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Bone marrow-derived c-jun N-terminal kinase-1 (JNK1) mediates liver regeneration

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ABSTRACT

Liver regeneration is controlled by a complex network of signaling molecules, and a prominent role for c-jun N-terminal kinase has been suggested during this process. In the present study, we aimed to characterize and define the cell-type-specific contribution of JNK1 activation during liver regeneration. We used hepatocyte-specific JNK1 knockout mice ($JNK1^{\Delta hepa}$) using the *cre/lox-P* system. We performed partial hepatectomy (PH) in WT, $JNK1^{\Delta hepa}$ and $JNK1^{-/-}$ animals and investigated time-points up to 72 h after PH. Additionally, bone marrow transplantation experiments were conducted in order to identify the contribution of hematopoietic cell-derived JNK1 activation for liver regeneration. Our results show that liver regeneration was significantly impaired in $JNK1^{-/-}$ compared to $JNK1^{\Delta hepa}$ and WT animals. These data were evidenced by lower BrdU incorporation and decreased cell cycle markers such as Cyclin A, Cyclin D, E2F1 and PCNA 48 h after PH in $JNK1^{-/-}$ compared with $JNK1^{\Delta hepa}$ and WT livers. In $JNK1^{-/-}$ mice, our findings were associated with a reduced acute phase response as evidenced by a lower activation of the IL-6/STAT3/SAA-1 cascade. Additionally, $CD11b^{+}Ly6G^{+}$-cells were decreased in $JNK1^{-/-}$ compared with $JNK1^{\Delta hepa}$ and WT animals after PH. The transplantation of bone marrow-derived $JNK1^{-/-}$ into WT recipients caused significant reduction in liver regeneration. Interestingly, the transplantation of $JNK1^{-/-}$ into mice lacking JNK1 in hepatocytes only partially delayed liver regeneration. In summary, we provide evidence that (1) JNK1 in hematopoietic cells is crucial for liver regeneration, and (2) a synergistic function between JNK1 in hepatocytes and hematopoietic-derived cells is involved in the hepatic regenerative response.

1. Introduction

The regenerative capacity is dormant in a healthy liver. However, surgical resection, drug intoxication or viral infections trigger a complex process with the ultimate aim to restore liver mass and cell function. In rodents, the preferred experimental model to study liver regeneration is two-thirds partial hepatectomy (PH). This model is highly reproducible, consists of a controlled sequence of events and has direct clinical implications [1]. In fact, in humans, liver resection is frequently performed during surgery of liver metastases or repair after liver trauma [2].

Partial resection of the liver triggers activation of the c-Jun N-terminal kinases (JNK), members of the mitogen-activated protein kinase (MAPK) family [3,4]. The TNF-mediated activation of JNK within 1 h after PH leads to the activation of AP-1, which, in turn, promotes the expression of Cyclin D, initiating G0-G1 transition and, thus hepatocyte proliferation [3–7]. In contrast to the central role of JNK in promoting liver regeneration, persistent JNK activation attenuates liver regeneration, clearly suggesting that the magnitude and duration of JNK activation is a critical regulator of liver homeostasis.[3]

Cell-type-specific ablation using the *cre/lox-P* system has helped to improve the understanding of the specific functions of JNK in distinct tissues and the interaction between the liver and other organs [7,8]. This is of clinical relevance since targeted pharmacological modulation of JNK function might be beneficial for patients with malignant liver tumors.

Abbreviations: ANOVA, analysis of variance; ALT, alanine aminotransferase; AP-1, activator protein 1; APR, acute phase response; AST, aspartate aminotransferase; BM, bone marrow; BMT, bone marrow transplantation; BrdU, 5-bromo-2′-deoxyuridine; KC, Kupffer cells; FXR, farnesoid X receptor; GR, glucocorticoid receptor; H&E, hematoxylin and eosin; HRP, horseradish peroxidase; IL-6, interleukin-6; $JNK1^{\Delta hepa}$, hepatocyte-specific deletion of JNK1; LXR, liver X receptor; MAPK, members of the mitogen-activated protein kinase family; PH, partial hepatectomy; PCNA, proliferating cell nuclear antigen; PFA, paraformaldehyde; PPARγ1, peroxisome proliferator-activated receptor γ1; qPCR, quantitative real-time PCR analysis; RXR and RARγ, retinoic acid receptors; SAA-1, serum amyloid-1; STAT3, signal transducer and activator of transcription-3; TNFα, tumor necrosis factor-α; WT, wild type

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Therefore, in the present study, we aimed to characterize and define the cell-type-specific contribution of JNK1 activation during liver regeneration using the two-thirds PH model. We addressed this task by combining global and hepatocyte-specific JNK1 knockout animals. Additionally, we performed bone marrow transplantation (BMT) to finally establish that whereas JNK1 in hematopoietic cells is crucial for liver regeneration, JNK1 in hepatocytes alone is irrelevant. However, a synergistic function between JNK1 in hematopoietic cells and hepatocytes is necessary for restoring liver mass.

2. Methods

2.1. Housing, generation of knockout mice and bone marrow transplantation

Animals were maintained in the animal facility of the University Hospital RWTH Aachen according to the German legal requirements. Wild-type (WT), constitutive JNK1$^{-/-}$ animals as well as hepatocyte-specific knockout mice JNK1$^{\Delta hepa}$ were bred in a defined C57BL/6 background. Genotypes were confirmed via PCR for the respective genes. Genotypes were confirmed by PCR analysis of genomic DNA. We transferred bone marrow from JNK$^{\Delta hepa}$ and JNK1$^{-/-}$ mice into 6- to 7-week-old JNK1$^{-/-}$ and WT isogeneic recipients ($n = 6$–7 mice per group) after ablative γ-irradiation, as described previously [7,9].

2.2. Partial hepatectomy

Two thirds partial hepatectomy (PH) as described previously[1] was performed in male mice in the age of 7 to 8 weeks. Animals were euthanized after varying periods of time. Control samples were taken from explanted livers of the respective strains at the time of PH.

