Loss of NF-κB activation in Kupffer cell-depleted mice impairs liver regeneration after partial hepatectomy

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Abshagen K, Eipel C, Kalff JC, Menger MD, Vollmar B. Loss of NF-κB activation in Kupffer cell-depleted mice impairs liver regeneration after partial hepatectomy. Am J Physiol Gastrointest Liver Physiol 292: G1570 –G1577, 2007. First published February 22, 2007; doi:10.1152/ajpgi.00399.2006.—Kupffer cells (KCs) are located in the liver sinusoids adjacent to hepatocytes and are capable of producing important growth-regulating mediators that exert both stimulatory and inhibitory influences on hepatocyte proliferation by paracrine mechanisms. To elucidate the overall effect of KC depletion on liver regeneration, mice were selectively and long-standing depleted of KCs by liposome-encapsulated dichloromethylene diphosphonate. Using in vivo fluorescence microscopy, immunohistochemistry, Western blot analysis, and NF-κB transcription factor DNA binding activity and cytokine assays, we analyzed livers of KC-depleted and KC-competent mice at days 3, 5, and 8 after partial (i.e., 68%) hepatectomy (PH). Selective KC elimination delayed cell proliferation, as indicated by significantly reduced PCNA and cyclin B1 protein expression in liver tissue at day 3 after PH. This was associated with a lower liver weight at day 8 upon PH. Resection-associated activation of NF-κB with translocation into parenchymal and nonparenchymal cell nuclei was diminished in livers of KC-depleted mice, primarily at day 3 after PH. KC-depleted mice further lacked the resection-induced rise in TNF-α and IL-6 serum concentrations. These findings imply that KCs play a stimulatory role in liver regeneration, mainly by activating NF-κB with influence on the cell cycle and by enhancing expression of the proliferative cytokines TNF-α and IL-6.

in vivo fluorescence microscopy; hepatocytes; clodronate; tumor necrosis factor-α; interleukin-6; proliferating cell nuclear antigen; nuclear factor-κB

LIVER REGENERATION IS A COMPLEX and evolutionarily conserved process that involves paracrine interactions of numerous hepatic cell types. Kupffer cells (KCs) are the liver macrophages predominantly located in the lumen of hepatic sinusoids, where they mainly clear particulate and foreign materials from the portal circulation (28). Because KCs are known to produce a variety of growth- and immunomodulating mediators that have stimulatory and inhibitory effects on liver regeneration, they have been postulated to play a key role in liver regeneration (35). However, their function in liver regeneration after partial hepatectomy (PH) and the underlying mechanisms are not fully understood (12).

Macrophage depletion constitutes a widely accepted and useful approach to elucidate their functional aspects in vivo. Liver tissue macrophages can selectively be depleted by intravenous administration of liposome-encapsulated dichloromethylene diphosphonate (Cl2MDP) without activation or damage to other liver cells (36, 37). So far, there are only a limited number of studies available using this KC-depleting approach to clarify their role in liver regeneration (7, 26, 35). Although Boulton et al. (7) noted an augmentation of the early phase of liver regeneration following KC depletion, it has also been reported that KC depletion exerts an inhibitory effect on liver regeneration by alteration of hepatic cytokine expression (26, 35). Most recently, KCs have been shown to express NF-κB (30, 42), which in turn is advocated to be a critical effector of the cascade initiating the regenerative process (23). Experiments in hepatectomized animals have shown that inhibition of NF-κB results in massive hepatocyte apoptosis and decreased mitosis with impairment of liver function (19). The so-far contradictory data on KCs in liver regeneration prompted us to precisely study the process of liver regeneration in KC-depleted animals with the emphasis on NF-κB activation and its downstream signaling.

MATERIALS AND METHODS

Liposome preparation and administration. Cl2MDP was kindly provided as gift from Roche Diagnostics (Mannheim, Germany). Cl2MDP encapsulated in multilamellar liposomes (400 nm) was prepared using the method described by van Rooijen and Sanders (36) with minor modifications. Inactive PBS-containing liposomes were prepared under identical conditions at the same time. All liposome preparations were stored under nitrogen gas and used within 14 days.

Experimental groups and protocol. For depletion of KCs, mice were applied Cl2MDP-liposomes (0.1 ml/10 g body wt) via the tail vein under light ether anesthesia at 24 h before PH and at every 72 h after PH. Control animals received equivalent volumes of PBS-liposomes (0.1 ml/10 g body wt). In KC-depleted and KC-competent mice (controls), livers were analyzed at days 3, 5, and 8 after PH (n = 6 animals per group and time point) as well as in nonresected animals (day 0; n = 6 animals per group and time point).

