatocytes in sham operated rats. **$P < .01$ vs. hepatocytes in nonligated lobes. (Immunostain with BrdU-antibodies and hematoxylin; original magnification ×400.)

the regional biliary obstruction experiments showed the intelligence with which one part of the liver communicates with another. This has been noted before in auxiliary liver transplant models[38] and in "double liver" preparations in which one liver fragment is provided a physiologic advantage such as splanchnic venous blood that is rich in hepatotrophic factors.[39] Under such circumstances, there is increased proliferation by the "disadvantaged" liver or fragment, but this is minor in the long run compared with the compensatory hyperplasia and hypertrophy in the advantaged hepatic tissue.[40-42] The coregulation between the coexisting livers or liver fragments results in the eventual atrophy of the damaged liver fragment, regeneration of the healthy one, and maintenance of the original total hepatic mass. Although slower in evolution than with regional portal blood deprivation, this also is the well-known outcome weeks or months after lobar duct ligation in animals[28,43] and humans.[44-46]

The specificity of the cross-talk between liver fragments was evident in the regional duct ligation experiments. Here, the acute primary insult to the duct cells in one part of the liver was promptly sensed and responded to by duct cell hyperplasia in the affected as well as the unaffected liver. This consequence could reflect a unique humorally transmitted signal from a stimulus such as but not limited to increased intraductal hydrostatic pressure,[3-5] in which inflammatory cells could play an intermediary role. Alternatively, the possibility cannot be ruled out that there are separate and highly specific growth factors other than cytokines governing duct cell regeneration.

The dissociation of messenger RNA expression of the four growth factors studied was striking and correlated with the physiologic end points. The complete absence of TGF$\alpha$ expression was striking in animals whose global DNA response to BDO was essentially equivalent to that of animals with 70% PH, but with a different temporal and cell target profile. The early expression of c-*erb*-B2 with BDO only was of particular interest because of the association of this oncogene with obstructing cholangiocarcinoma.[27] It will be worthwhile to do systematic studies of this oncogene in biopsy specimens of other duct-specific diseases. In addition, it will be important to determine the more complete range of gene activation caused by BDO, which may prove to be multiple, as has already been shown after PH.[47]