2.3. Immunoblot analysis

Protein extracts were electrophoresed and then blotted following standard procedures. Blots were incubated with primary antibodies anti-Ccn D1, anti-Ccn A2 (Santa Cruz Biotechnology, Santa Cruz, CA) and anti-pSTAT3 from Cell Signaling (Beverly, MA). As secondary antibodies, anti-rabbit-HRP (Cell Signaling) and anti-mouse-HRP (Santa Cruz) were used. GAPDH from AbD SeroTec (Düsseldorf, Germany) was used as loading control.

2.4. Quantitative real-time PCR

Total RNA was purified from liver tissue using Trizol reagent (Invitrogen, Karlsruhe, Germany). Total RNA (1 μl) was used to synthesize cDNA using SuperScript first-Stand Synthesis System (Invitrogen, Karlsruhe, Germany) and was resuspended in 100 μl of H_2O. Quantitative real-time PCR was performed using SYBR Green Reagent (Invitrogen, Karlsruhe, Germany) in 7300 real-time PCR system (Applied Biosystem, Darmstadt, Germany). GAPDH expression was used to normalize gene expression in a given sample which is represented as fold induction *versus* WT basal expression in control samples. Primers can be provided upon request.

2.5. Histological, immunofluorescence analysis

Livers from mice were harvested and, following fixation with 4% PFA, were embedded in paraffin for further histological evaluation. H&E staining was performed on paraffin-embedded liver sections. Samples were reviewed by a blinded pathologist who analyzed the degree of liver injury. For immunofluorescence staining, liver cryosections of 5 μm were stained with CD11b (Santa Cruz, Heidelberg, Germany), Oil Red O or anti-BrdU antibody as previously described [1]. Examination was performed using a Leica automatic stainer (Wetzlar, Germany). Mounting solution containing 4′,6′-diamidino-2-phenylindole (DAPI) (Vector Laboratories, Burlingame, CA) was used to counterstain the nuclei of hepatocytes and incubated with fluorescence labeled secondary antibodies (AlexaFluor 488 and 564, Invitrogen, Carlsbad, CA, USA).

2.6. Flow cytometry analysis

Immune cells from whole liver were isolated and stained with fluorochrome-conjugated antibodies (CD4-PE, CD3-APC, NK1.1/PE/Cy7, CD8-FITC, CD45-APC-Cy7, CD11b-PE, CD11c-APC, Ly6G-FITC, CD19-PERCP/Cy5.5, Gr1.1 PERCP/Cy5.5 and F4/80-Bio/Streptomycin/Cy7) (BD Biosciences, Heidelberg, Germany). All samples were acquired by flow cytometry (FACS Canto II; BD Biosciences) and analyzed using the Flowjo® software.

2.7. General procedures

Serum ALT, AST, cholesterol, glucose and triglycerides were processed by the Central Laboratory Facility at University Hospital RWTH Aachen. Blood counts were performed by the Central Laboratory Facility for laboratory animals at University Hospital RWTH Aachen.

2.8. Statistical analysis

Data are expressed as the mean ± SD. Statistical significance was determined via one- and two-way analysis of variance (ANOVA) followed by a Bonferroni test. Statistics regarding the BrdU stainings were determined via unpaired *T*-test.

3. Results

3.1. Liver regeneration after partial hepatectomy is independent of hepatocyte-derived JNK1

Following liver resection, hepatocyte proliferation starts in areas of the lobules surrounding the portal triads and then proceeds to the pericentral areas 36 to 48 after PH [10]. Thus, we studied DNA synthesis 48 h after surgery and first examined bromodeoxyuridine incorporation (BrdU). As expected, BrdU incorporation was significantly decreased in JNK1$^{-/-}$ compared with WT and JNK1$^{\Delta hepa}$ mice, 48 h after PH (Fig. 1a). This reduction was consistent with the lower number of mitotic figures we observed at this time point in the H&E staining (Supplementary Fig. 1). Moreover, we analyzed cyclin A protein expression (Fig. 1b) and also included other cell cycle markers such as Cyclin D, E2F1 and PCNA, which showed that hepatic regeneration is strongly reduced in JNK1$^{-/-}$ compared with JNK1$^{\Delta hepa}$ and WT mice (Fig. 1c–e).

To better characterize the differences between JNK1$^{\Delta hepa}$ and JNK1$^{-/-}$ livers, we examined in more detail the course of liver regeneration up to 72 h after PH (Suppl. Fig. 1). We observed that JNK1$^{-/-}$ and JNK1$^{\Delta hepa}$ knockout mice displayed lower BrdU incorporation compared with WT mice 72 h after PH (Fig. 2a + b). Furthermore, liver injury as evidenced by serum transaminases was less prominent in JNK1$^{-/-}$ compared with JNK1$^{\Delta hepa}$ and WT mice (Fig. 2c). However, we found no differences in liver *versus* body weight (LW/BW) ratio at this time point (Fig. 2d).

3.2. IL-6/SAA-1/STAT-3 activation is decreased in JNK1$^{-/-}$ mice after partial hepatectomy

Partial hepatectomy (PH) causes JNK activation and a robust regenerative response to restore liver mass [5]. First, Hui [11] and later Das [8] have clearly demonstrated that JNK1$^{-/-}$ knockout mice show impaired liver regeneration. IL-6-dependent STAT-3 activation plays a physiological role in promoting hepatic survival by stimulating proliferation of hepatocytes in a paracrine manner, specifically via inducing the acute phase response [12–14]. Thus, we examined whether the defect in liver regeneration in JNK1$^{-/-}$ mice was associated with changes in STAT-3/IL-6 activation (Fig. 3).