Liver regeneration model. Upon approval by the local government, all experiments were performed in accordance with the German legislation on protection of animals and the National Institutes of Health “Guides for the Care and Use of Laboratory Animals” (DHEW Publication No. (NIH) 86-23, Revised 1985). Female C57BL/6J mice (10–12 wk old; Charles River Laboratories, Sulzfeld, Germany) were anesthetized by breathing isoflurane (1.5 vol%) and subjected to a 68% PH (13, 17). The animals were allowed to recover from anesthesia and surgery under a red warming lamp and were held in single cages until the subsequent experiments followed postoperative days 3, 5, and 8 (n = 6 animals per time point). Sham-operated animals without hepatic resection served as group day 0.

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In vivo fluorescence microscopy for analysis of KC phagocytic activity. Under ketamine-xylazine anesthesia (90:25 mg/kg body wt ip), sham-treated controls and heptatectomized animals were subjected to intravital fluorescence microscopy, as previously described in detail by our group (1). Animals were placed in supine position on a heating pad and underwent insertion of a polyethylene catheter (PE-50, inner diameter 0.58 mm; Portex, Hythe, UK) in the right carotid artery. After laparotomy and exteriorization of the left liver lobe, KC phagocytic activity was assessed by intra-arterial injection of plain fluorescent latex particles (3 × 10^9 beads/10 g body wt in isotonic saline; diameter 1.1 μm; Polysciences, Warrington, PA) (38). For assessment of kinetics of particle adherence, 20–25 selected observation areas per experiment were videotaped successively within 10 min after injection with the use of blue light epi-illumination (excitation/emission wavelength 450–490 nm/520 nm) and a water-immersion objective (4×/200, 0.50W; Zeiss). The kinetics of adherence were quantified offline by counting the number of particles moving in sinusoids as a percentage of all particles visible in the area during observation for 10 s (38). After the microscopy, liver tissue was sampled for subsequent histochemical and molecular biology analysis (n = 6 animals per time point and group).

Regeneration study. Following in vivo microscopy, animals were killed and the remnant livers were harvested, weighed, and processed for subsequent analysis. The weight of regenerated liver was used to calculate the growth of residual liver lobes according to weight of regenerated liver/preoperative liver weight × 100 (%). Preoperative liver weight was assumed to be 4.3% of body weight.

Being aware that liver weight is influenced by various extrinsic and intrinsic factors that are often unrelated to hepatic regeneration, we additionally used 5-bromo-2-deoxyuridine (BrdU) incorporation to study DNA synthesis upon liver resection by immunohistochemistry. For this purpose, additionally pretreated mice were given BrdU (50 mg/kg body wt ip) 1 h before harvest of liver tissue (n = 4 animals per group and time point) (4, 5, 13).

Western blot analysis of liver tissue. For Western blot analysis of PCNA, cyclin B1, and NF-κB, liver tissue was homogenized in lysis buffer (1 M Tris, pH 7.5, 5 M NaCl, 250 mM EDTA, 10% Triton-X 100, 4% NaN₃, and 100 mM PMSF), incubated for 30 min on ice, and centrifuged for 15 min at 10,000 g. Before use, all buffers received a protease inhibitor cocktail (1:100 vol/vol; Sigma, St. Louis, MO).

Protein concentrations were determined using the BCA protein assay (Pierce, Rockford, IL) with bovine serum albumin as standard. Equal amounts of whole protein extracts (PCNA: 20 μg; cyclin B1 and NF-κBp65: 60 μg) were separated discontinuously on sodium dodecyl sulfate polyacrylamide gels (12% SDS-PAGE) and transferred to a polyvinylidene difluoride membrane (Immobilon-P transfer membrane; Millipore, Billerica, MA). After blockage of nonspecific binding sites, membranes were incubated for 2 h at room temperature with monoclonal anti-PCNA (1:2,000; Santa Cruz Biotechnology, Santa Cruz, CA), polyclonal rabbit anti-cyclin B1 (1:1,000; Santa Cruz), and polyclonal goat anti-NF-κBp65 (1:500; Santa Cruz) followed by peroxidase-conjugated goat anti-rabbit IgG antibody (PCNA: 1:8,000; cyclin B1: 1:10,000; Cell Signaling Technology, Frankfurt, Germany) or rabbit anti-goat IgG antibody (NF-κBp65: 1:40,000; Santa Cruz) as secondary antibodies. Protein expression was visualized by means of luminal-enhanced chemiluminescence (ECL Plus; Amersham Pharmacia Biotech, Freiburg, Germany) and exposure of the membrane to a blue light-sensitive autoradiography film (Kodak BioMax light film; Kodak-Industrie, Chalon-sur-Saone, France). Exposure was densitometrically assessed (Quantity One, Gel Doc XR; Bio-Rad Laboratories, Munich, Germany) and normalized to the β-actin signals (monoclonal mouse anti-β-actin antibody, 1:20,000; Sigma) or β-tubulin signals (polyclonal rabbit anti-β-tubulin antibody, 1:500; Santa Cruz).

