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Interplay of hepatic and myeloid STAT3 in facilitating liver regeneration via tempering innate immunity

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Abstract

Liver regeneration triggered by 2/3 partial hepatectomy is accompanied by elevated hepatic levels of endotoxin, which contributes to the regenerative process, but liver inflammation and apoptosis remain paradoxically limited. Here we show that STAT3, an important anti-inflammatory signal, is activated in myeloid cells after partial hepatectomy and its conditional deletion results in an enhanced inflammatory response. Surprisingly, this is accompanied by an improved rather than impaired regenerative response with increased hepatic STAT3 activation, which may contribute to the enhanced liver regeneration. Indeed, conditional deletion of STAT3 in both hepatocytes and myeloid cells results in elevated activation of STAT1 and apoptosis of hepatocytes, and a dramatic reduction in survival after partial hepatectomy, whereas additional global deletion of STAT1 protects against these effects. *Conclusions:* An interplay of myeloid and hepatic STAT3 signaling is essential to prevent liver failure during liver regeneration through tempering a strong innate inflammatory response mediated by STAT1 signaling.

Keywords

Liver regeneration; STAT3; myeloid cells; inflammatory response

Introduction

The liver has great ability to regenerate after injury or tissue loss, which is tightly controlled by multiple signaling pathways induced by a wide variety of cytokines, growth factors, and hormones.¹⁻⁴ Liver regeneration triggered by 2/3 partial hepatectomy (PHx), a widely used experimental model, proceeds initially by proliferation of hepatocytes and then by proliferation of nonparenchymal cells, including biliary epithelial, sinusoidal endothelial, and hepatic stellate cells.¹⁻⁴ Emerging evidence suggests that a variety of factors contribute to the initiation and progression of liver regeneration. These include hepatocyte growth factors, platelet-derived serotonin, stem cell factor, complements, and the innate inflammatory response.¹⁻⁴ Among these, the role of the innate inflammatory response has been extensively investigated.

It is generally accepted that PHx leads to elevation of serum levels of bacterial endotoxin (LPS),⁵ which stimulates Kupffer cells to produce TNF- α and IL-6. The latter then targets the IL-6 receptor complex (gp80/gp130) in hepatocytes, triggering the activation of signal transducer and activator of transcription 3 (STAT3), which promotes hepatocyte survival and proliferation.¹⁻⁴ However, a recent study suggests that MyD88 rather than LPS acting via TLR4 and CD14 contributes to IL-6/STAT3 activation after PHx, and the role of MyD88 in liver regeneration has been controversial.^{6, 7} IL-6 knockout mice display acute liver failure and impaired liver regeneration after PHx,⁸ although recent studies suggest that IL-6 may play a more important role in hepatoprotection during liver regeneration.^{9, 10} IL-6 activation of STAT3 also induces expression of suppressor of cytokine signaling 3 (SOCS3), which in turn terminates STAT3 signaling and negatively regulates liver regeneration.¹¹ Although mice with global knockout of IL-6 develop acute liver failure following PHx,^{8, 9} conditional deletion of the IL-6 downstream signaling molecule gp130 or STAT3 in hepatocytes did not result in acute liver failure, resulting in either no effect or only impaired liver regeneration after PHx.^{10, 12} The more severe liver damage following IL-6 knockout may be due to STAT3-independent signaling of IL-6, to extrahepatic actions of IL-6, or both.

After PHx, portal and systemic plasma concentrations of LPS are significantly elevated with peak levels reaching 140 pg/ml.⁵ Despite such high circulating levels of LPS, hepatocyte apoptosis and hepatic and systemic inflammation remain minimal.¹⁻³ For example, PHx reportedly induced only slight or no elevation of hepatocyte apoptosis,¹³ and even made hepatocytes resistant to Fas- and LPS-induced apoptosis.^{13, 14} PHx is associated with elevated serum levels of proinflammatory cytokines, but except for IL-6, these changes are minimal (supplemental Table S1),¹⁵ and there are no obvious inflammatory foci in the liver after PHx.¹⁻³ At present, the mechanisms that temper liver inflammation and apoptosis post PHx remain obscure. STAT3, a key signal for cell survival,¹⁶ is activated by PHx in the liver. However, deletion of STAT3 in hepatocytes only moderately impaired PHx-induced liver regeneration without inducing hepatocyte apoptosis.¹² Here we demonstrate for the first time that STAT3, an important anti-inflammatory signal,¹⁷ is also markedly activated in immune cells by PHx. Conditional deletion of STAT3 in myeloid lineage cells resulted in an enhanced inflammatory response and increased liver regeneration. Combined conditional ablation of STAT3 in both hepatocytes and myeloid cells, but not in either cell type alone, resulted in a dramatic reduction in survival with elevated activation of STAT1 and hepatocyte apoptosis after PHx. These findings suggest that an interplay of STAT3 in myeloid cells and hepatocytes plays a critical role in ensuring normal liver regeneration via tempering systemic and hepatic innate inflammatory responses.