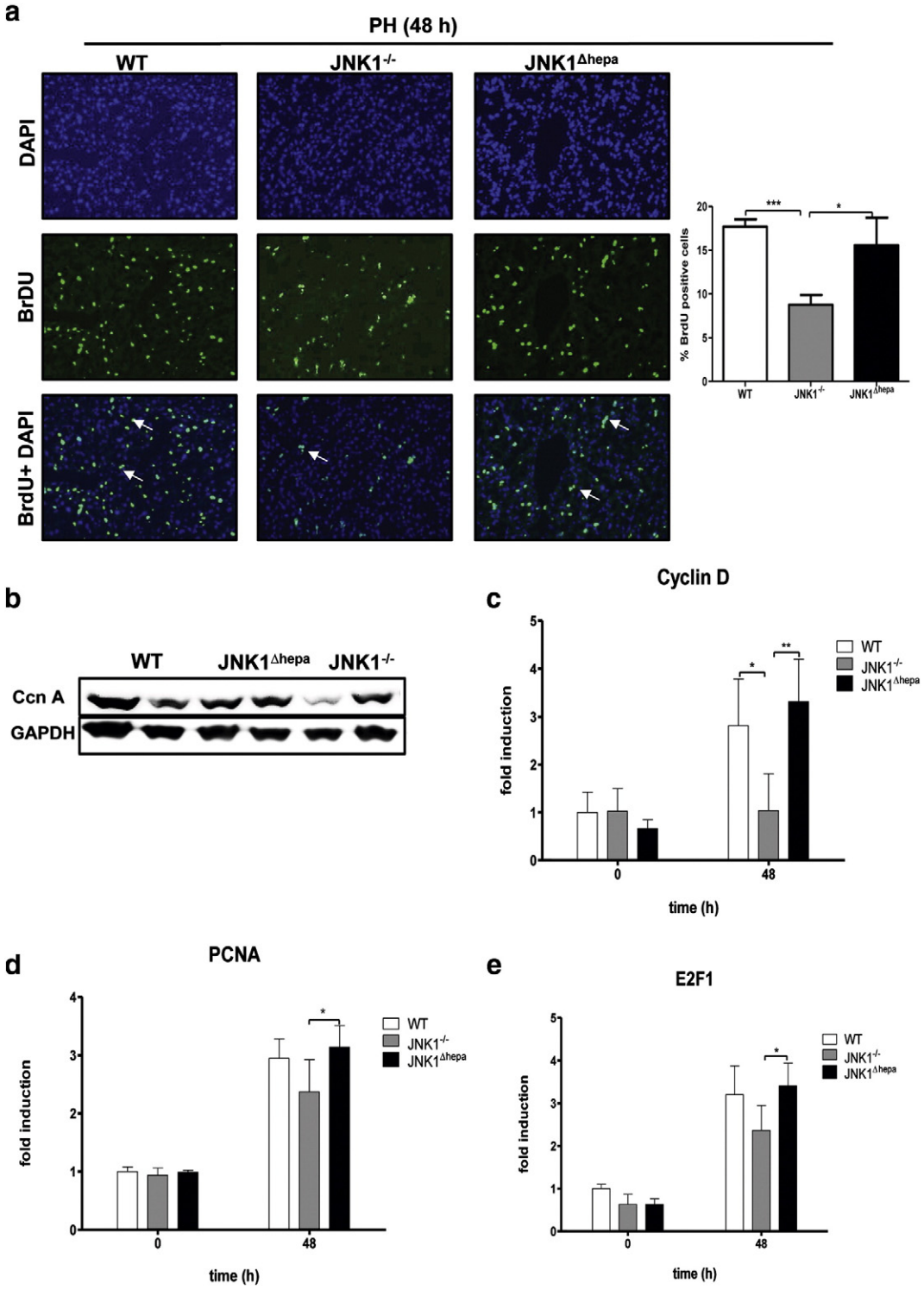

Fig. 1. Liver regeneration after partial hepatectomy is independent of hepatocyte-derived JNK1. (a) BrdU immunofluorescence staining was performed in WT, JNK1$^{-/-}$ and JNK1$^{\Delta hepa}$ mice, 48 h after PH (20×). Quantification of BrdU-positive cells was graphed and presented. (b) Western blot was performed to detect cyclin A expression in liver extracts isolated from WT, JNK1$^{-/-}$ and JNK1$^{\Delta hepa}$ livers 48 h after PH. GAPDH was used as loading control. mRNA levels of cyclin D1 (c), PCNA (d) and E2F1 (e) were determined using qRT-PCR expression of GAPDH mRNA was used to normalize gene expression (***$P < 0.001$; **$P < 0.01$; *$P < 0.05$, $n = 4$).

First, we studied STAT3 activation 6 h after PH. Protein levels of p-STAT3 in JNK1$^{-/-}$ were significantly decreased compared with JNK1$^{\Delta hepa}$ and WT mice 6 h after PH (Fig. 3a). Concomitant with STAT3 activation, we observed decreased SAA-1 mRNA levels in JNK1$^{-/-}$ compared with JNK1$^{\Delta hepa}$ mice (Fig. 3b). Furthermore, the mRNA expression of IL-6 was reduced in JNK1$^{-/-}$ compared with JNK1$^{\Delta hepa}$ and WT mice (Fig. 3c). Hence, these data showed an impaired regulation of the acute phase response (APR) in JNK1 knockout livers.