Immunohistochemistry. Liver tissue of animals at days 0, 3, 5, and 8 after resection was excised, fixed in 4% phosphate-buffered formalin for 2–3 days, and then embedded in paraffin. From the paraffin-embedded tissue block, 5-μm sections were cut.

The degree of KC depletion was analyzed immunohistochromically by using the F4/80 antigen (1:10; Serotec, Oxford, UK), a marker for resident tissue macrophages (35). Overnight incubation (4°C) with the first antibody (polyclonal rat anti-F4/80) was followed by alkaline-phosphatase (AP)-conjugated mouse anti-rat immunoglobulin (1:200; Dako). The sites of AP binding were detected using fuchsin (Dako Cytomation, Hamburg, Germany).

For the demonstration of DNA-incorporated BrdU in liver cells, sections collected on poly-L-lysine-coated glass slides were incubated with monoclonal mouse anti-BrdU antibody (1:50; Dako) overnight at 4°C, followed by horseradish peroxidase (HRP)-conjugated goat anti-mouse immunoglobulin (LSAB kit plus; Dako). The sections were counterstained with hemaluma. The sites of peroxidase binding were detected using 3,3′-diaminobenzidine. BrdU-positive hepatocellular nuclei were counted within 50 consecutive high-power fields (HPF; ×40 objective, numerical aperture 0.65), and counts are given as cells per HPF (13). In analogy, BrdU-expressing nonparenchymal cells were assessed, and counts are also given as cells per HPF.

The detection of active NF-κB was performed using a polyclonal goat antibody directed against the epitope mapping within the amino-terminal domain of NF-κBp65 (1:100; Santa Cruz) overnight at 4°C, followed by HRP-conjugated rabbit anti-goat immunoglobulin (LSAB kit plus). Cell-specific expression of NF-κBp65 was analyzed by counting NF-κBp65-positive hepatocytes and nonparenchymal cells within 50 HPF with discrimination of the intracellular distribution serving as an indicator of NF-κB nuclear translocation. The nuclear-to-cytoplasmic ratio of parenchymal and nonparenchymal cells was calculated by dividing the number of cells with nuclear NF-κBp65 positivity by the number of cytoplasmic NF-κBp65 positive cells.

Bioplex protein array system. A panel of serum cytokines was measured in duplicate using the Bioplex protein array system (Bio-Rad), according to the instructions of the manufacturer. This novel multiplexed, particle-based flow cytometric assay, which utilizes anti-cytokine monoclonal antibodies linked to microspheres incorporating distinct proportions of two fluorescent dyes, enables quantification of several mediators in a sample volume as small as 12.5 μl.

With the Bioplex cytokine assay kit in combination with the Bioplex Manager software, serum TNF-α, IL-6, and IL-10 levels were assessed. For each cytokine, standards ranged from 1.5 to 32,000 pg/ml serum. Values of the respective serum cytokine levels of mice at day 0 were set as 1, and all other data are given as relative increases.

NF-κB transcription factor assay. DNA-binding activity of hepatic NF-κBp65 was measured in duplicate using a nonradioactive transcription factor assay (Chemicon International, Temecula, CA) according to the instructions of the manufacturer. This method combines the principle of the electrophoretic mobility shift assay with the 96-well-based enzyme-linked immunosorbent assay. During the assay, a double-stranded biotinylated oligonucleotide containing the flanked DNA binding consensus sequence for NF-κB (5′-GG-GACTTTCCC-3′) was mixed with nuclear extract of liver tissue (50 μg/well) and transferred to the streptavidin-coated plate well. The bound NF-κB transcription factor subunit p65 was detected with a specific primary antibody, followed by a highly sensitive HRP-conjugated secondary antibody, for which colorimetric signals were detected in a spectrophotometric plate reader (Magellan; Tecom Sunrise, Tecan Trading, Männedorf, Switzerland).