## REFERENCES


1. Tramas EG, Symeonidis A. Morphologic and functional changes in the livers of rats after ligation or excision of the common bile duct. Am J Pathol 1957;33:13-27.
2. Accatino L, Contreras A, Fernandez S, Quintana C. The effect of complete biliary obstruction on bile flow and bile acid excretion: postcholestatic choleresis in the rat. J Lab Clin Med 1979;93:706-717.
3. Alpini G, Lenzi R, Sarkozi L, Tavoloni N. Biliary physiology in rats with bile ductular cell hyperplasia: evidence for a secretory function of proliferated bile ductules. J Clin Invest 1988;81:569-578.
4. Slott PA, Liu MH, Tavoloni N. Origin, pattern and mechanism of bile duct proliferation following biliary obstruction in the rat. Gastroenterology 1990;99:466-477.
5. Shibayama Y. Factors producing bile infarction and bile duct proliferation in biliary obstruction. J Pathol 1990;160:57-62.
6. Fausto N. Liver regeneration. In: Zakim D, Boyer TD, eds. *Hepatology: a textbook of liver disease.* (Ed 2). Philadelphia: Saunders, 1990:49-65.
7. Michalopoulos GK. Liver regeneration: molecular mechanism of growth control. FASEB J 1990;4:176-87.
8. Francavilla A, Starzl TE, Van Thiel DH, Barone M, Polimeno L. Hepatic regeneration. In: Lebouton AV, ed. *Molecular and cell biology of the liver.* Boca Raton, FL: CRC Press, 1993:309-346.
9. Makowka L, Scanas G, Esquivel C, Venkataramanan R, Todo S, Iwatsuki S, Van Thiel DH, et al. The effect of cyclosporine on hepatic regeneration. Surg Forum 1986;37:352.
10. Francavilla A, Barone M, Todo S, Zeng DH, Porter KA, Starzl TE. Augmentation of rat liver regeneration by FK 506 compared with cyclosporine. Lancet 1989;2:1248-1249.
11. Francavilla A, Starzl TE, Carr B, Azzarone A, Carrieri G, Zeng QH, Porter KA. The effects of FK 506, cyclosporine and rapamycin on liver growth *in vitro* and *in vivo.* Transplant Proc 1991;23:2817-2820.
12. Mazzaferro V, Porter KA, Scotti-Foglieni CL, Venkataramanan R, Makowka L, Rossaro L, Francavilla A, et al. The hepatotrophic influence of cyclosporine. Surgery 1990;107:533-539.
13. Starzl TE, Porter KA, Mazzaferro V, Todo S, Fung J, Francavilla A. Hepatotrophic effects of FK506 in dogs. Transplantation 1991;51:67-70.
14. Higgins GM, Anderson RM. Experimental pathology of the liver. I. Restoration of the liver of the white rat following partial surgical removal. Arch Pathol 1931;12:186-202.
15. Francavilla A, Eagon PK, Di Leo A, Polimeno L, Panella C, Aquilino AM, Ingrosso M, et al. Sex hormone related functions in regenerating male rat liver. Gastroenterology 1986;91:1263-1270.
16. Brandbury P, Gordon KC. Connective tissue and stain. In: Bancroft JD, Stevens A, eds. *Theory and Practice of Histological Techniques.* New York: Churchill Livingstone, 1990:119-142.
17. Morstyn AG, Hsu SM, Kinsella T, Russo A, Gratzner H, Mitchell J. Bromodeoxyuridine in tumors and chromosomes detected with a monoclonal antibody. J Clin Invest 1983;73:1844-1850.
18. Wilson GD. Assessment of human tumor proliferation using bromodeoxyuridine-current status. Acta Oncol 1991;30:903-910.
19. Sasaki A, Naganuma H, Kimura R, Isoe S, Nakano S, Nukui H, Suzuki K, et al. Proliferating cell nuclear antigen (PCNA) immunostaining as an alternative to bromodeoxyuridine (BrdU) immunostaining for brain tumours in paraffin embedded sections. Acta Neurochir 1992;117:178-181.
20. Graham RC, Lindholm V, Karnovsky MJ. Cytochemical demonstration of peroxidase activity with 3-amino-9-ethylcarbazole. J Histochem Cytochem 1965;13:150-156.
21. Moll R, Franke WW, Schiller DL. The catalog of human cytokeratins: patterns of expression in normal epithelia, tumors, and cultured cells. Cell 1982;31:11-24.
22. Van Eyken P, Sciot R, Von Damme B, DeWolf-Peeters C, Desmet VJ. Keratin immunohistochemistry in normal human liver: cytokeratin pattern of hepatocytes, bile ducts and acinar gradient. Virchows Arch [A] 1987;412:63-72.
23. Alpini G, Lenzi R, Zhai WR, Slott PA, Liu MH, Sarkozi L, Tavoloni N. Bile secretory function of infrahepatic biliary epithelium in the rat. Am J Physiol 1989;257:G124-G133.
24. Alpini G, Lenzi R, Zhai WR, Liu MH, Slott PA, Paronetto F, Tavoloni N. Isolation of a nonparenchymal liver cell fraction enriched in cells with biliary epithelial phenotypes. Gastroenterology 1989;97:1248-1260.
25. Carr BI, Huang TH, Itakura K, Neel M, Marceau N. TGF$\beta$ gene transcription in normal and neoplastic liver growth. J Cell Biochem 1989;39:477-487.
26. Silverman JA, Zurlo J, Watson MA, Yager JD. Expression of c-*raf*-1 and A-*raf*-1 during regeneration of rat liver following surgi-

cal partial hepatectomy. Molecular Carcinogenesis 1989;2:63-67.