Materials and Methods

Mice

Eight- to 10-week-old male mice were used in this study. Hepatocyte-specific STAT3KO (STAT3^{Hep^{-/-}}) and Myeloid cell-specific STAT3KO (STAT3^{Mye^{-/-}}) mice were described previously.¹⁸ Littermate wild-type mice (STAT3^{flox/flox}) were used as controls. STAT3^{Mye^{-/-}} mice have been proved to be a valuable tool in analyzing the physiologic role of STAT3 in monocytes/macrophages and neutrophils.¹⁷ Male STAT3^{Mye^{-/-}} mice were bred with female STAT3^{Hep^{-/-}} mice to generate four lines of mice: wild-type littermates (STAT3^{flox/flox}), STAT3^{Mye^{-/-}}, STAT3^{Hep^{-/-}}, STAT3^{Mye^{-/-}Hep^{-/-}} mice in which the STAT3 gene was deleted in both myeloid cells and hepatocytes.

STAT3^{Hep^{-/-}}STAT1^{-/-} and STAT3^{Mye^{-/-}}STAT1^{-/-} mice were developed via several steps of crossing STAT3^{Hep^{-/-}} mice with STAT1^{-/-} mice, and STAT3^{Mye^{-/-}} mice with STAT1^{-/-} mice, respectively. Male STAT3^{Mye^{-/-}}STAT1^{-/-} mice were bred with female STAT3^{Hep^{-/-}}STAT1^{-/-} mice to generate STAT3^{Mye^{-/-}Hep^{-/-}}STAT1^{-/-} triple KO mice, in

which the STAT3 gene was deleted in myeloid cells and hepatocytes while the STAT1 was deleted globally. All knockout strains mentioned above were developmentally normal and have normal life spans. All animal studies were approved by the Institutional Animal Care and Use Committees of the NIAAA, NIH.

Partial hepatectomy (PHx) model

For 2/3 partial hepatectomy (PHx) surgery, mice were anesthetized with sodium pentobarbital, followed by laparotomy, ligation of the median and left lateral lobes of the liver at their stem and excision under aseptic conditions, as described previously.¹⁹ For sham operation, mice were anesthetized and then subjected to laparotomy, followed by brief manipulation of the intestines, but not the liver, with cotton swabs before wound closure. The animals were killed by decapitation at the indicated times following surgery.

Statistical Analysis

Data are expressed as mean±SD. To compare values obtained from 2 groups, the Student's *t* test was performed. To compare values obtained from three or more groups, 1-factor analysis of variance (ANOVA) was used, followed by Tukey's post hoc test. Statistical significance was taken at the *P* <0.05 level.

Other methods

All other methods are described in the supporting document.

Results

Activation of STAT3 in myeloid cells after PHx

Figs. 1A and 1B show that phospho-STAT3 was markedly elevated by PHx in the liver and spleen tissues (Fig. 1A) as well as in liver leukocytes (Fig. 1B). Flow cytometric analyses show that phospho-STAT3 was elevated in both Gr1^{high} neutrophils and F4/80⁺ macrophages in the liver post PHx (Fig. 1C and Fig. S1). In addition, flow cytometric analyses reveal that the percentage of Gr1^{high} neutrophils was significantly increased in the liver post PHx while the percentage of F4/80⁺ macrophages was slightly increased (Fig. 1D). The total number of leukocytes, neutrophils, and macrophages was markedly elevated in the liver post PHx compared to the sham group (Fig. 1E). Increased levels of pSTAT3 were also detected in the liver but not in the spleen after sham operation to a lesser extent (Fig. 1A), which is in agreement with earlier findings.⁸

Deletion of STAT3 in myeloid cells and hepatocytes results in enhanced and reduced liver regeneration, respectively, while deletion of STAT3 in both cell types results in liver failure after PHx

To explore whether STAT3 activation in neutrophils/macrophages plays a role in controlling liver inflammation after PHx, we generated myeloid cell-specific STAT3 knockout mice (STAT3^{Mye^{-/-}}), in which the STAT3 gene had been deleted in myeloid lineage cells, including neutrophils, monocytes and macrophages.¹⁷ To further understand the interaction of myeloid and hepatic STAT3 in controlling liver inflammation and regeneration, we also generated hepatocyte-specific STAT3 knockout (STAT3^{Hep^{-/-}}) and hepatocyte/myeloid cell-specific double knockout (STAT3^{Mye^{-/-}Hep^{-/-}}) mice. After PHx, STAT3^{Mye^{-/-}} and STAT3^{Hep^{-/-}} mice showed no obvious adverse phenotype and no mortality, and their liver/body weight ratios were similar to that in wild-type mice (Fig. S2a). In contrast, 75% of STAT3^{Mye^{-/-}Hep^{-/-}} mice died between 24 and 40 hrs post PHx (Fig. 2A), with the remaining 25% surviving for at least 1 month after PHx. Liver histology showed that the number of inflammatory foci was greater in STAT3^{Mye^{-/-}} and STAT3^{Mye^{-/-}Hep^{-/-}} mice compared to wild-type and STAT3^{Hep^{-/-}} mice