Statistical analysis. All data are expressed as means ± SE. After testing for normality and equal variance across groups, we assessed differences between the groups using one-way ANOVA followed by the appropriate post hoc comparison test, including Bonferroni probabilities to compensate for multiple comparisons. Statistical significance was set at P < 0.05. Statistics were performed using the software package SigmaStat (Jandel, San Rafael, CA).
RESULTS

Evidence of KC depletion. In all experiments we verified the completeness of KC depletion by immunostaining for the F4/80 antigen. Treatment with Cl2MDP-liposomes at −24 h and subsequently every 72 h guaranteed absence of KCs at the time point of resection as well as during the whole 8-day period of regeneration (Fig. 1, A and B). There was no histological evidence of damage to hepatocytes or other cell subpopulations (Fig. 1, A and B).

Uptake of fluorescent latex particles was used as a functional parameter of KC phagocytosis. Following Cl2MDP-liposome treatment, there was a distinct attenuation in clearance of latex particles from blood; however, this was without complete blockade of particle adherence (Fig. 1).

Effect of KC depletion on liver regeneration. There was a constant increase of liver weight upon PH with return to almost preoperative values at day 8 after resection in KC-competent mice, whereas KC-depleted animals showed only a limited restoration, up to 79% of the initial weight (Table 1).

Effect of KC depletion on proliferation markers in liver regeneration. The cumulative hepatocyte DNA synthesis, as histochemically determined in liver sections by BrdU incorporation, was slightly decreased at day 3 after PH in KC-depleted mice compared with control mice, with a displacement of the proliferation maximum of parenchymal cells from day 3 in KC-competent mice to day 5 in KC-depleted mice (Fig. 2, A and B). In contrast, the DNA synthesis phase of nonparenchymal cells was not found altered in KC-depleted animals; however, a second proliferation peak was observed at day 8 after resection (Fig. 2, C and D).

To exclude the possibility that the proliferating nonparenchymal cells are KCs, we performed double immunostaining for BrdU and F4/80. The immunohistochemical images of KC-depleted animals did not show cells double positive for F4/80 and BrdU, confirming that hepatectomy of these animals did not induce KC repopulation and proliferation (data not shown). This indicates that BrdU-positive nonparenchymal cells in KC-depleted livers can most probably be attributed to proliferation of sinusoidal endothelial cells and stellate cells.

Densitometric analysis of PCNA protein expression served as a parameter for proliferation and showed a significant reduction in KC-depleted mice. In particular, at days 3 and 5 after resection, hepatic PCNA expression in KC-depleted mice was <50% of that found in KC-competent control mice (Fig. 3, A and B).

Densitometric analysis of cyclin B1 protein expression, an M-phase-specific protein of the cell cycle (40, 43), served to distinguish the mitotic rate in KC-competent and KC-depleted livers. Cyclin B1 revealed an expression pattern comparable to that of PCNA, characterized by significantly reduced values in KC-depleted mice at day 3 after PH (Fig. 3, C and D).

Effect of KC depletion on transcriptional factor NF-κB during liver regeneration. Because of the lack of KC-specific NF-κBp65 expression, densitometric analysis of total NF-κBp65 levels in whole liver protein extracts showed a marked decrease at day 3 after resection in KC-depleted mice (Fig. 4, A and B). Furthermore, DNA-binding activity of NF-κBp65 in hepatic nuclear extracts was significantly reduced to 25% at day 3.
this early time point after resection compared with extracts of KC-competent mice (Fig. 4C).

Following immunohistochemistry with subsequent quantitative analysis of the intracellular distribution of NF-κBp65, sham-treated nonresected animals showed little NF-κBp65 staining in the cytoplasmic and, particularly, the nuclear compartment of liver cells (Fig. 5, B and D). On the contrary, NF-κBp65 expression was significantly enhanced upon resection with a preferential location in nuclei of hepatocytes at day 3 and of nonparenchymal cells, namely, KCs, at days 3 and 5 (Fig. 5, A–D). C12MDP-liposome application strikingly attenuated this increase of NF-κBp65 expression in hepatocytes and nonparenchymal cells (Fig. 5, A–D). In these animals, NF-κBp65 staining revealed a perinuclear pattern and was rarely

Fig. 2. Representative immunohistochemical images (A and C; original magnification, ×400) and quantitative analysis (B and D) of BrdU-stained parenchymal (PCs) and nonparenchymal cells (NPCs) in tissue sections at multiple time points (3d, day 3; 8d, day 8) upon PH in controls (solid bars) and KC-depleted mice (shaded bars). Values are means ± SE of 4 independent experiments per group and time point and are expressed as the number of cells per high-power field (n/HPF). ANOVA and post hoc comparison; *P < 0.05 vs. PBS.