27. Voravud N, Foster S, Gilbertson JA, Sikora K, Waxman J. Oncogene expression in cholangiocarcinoma and in normal hepatic development. Hum Pathol 1989;20:1163-1168.
28. Kountouras J, Billing TH. Prolonged bile duct obstruction: a new experimental model for cirrhosis in the rat. Br J Exp Pathol 1984;65:305-311.
29. Marucci L, Baroni GS, Mancini R, Benedetti A, Jezequel AM, Orlandi F. Cell proliferation following extrahepatic biliary obstruction: evaluation by immunohistochemical method. J Hepatol 1993;17:163-169.
30. Francavilla A, Starzl TE, Porter K, Foglieni CS, Michalopoulos GK, Carrieri G, Trejo J, et al. Screening for candidate hepatic growth factors by selective portal infusion after canine eck fistula. HEPATOLOGY 1991;14:665-670.
31. Grisham JW. Morphologic study of deoxyribonucleic acid synthesis and cell proliferation in regenerating rat liver: autoradiography with 3H-thymidine. Cancer Res 1962;22:842-847.
32. Nakamura T, Arakaki R, Ichihara A. Interleukin-1B is a potent growth inhibitor of adult rat hepatocytes in primary culture. Exp Cell Res 1988;179:488-497.
33. Buyssens N. Ductular proliferation. Gastroenterology 1965;49:702-706.
34. Vane JR. Inhibition of prostaglandin synthesis as a mechanism of action for aspirin-like drugs. Nature (Lond) 1971;231:232-235.
35. Spector AA, Gordon JA, Moore SA. Hydroxyeicosatetraenoic acids (HETEs). Prog Lipid Res 1988;27:271-323.
36. Ford-Hutchinson AW. Leukotrienes: their formation and role as inflammatory mediators. FASEB Monogr 1985;44:25-29.
37. Travis J. The search for liver stem cells picks up. Science 1993;259:1829.
38. Marchioro TL, Porter KA, Dickinson TC, Faris TD, Starzl TE. Physiologic requirements for auxiliary liver homotransplantation. Surg Gynecol Obstet 1965;121:17-31.
39. Starzl TE, Francavilla A, Halgrimson CG, Francavilla FR, Porter KA, Brown TH, Putnam CW. The origin, hormonal nature, and action of hepatotrophic substances in portal venous blood. Surg Gynecol Obstet 1973;137:179-199.
40. Starzl TE, Lee IY, Porter KA, Putnam CW. The influence of portal blood upon lipid metabolism in normal and diabetic dogs and baboons. Surg Gynecol Obstet 1975;140:381-396.
41. Starzl TE, Porter KA, Kashiwagi N, Lee IY, Russell WJI, Putnam CW. The effect of diabetes mellitus on portal blood hepatotrophic factors in dogs. Surg Gynecol Obstet 1975;140:549-562.
42. Starzl TE, Porter KA, Kashiwagi N, Putnam CW. Portal hepatotrophic factors, diabetes mellitus and acute liver atrophy, hypertrophy and regeneration. Surg Gynecol Obstet 1975;141:843-858.
43. Hardison WGM, Weiner RG, Hattoff DE, Miyai K. Similarities and differences between models of extrahepatic biliary obstruction and complete biliary retention without obstruction in the rat. HEPATOLOGY 1983;3:383-390.
44. Christofferson P, Poulsen H. Histological changes in human liver biopsies following extrahepatic biliary obstruction. Acta Pathol Microbiol Scand 1970;212(suppl):150-157.
45. International Group. Histopathology of the intrahepatic biliary tree. Liver 1983;3:161-175.
46. Desmet VJ. Cholestasis: extrahepatic obstruction and secondary biliary cirrhosis. In: MacSween RNM, Anthony PP, Scheuer PJ, eds. *Pathology of the Liver.* (Ed 2). New York: Churchill Livingstone, 1987:364-423.
47. Mohn K, Laz TM, Hsu J-C, Melby AE, Bravo R, Taub R. The immediate early-growth response in regenerating liver and insulin-stimulated H-35 cells: comparison with serum-stimulated 3T3 cells and identification of 41 novel immediate-early genes. Mol Cell Biol 1991;11:381-390.