During liver regeneration in rodents, there is a sequential expression of several proto-oncogenes such as c-Jun and c-Myc during the first hours

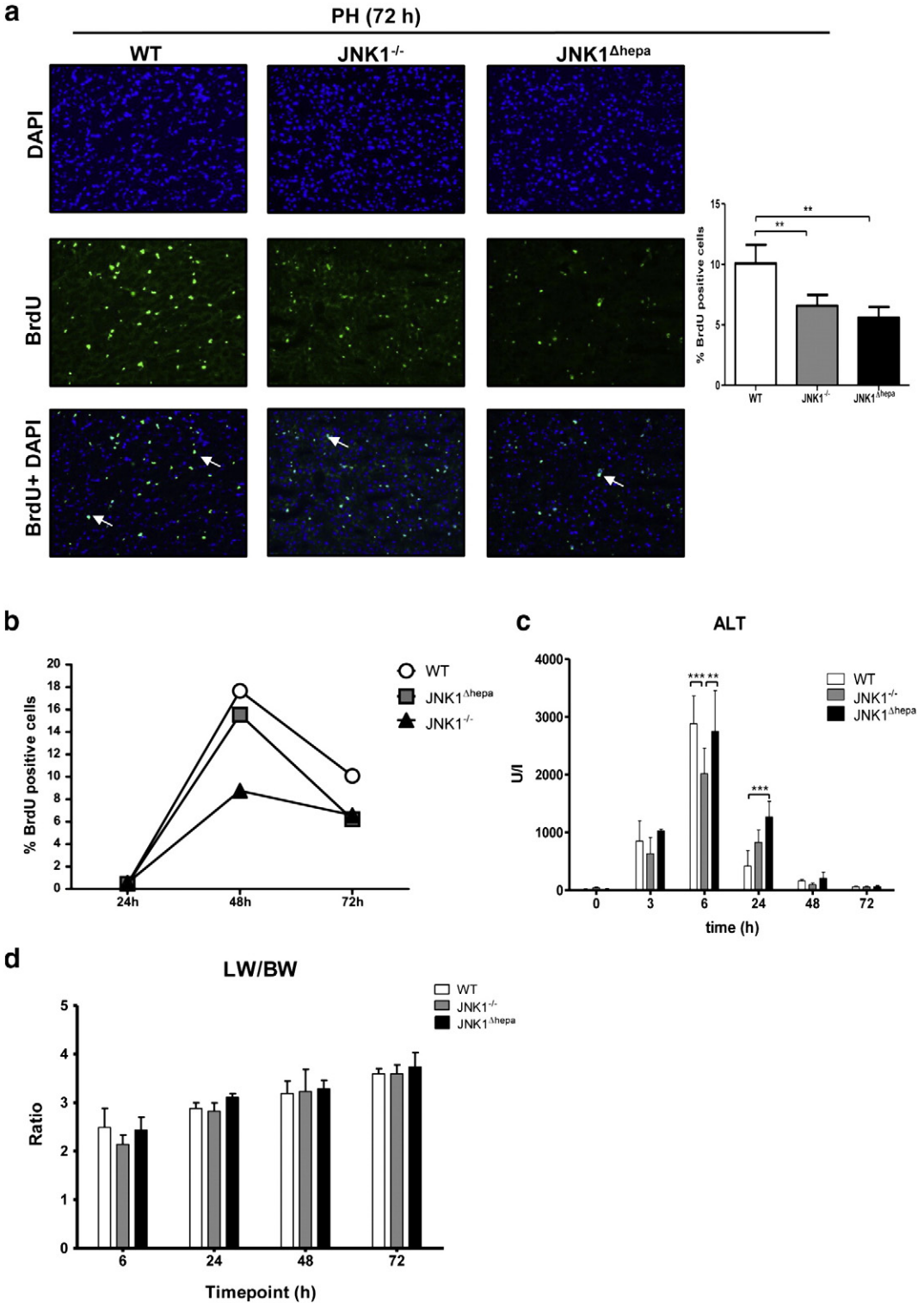

Fig. 2. Time-course study of liver regeneration after PH (a) BrdU immunofluorescence staining at 72 h after PH was performed, quantified and represented in a graph. (b) BrdU incorporation was evaluated throughout liver regeneration in WT, JNK1$^{-/-}$ and JNK1$^{\Delta hepa}$ mice after PH (20×). (c) ALT levels were collected upon euthanasia. (d) The ratio between the liver and the body weight of partial-hepatectomized mice after euthanasia is presented (***$P < 0.001$; **$P < 0.01$).

after PH [15]. Thus, we evaluated the expression of c-Jun and c-Myc after PH. We observed that c-Jun expression was down-regulated in JNK1$^{-/-}$ compared with JNK1$^{\Delta hepa}$ and WT mice 6 h after PH (Fig. 3d). Liver regeneration is accompanied by an increase in c-myc expression which precedes the synchronous entry of the remaining hepatocytes into the cell cycle [16]. Interestingly, we detected higher c-Myc mRNA levels in JNK1$^{-/-}$ livers—albeit not significant—indicating delayed liver regeneration in these mice (Fig. 3e). Altogether, these results show that after PH in JNK1$^{-/-}$ livers the delayed regenerative response is associated with impaired IL-6/STAT-3 activation.