Fig. 3. Representative Western blot analysis (A and C) and densitometric analysis (B and D) of PCNA (A and B) and cyclin B1 expression (C and D) in liver tissue at multiple time points upon PH in controls (solid bars) and KC-depleted mice (shaded bars). Western blot signals were corrected with those of β-actin (B) and β-tubulin (D) serving as internal control. Values are means ± SE of 6 independent experiments per group and time point. ANOVA and post hoc comparison; *P < 0.05 vs. PBS.
present in the nuclei of parenchymal and nonparenchymal cells (Fig. 5, B–D).

**Effect of KC depletion on cytokine expression pattern in liver regeneration.** To examine the role of KCs in PH-evoked cytokine production, we measured serum concentrations of several cytokines using a multiplex cytokine assay. As indicated in Fig. 6, serum TNF-α and IL-6 concentrations increased up to day 5 in KC-competent mice, whereas in KC-depleted mice cytokine expression failed to rise or was even found repressed at days 3 and 5 after resection (Fig. 6, A and...
Serum IL-10 concentrations of KC-competent and KC-depleted mice revealed a pattern similar to that of TNF-α (data not shown).

**DISCUSSION**

In recent years, a number of in vivo and in vitro methods have been used to study the role and function of KCs under physiological and pathological conditions. These studies have led to controversial findings without any conclusive evidence of whether the net influence of KCs in liver regeneration is to stimulate or to diminish hepatocyte proliferation. This study now provides direct in vivo evidence for the stimulatory role of KCs in parenchymal cell proliferation upon hepatectomy.

**Methodological considerations.** Various models have been employed to clarify the functional role of KCs. However, many agents, such as gadolinium chloride (GdCl₃), might be inappropriate for this purpose, since they activate macrophages to secrete biologically active substances (35). The rare earth metal GdCl₃ itself activates intrahepatic cytokines, is retained in hepatocytes, and is indeed highly toxic (31). Using this substance to deplete KCs in vivo, Rai et al. (31) reported that GdCl₃ enhances the expression of hepatic TNF-α mRNA and promotes liver regeneration after subsequent PH. Given that TNF-α has been shown to promote hepatocyte mitosis upon PH (21), increased expression of hepatic TNF-α mRNA in GdCl₃-treated animals might account for the observation of Rai et al. (31). IL-10, a cytokine that downregulates TNF-α, was found to be abolished in GdCl₃-treated mice. Thus GdCl₃ treatment might enhance liver regeneration due to the absence of IL-10 in limiting the TNF-α surge (31). Apart from this, the use of GdCl₃ as an agent to induce KC inhibition provides further substantial difficulties in interpretation. For example, there is ongoing controversy as to whether GdCl₃ eliminates KCs or just alters macrophage function, phenotype (25), and acinar distribution (24).

In contrast, application of liposome-encapsulated Cl₂MDP seems to be a more appropriate approach for investigating the effect of KC depletion on liver regeneration. Encapsulated Cl₂MDP is nontoxic, and upon intracellular release of the substance, KCs are selectively eliminated without activation (29, 36, 37). Therefore, we chose this approach for KC depletion in the present study. Our data show that KCs were eliminated within 24 h and during the whole observation period of Cl₂MDP-liposome treatment, as demonstrated by the absence of F4/80-expressing cells in the present study as well as in studies of other groups (28, 35). In addition, the marked attenuation of phagocytosis of latex particles underlines the effectiveness of KC depletion in animals receiving Cl₂MDP-liposomes, because the remaining latex particle adherence can be attributed to sinusoidal endothelial cells (7, 39).

**Effect of KC depletion on liver regeneration.** The present study shows unequivocally that KC depletion by Cl₂MDP-liposomes resulted in reduced liver weights at days 5 and 8 after PH. With respect to the fact that KC-depleted mice revealed an increase of cumulative BrdU incorporation not only in hepatocytes but also in nonparenchymal cells, limited liver regeneration might be attributed to a delay rather than to a reduction in proliferation (26). In line with this view, Takeishi et al. (35) applied Cl₂MDP-liposomes first at day 3 after resection and, consequently, observed a reduction in the rate of hepatocyte proliferation at later stages of liver regeneration. However, the proliferation marker PCNA, which arises during the entire cell cycle (11), has been found to be markedly decreased over the whole observation period in KC-depleted mice. Additional analysis of the M-phase-specific protein cyclin B1 (40, 43) revealed an expression pattern comparable to that of PCNA, characterized by a significantly decreased protein expression at day 3 after PH in KC-depleted mice. Thus it might be speculated that KC-depletion interferes with the cell cycle at the transition from the S to the G₂/M phases, because BrdU incorporation only reflects the synthesis phase of the cell cycle.