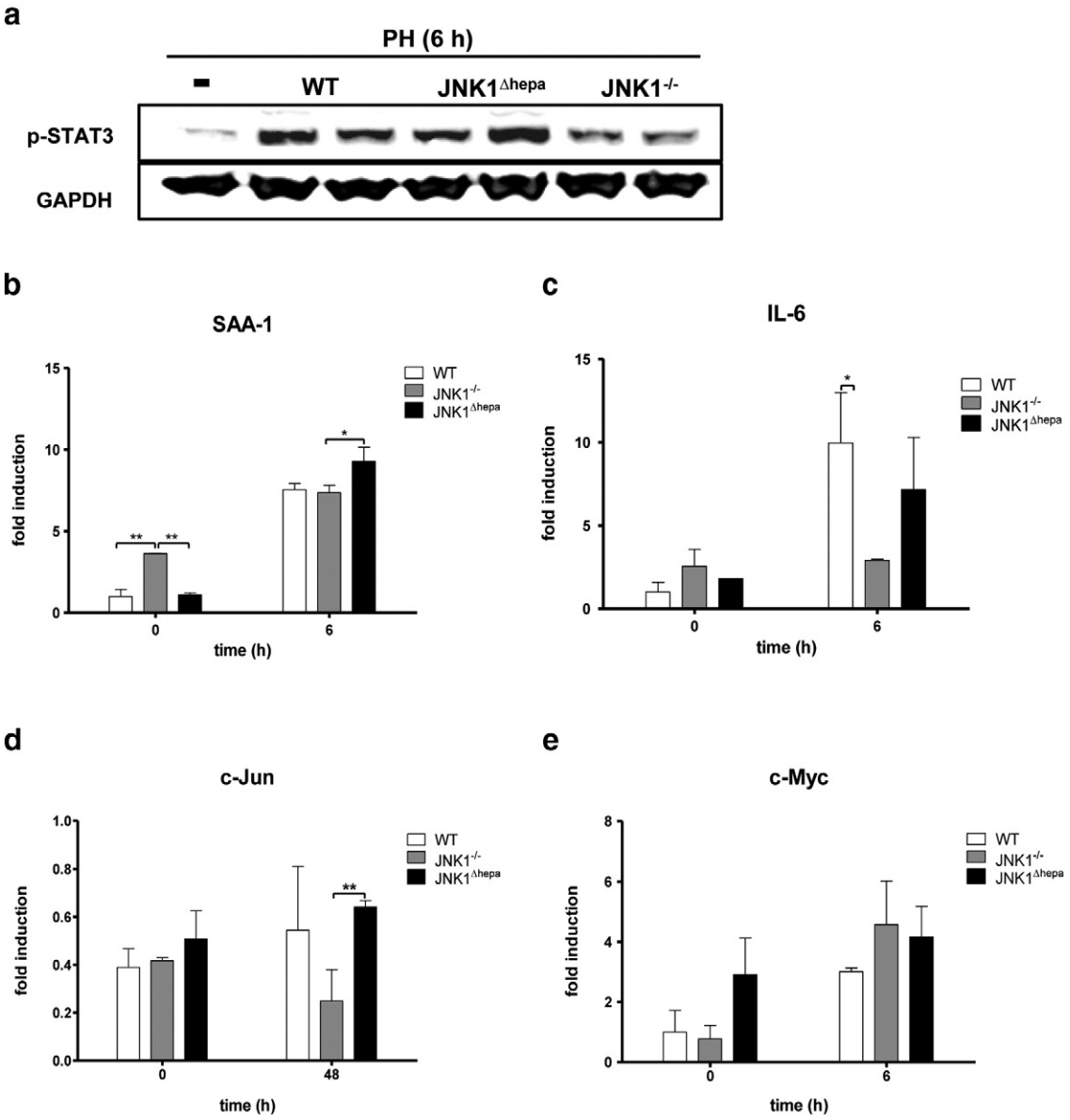

Fig. 3. The IL-6/SAA-1/STAT-3 pathway is decreased in JNK1$^{-/-}$ mice after partial hepatectomy. (a) Western Blot was performed to detect STAT3 expression in liver extracts isolated from WT, JNK1$^{-/-}$ and JNK1$^{\Delta hepa}$ livers at 6 h after PH. GAPDH was used as loading control. mRNA levels of SAA-1 (b), IL-6 (c), c-Jun (d) and c-Myc (e) were determined by qRT-PCR. GAPDH mRNA was used to normalize gene expression ($^{**}P < 0.01$; $^{*}P < 0.05$, $n = 4$).

3.3. Infiltrating macrophages and T-cells are reduced in JNK1$^{-/-}$ livers after PH

Infiltrating macrophages together with Kupffer cells (KC) orchestrate liver regeneration after PH [2]. Macrophages play a critical role in overall tissue recovery after PH and the impact of immune cell infiltration on the acute phase response (APR) has been shown previously [17]. In order to study differences in resident and/or infiltrating macrophages in JNK1-deleted animals, we performed FACS analysis and stained for CD11b^{+} and Ly6G^{+}-positive cells 48 h after PH (Fig. 4a + b). Our results showed that CD11b^{+} Ly6G^{+}-cells are decreased in JNK1$^{-/-}$ compared with JNK1$^{\Delta hepa}$ and WT animals 48 h after PH.

It has been shown that during liver regeneration, extrathymic T cells in the liver are significantly activated [18]. Surprisingly, we found higher amounts of CD4^{+}, NK cells and leukocytes but decreased CD8^{+} lymphocytes and NK1.1 cells in JNK1$^{-/-}$ compared with JNK1$^{\Delta hepa}$ and WT livers (Fig. 4c, Supplementary Fig. 2a–d).

Fat accumulation is a typical feature during PH-induced liver regeneration [19]. We examined lipid accumulation in regenerating livers by Oil Red O staining. We observed reduced fat accumulation in JNK1$^{-/-}$ compared with JNK1$^{\Delta hepa}$ and WT livers. This observation was associated to lower serum glucose levels (Fig. 4d, Supplementary Fig. 3).

3.4. Bone marrow-derived JNK1 is essential for liver regeneration after PH

Since our results demonstrated that JNK1$^{-/-}$ but not JNK1$^{\Delta hepa}$ livers exhibited impaired liver regeneration, we performed bone marrow transplantation (BMT) to examine whether JNK1 in bone marrow (BM)-derived cells might be involved to explain impaired liver regeneration as evidenced in JNK1$^{-/-}$ livers after PH. Bone marrow (BM) derived from WT, JNK1$^{-/-}$ or JNK1$^{\Delta hepa}$ mice was transplanted in JNK1$^{-/-}$, WT or JNK1$^{\Delta hepa}$ animals. After BM, we waited for 8 weeks to allow complete restoration of Kupffer cell (KC) with BM-derived cells as previously described [20]. We then performed PH and investigated the 48 h time-point after surgery (Fig. 5a–c, Supplementary Fig. 4).

The transplantation of BM derived from WT or JNK1$^{\Delta hepa}$ animals into JNK1$^{-/-}$ mice (WT/ JNK1$^{\Delta hepa}$ JNK1$^{-/-}$) completely restored liver regeneration as evidenced by BrdU staining 48 h after PH (Fig. 5a; left

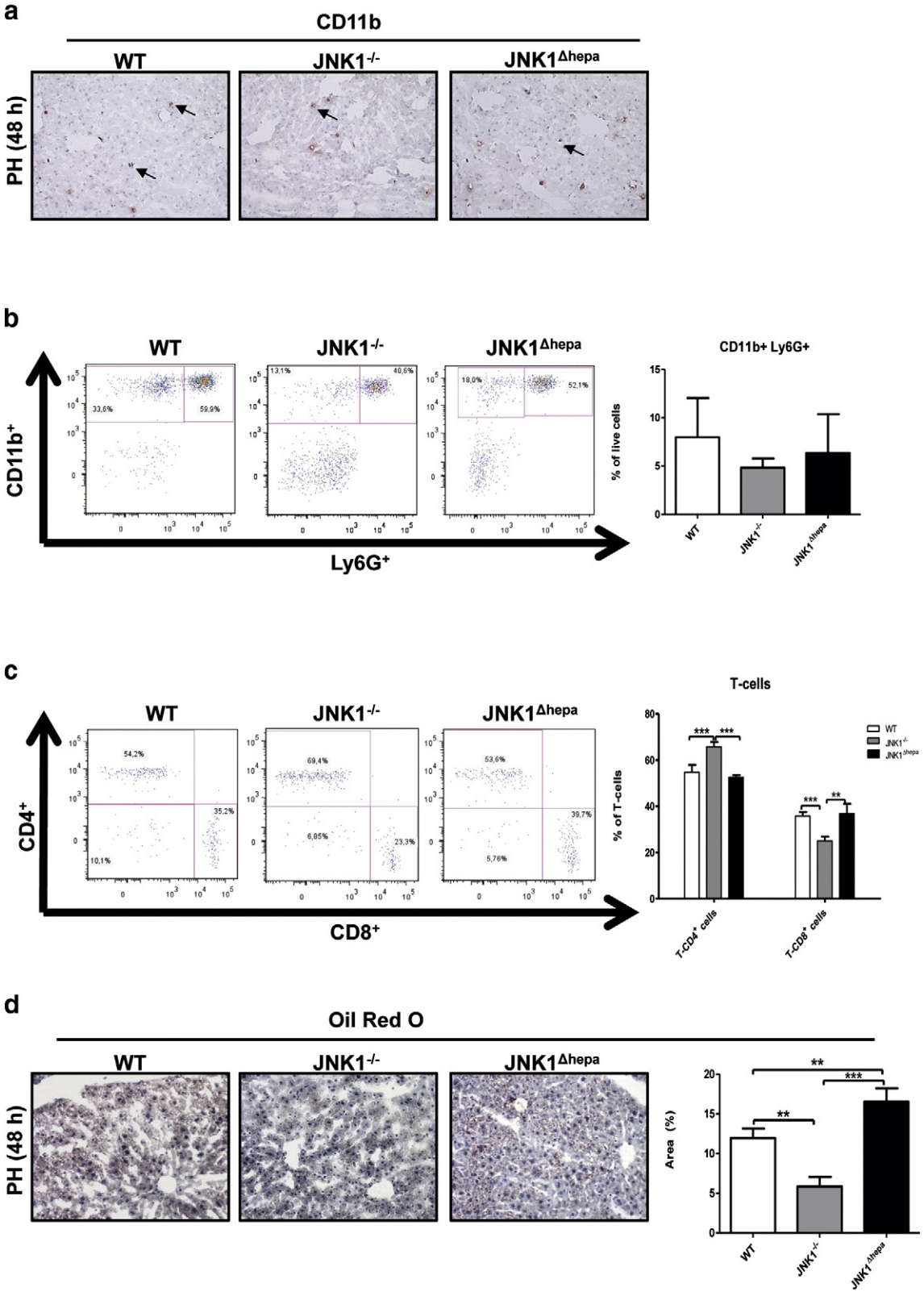

Fig. 4. Infiltrating macrophages and T-cells are reduced in JNK1$^{-/-}$ livers after PH. (a) CD11b immunofluorescence on frozen sections was performed to detect resident monocytes in livers of WT, JNK1$^{-/-}$ and JNK1$^{\Delta hepa}$ mice, 48 h after PH (20×). (b) 48 h samples from WT, JNK1$^{-/-}$ and JNK1$^{\Delta hepa}$–hepatectomized livers were subjected to FACS analysis to stain for the myeloid populations. The relative amount of CD11b^{+} Ly6G^{+} cells was quantified and represented in FACS blots. (c) 48 h samples from WT, JNK1$^{-/-}$ and JNK1$^{\Delta hepa}$–hepatectomized livers were subjected to FACS analysis to stain for the lymphoid populations. The relative amount of CD4^{+} *vs* CD8^{+} cells was quantified and represented in FACS blots. (d) Oil Red O staining was performed in frozen sections (20×) of WT, JNK1$^{-/-}$ and JNK1$^{\Delta hepa}$ livers 48 h after PH and the percentage of lipid droplets per area was quantified (***$P < 0.001$; **$P < 0.01$).