KC's are known to produce several kinds of biologically active mediators and seem to be the most important source of TNF-α (10). In contrast to KC-competent animals revealing a rise of TNF-α and IL-6 serum concentrations upon liver resection, KC-depleted mice failed to mount an equivalent cytokine response. Consistent with the ability of TNF-α to induce IL-6 (14, 27), the rise of TNF-α in KC-competent mice was paralleled by an increase of serum IL-6, whereas this...
autocrine effect was missing in KC-depleted mice. Although TNF-α-deficient mice showed normal liver regeneration (16, 18), TNF-α has been regarded as an important promoter of hepatocyte proliferation (22). In support of this view, studies using antibodies against TNF-α have demonstrated a reduction of hepatocyte proliferation (2, 33). In addition, livers of MyD88-deficient mice exhibited an impaired regeneration also accompanied by blunted TNF-α and IL-6 expression (32). With the use of TNF receptor type 1-deficient mice, it has been shown that impaired liver regeneration resulted from the failure of NF-κB activation and subsequent suppression of IL-6 production in the liver of these mice after PH (41). Furthermore, using a murine model of bone marrow transplantation, Aldegue et al. (3) showed clearly an importance of KC-derived NF-κB in adequate liver regeneration.

PH induces an early cellular response involving the transcription factor molecule NF-κB (15), which has been attributed a key role in the initiation of hepatocellular proliferation (23). Upon nuclear translocation, NF-κB induces the transcription of a large set of immediate-early genes (20, 34), which are particularly involved in the cell cycle regulation (6). Moreover, NF-κB is responsible for the expression of numerous proinflammatory mediators, including TNF-α (6, 20). In KC-competent mice we observed the highest NF-κBp65 nuclear staining in hepatocytes at day 3 after resection, whereas in non-parenchymal cells NF-κBp65 nuclear staining occurred over day 5. In line with this finding, it has most recently been shown that after PH, NF-κB expression mainly occurs in KCs (42). Presently, we have demonstrated that in KC-depleted mice both, hepatocytes and nonparenchymal cells almost completely lack the initial nuclear shuttle of NF-κBp65. In parallel, DNA-binding activity of nuclear NF-κBp65 was significantly reduced. This indicates that KC-depleted liver tissue desiderates appropriate NF-κB activation and subsequent TNF-α release. In support of this view, it has been shown that inhibition of NF-κB activation by overexpression of the inhibitor IκB reduces hepatocyte proliferation after PH by downregulation of IL-6 as a downstream event (9, 19, 42). Most notably, however, specific inhibition of hepatocellular NF-κB by using a transgenic mouse model did not impair DNA synthesis (8), indicating that KC-confined NF-κB expression in liver regeneration is more important than hepatocellular NF-κB activation. These data are consistent with the results of our study showing that decreased NF-κB activation is due to the absence of KCs, which in turn results in retardation of liver regeneration. Therefore, we finally suggest that hepatocyte proliferation during liver regeneration can be triggered by a signal transduction pathway that involves KC-specific NF-κB, TNF-α, and IL-6.

At first sight, increased BrdU staining in the present study contrasts the finding of reduced PCNA and cyclin B1 protein expression. However, it has been reported that inhibition of NF-κB results in a significant decrease in the number of mitotic figures in the regenerating liver without impairment of DNA synthesis (6). Because of this finding and the present results of decreased cyclin B1 expression in KC-depleted mice, we suppose a NF-κB-dependent alteration of the cell cycle at the S-to-G2/M phase transition. This might explain the observation that KC-depleted mice present with reduced liver regeneration despite increased DNA synthesis. However, further investigations of KC-depleted livers are necessary to specify the impact of KC-specific NF-κB on the cell cycle.

Taking all findings together, KCs turn out to be essential for intact liver regeneration by their NF-κB activation and effector downstream signals, such as TNF-α and IL-6 release. Thus KCs play a crucial role in liver regeneration by supporting cellular proliferation.

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REFERENCES

LIVER REGENERATION AND KUPFFER CELLS