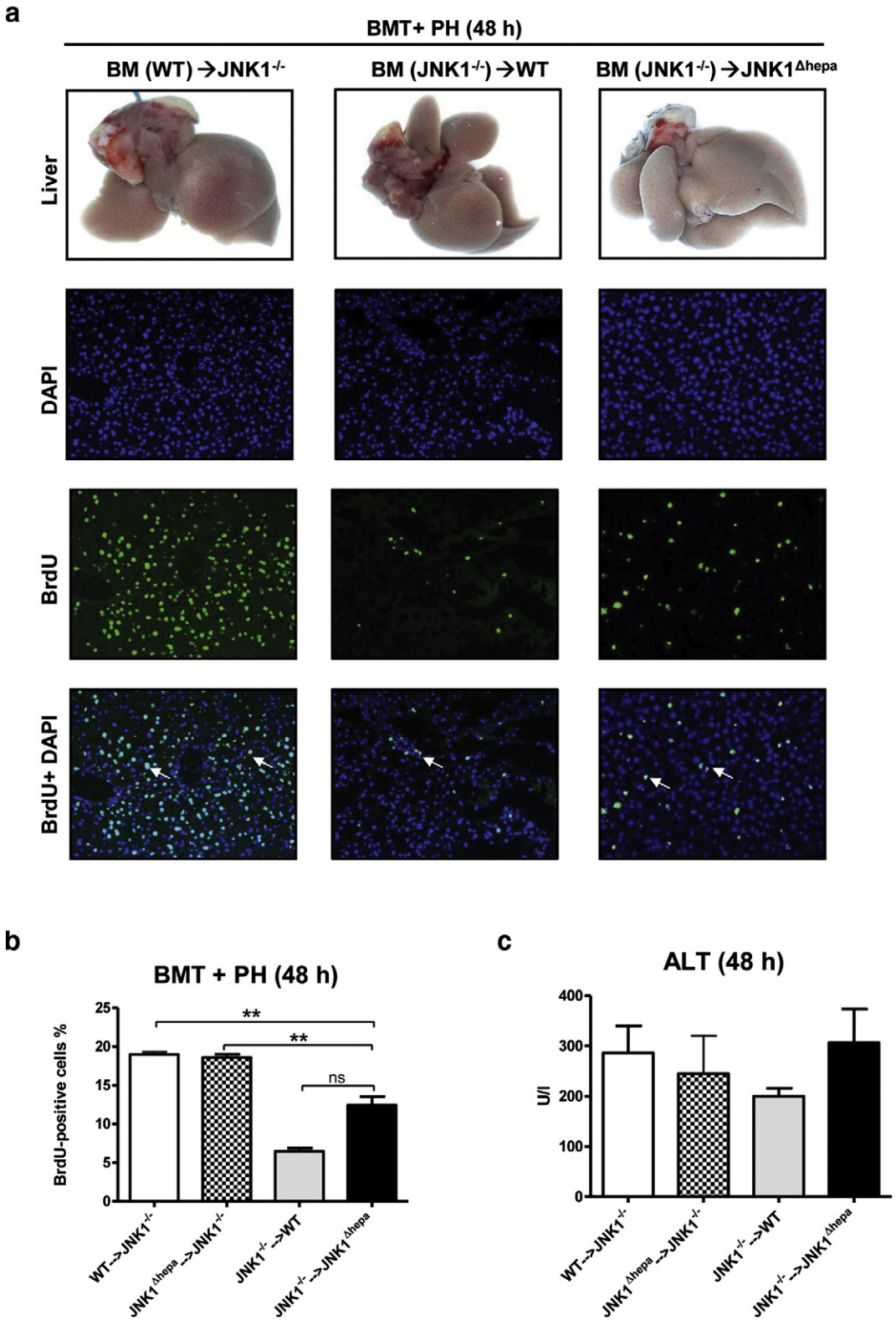

Fig. 5. Bone marrow-derived JNK1 is essential for liver regeneration. (a) WT bone marrow-derived cells were transplanted into JNK1$^{-/-}$ recipients (left panel). In parallel, JNK1$^{-/-}$ bone marrow-derived cells were transplanted into WT (center panel) and JNK1$^{\Delta hepa}$ recipients (right panel). In all groups, 8 weeks after irradiation, mice were subjected to PH and were sacrificed in the following 48 h. BrdU was performed on frozen sections and counterstained with DAPI. (b) BrdU incorporation was quantified and graphed for comparison (20×). (c) Serum transaminases (ALT) from the same mice were measured (**$P < 0.01$).

panel, Fig. 5b, Supplementary Fig. 4). However, WT animals transplanted with JNK1$^{-/-}$ BM (JNK1$^{-/-}$ WT) displayed a major reduction in liver regeneration and no significant differences in serum transaminases were found (Fig. 5a; center panel, Fig. 5b + c, Supplementary Fig. 4b). Interestingly, the transplantation of BM cells into mice lacking JNK1 in hepatocytes (JNK1$^{-/-}$ in JNK$^{\Delta hepa}$) partially reduced liver regeneration,

indicating that a synergistic function between JNK1 in hepatocytes and non-parenchymal cells (NPCs) seems involved in controlling the regenerative response.

4. Discussion

JNK activation has been shown to play an important role during liver regeneration. TNFR1$^{-/-}$ mice or rats injected with an anti-TNF antibody show reduced liver regeneration which was associated with impaired JNK activation [4,21] and more specifically a critical function of JNK during hepatic regeneration has been demonstrated by using JNK inhibitors after PH[4]. In agreement with previous reports [8,11], our observations in JNK1$^{-/-}$ livers indicated decreased liver regeneration 48 h after PH. In our present study, we focused to better characterize the molecular mechanisms mediating this effect.

Earlier results demonstrated that after PH, IL-6-dependent pathways trigger the APR and thereby hepatocyte proliferation. Thus, we first examined IL-6 induction after PH. Interestingly, we observed significantly increased basal expression of IL-6 and SAA-1 in the livers of JNK1$^{-/-}$ mice. This observation might be explained by compensatory JNK2 expression, which is critical in controlling the production of inflammatory cytokines [22]. However, after PH, our results showed decreased IL-6 expression in JNK1$^{-/-}$ but not in JNK1$^{\Delta hepa}$ animals which correlated with a weaker activation of the transcription factor STAT3. Indeed, both IL-6 and the phosphorylation and activation of Stat-3 are dependent on the activity of JNK, mainly JNK1 [23–25]. This observation was associated with changes in the expression of immediate early genes such as c-Jun and myc but also affected genes involved in cell cycle control such as Cyclin A, Cyclin D1, E2F1 and PCNA [14,26–31].

Our results indicate that in JNK1$^{-/-}$ mice, diminished IL-6/STAT-3 activation might be involved in causing impaired G0/G1 phase transition and the decrease in hepatocyte proliferation after PH. IL-6$^{-/-}$ mice also display a reduced rate of DNA synthesis due to abnormalities in G phase after PH [14]. Additionally, JNK1$^{-/-}$ livers elicited lower mRNA levels of c-Jun associated with delayed regeneration, suggesting that besides a change in IL-6/STAT-3 activation other pathways might contribute to this finding. Our data indicate that the reduced proliferation observed in JNK1$^{-/-}$ livers cannot be only explained by lack of JNK1 expression in hepatocytes. In turn, the role of JNK2 in liver regeneration is less clear. Sabapathy et al. [32] demonstrated that loss of JNK2 accelerated liver regeneration, through compensatory proliferation via JNK1. However, Das' study [8] reported no role for JNK2 in liver regeneration. To further analyze the function, Davis' group performed PH in mice with compound deletion of JNK1 and JNK2 in hepatocytes. Whereas they observed reduced proliferation and number of mitotic figures in hepatocyte JNK-deficient mice, no differences in overall liver regeneration was found. These data together with our observations indicate that (1) the function of JNK2 in hepatocytes is dispensable during liver regeneration and (2) another tissue rather than the liver is essential for providing the JNK-specific function during hepatic reconstitution.

Lipids accumulate in the regenerating liver after PH [33]. We found that metabolic functions such as glucose levels are diminished in JNK1$^{-/-}$ mice. In fact, JNK1$^{-/-}$ mice have been reported to have decreased phosphorylation of insulin receptor substrate-1 (IRS-1) at Ser^{307}, which ameliorates insulin resistance, and are protected against HFD-induced hepatocyte injury and steatosis [34,35]. Thus, it seems very likely that JNK1 mediates insulin resistance independent of nuclear receptor interactions. In fact, peroxisome proliferator-activated receptor γ1 (PPARγ1), glucocorticoid receptor (GR) and the retinoic acid receptors (RXR and RARγ) are the only nuclear receptor described as JNK substrates rather than liver X receptor (LXR) or farnesoid X receptor (FXR), transcription factors activated after liver resection [36]. Overall, these data indicate that the lower lipid accumulation observed in JNK1$^{-/-}$ might contribute to improved insulin sensitivity and reduced mitotic rate as well as impaired hepatocyte proliferation after PH.

Our results using JNK1$^{\Delta hepa}$ animals demonstrated that the observations found in JNK1$^{-/-}$ livers cannot be explained by lack of JNK1 expression in hepatocytes. Similar results had been described previously. Despite a reduction in the mitotic rate, compound JNK1 and JNK2 deficiency in hepatocytes did not affect overall liver regeneration [8]. In contrast to our results, the same group found that compound JNK1 and JNK2 deficiency in hepatocytes plus in non-parenchymal cells did not prevent liver regeneration. However, they identified a protective role for JNK in hepatocytes but deleterious in NPCs after treating the same mice with DEN. These contradictory results may be explained by the differential expression of cytokines during liver regeneration and tumor development. However, it is very plausible that the function of JNK1 and JNK2 in hepatocytes or NPCs might be dependent on the experimental model used. Altogether, from our present study, we can conclude that hematopoietic-derived JNK1 is a crucial determinant for hepatocyte proliferation after PH.

Immune-mediated mechanisms are strongly activated during liver regeneration, e.g., via induction of the APR consequently contributing to hepatocyte proliferation. In JNK1$^{-/-}$ mice, we observed a decreased number of CD11b-positive—TNF-producing cells—and significant changes were evident in the T-cell ratio of CD4- and CD8-positive T-cells. It is now generally accepted that bone marrow-derived cells play a fundamental role during liver regeneration after PH. Indeed, Fuji et al. (2010) reported a strong increase in the CD11b population after partial resection. Furthermore, JNK is required for the differentiation of pro-inflammatory macrophages and the promotion of the Th1 response [37–39]. Concomitantly, the deletion of JNK1 in hematopoietic cells decreases cell infiltration as well as proliferation in an experimental model of chronic liver injury where the regenerative stimulus is the death of hepatocytes [22]. Thus, all these data suggest that JNK1 in hematopoietic cells may contribute to the recruitment, infiltration and proliferation of inflammatory cells in the regenerating liver.

Concomitant with the study of Das et al. [8], our results indicated that lack of JNK1 expression in hepatocytes alone is not required for hepatic regeneration. Thus, we next focused on the identification of the tissue mediating the JNK1-dependent effect required for physiological liver regeneration. The BMT experiments identified that JNK1 in hematopoietic cells mediate a pro-proliferative effect after PH. Different studies have indicated that BM-derived cells are required to restore liver mass during liver regeneration. Among them, deficiency of TNF in BM-derived cells seems to impair the regenerative response after PH [40].

Up to now, the identity of the tissue-derived function of JNK1 has remained unknown. Some authors have speculated that the hypothalamus might directly influence liver regeneration and thus might be a likely candidate [41]. The major physiological functions of JNK1 have been associated to peripheral tissues rather than to hepatic areas [7,8,42–44].

Our data suggest that JNK in hematopoietic cells has a dominant function in stimulating the proliferative response during liver regeneration after PH. Loss of JNK1 in BM-derived cells caused delayed regeneration. In contrast, JNK1 deletion in hepatocytes alone has no impact on the proliferative response but acts synergistically to JNK1 in hematopoietic cells. The contribution of JNK1 from different tissues to the overall process of liver regeneration after injury has been already reported by our lab [22]. However, we cannot exclude the possibility that cell-type-specific JNK-derived effects affect also the expression of c-Jun. In the present study, we show that global JNK1 deletion significantly decreased c-Jun expression, whereas JNK1 deficiency in hepatocytes did not affect c-Jun expression. Thus, other authors [45] have raised the possibility that JNK-dependent signaling has partial redundancy with other pathways such as p38.

In summary, the role of JNK1 during liver regeneration seems to be more complex than originally thought as different tissues in a JNK1-dependent manner contribute to liver regeneration after PH.

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Disclosures: The authors declare that they have no competing financial interests.

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.bbadis.2014.10.011.

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