(Figs. 2B-C). Compared to wild-type mice, hepatocyte proliferation as determined by BrdU incorporation and mitosis was significantly reduced in STAT3^{Hep^{-/-}} mice but was elevated in STAT3^{Mye^{-/-}} mice 40 hrs post PHx. The surviving STAT3^{Mye^{-/-}Hep^{-/-}} mice also had lower BrdU incorporation and mitosis in hepatocytes 40 hrs post PHx compared with wild-type mice (Figs. 2B, 2D, and Fig. S2b) and had reduced serum albumin compared with other groups (Fig. S2c). TUNEL analyses revealed that the number of apoptotic hepatocytes was much greater in STAT3^{Mye^{-/-}Hep^{-/-}} mice than in the other groups (Figs. 2B, 2E).

Deletion of STAT3 in myeloid cells enhances inflammatory response after PHx

Flow cytometric analyses show that in wild-type mice, the number of infiltrating Gr1^{high} cells, which represent neutrophils,²⁰ was elevated significantly 3 and 6 hrs post sham operations and maintained at slightly higher levels at 24 hrs, with such elevations being more pronounced and prolonged following PHx (Figs. S3 and Fig. 3A). The number of F4/80⁺ macrophages was slightly elevated post sham and PHx. Similar elevation of Gr1^{high} and F4/80⁺ cells was also observed in STAT3^{Hep^{-/-}} mice. In addition, the total number of infiltrating Gr1^{high} and F4/80⁺ cells was much higher in the livers of STAT3^{Mye^{-/-}} and STAT3^{Mye^{-/-}Hep^{-/-}} mice as compared to wild-type and STAT3^{Hep^{-/-}} mice, both before surgery, as well as post sham and PHx. This suggests that PHx-induced activation of STAT3 in myeloid cells inhibits their infiltration into the liver.

Fig. 3B summarized the serum levels of proinflammatory cytokines such as TNF- α , IL-6, and IFN- γ . In general, after sham operation, serum levels of IL-6 but not other cytokines was elevated in wild-type mice, while both STAT3^{Mye^{-/-}} and STAT3^{Mye^{-/-}Hep^{-/-}} mice had higher serum levels of IL-6 as well as TNF- α and IFN- γ . After PHx, serum levels of IL-6 were markedly elevated in wild-type and STAT3^{Hep^{-/-}} mice, such elevation was prolonged in STAT3^{Mye^{-/-}} and STAT3^{Mye^{-/-}Hep^{-/-}} mice. Serum levels TNF- α and IFN- γ were slightly elevated in wild-type and STAT3^{Hep^{-/-}} mice but were dramatically higher in STAT3^{Mye^{-/-}} and STAT3^{Mye^{-/-}Hep^{-/-}} mice. In addition, serum TNF- α levels were higher in STAT3^{Mye^{-/-}Hep^{-/-}} mice than STAT3^{Mye^{-/-}} mice 6 and 9 hrs post PHx

Deletion of STAT3 results in enhanced STAT1 in myeloid cells and hepatocytes after PHx

Next, we investigated the mechanisms underlying liver failure and impaired liver regeneration in STAT3^{Mye^{-/-}Hep^{-/-}} mice by analyzing the activation of the STAT3 pathway, which promotes hepatocyte survival and liver regeneration,^{12, 16} as well as STAT1 activation, which induces hepatocyte apoptosis and inhibits liver regeneration.²¹ As illustrated in Fig. 4A, STAT3 was activated by PHx in wild-type mice, as reflected by elevated levels of pSTAT3 that peaked 3-6 hr post-surgery. Compared to wild-type mice, in STAT3^{Mye^{-/-}} mice the STAT3 pathway was constitutively active, with both the STAT3 protein levels and pSTAT3 being elevated (PHx 0 hr), and increased slightly further following PHx. In contrast, hepatic STAT3 and pSTAT3 were both very low in STAT3^{Hep^{-/-}} and STAT3^{Mye^{-/-}Hep^{-/-}} mice, as expected.

STAT1 activation (pSTAT1) was not detected in the liver of wild-type and STAT3^{Mye^{-/-}} mice after PHx. However, in both STAT3^{Hep^{-/-}} and STAT3^{Mye^{-/-}Hep^{-/-}} mice hepatic levels of pSTAT1 were greatly increased following PHx, with peaks occurring 3-6 hrs post-PHx. A delayed increase in the expression of the STAT1 protein was observed 24 to 40 hrs post PHx in STAT3^{Hep^{-/-}} mice, whereas in STAT3^{Mye^{-/-}Hep^{-/-}} mice, STAT1 protein levels were constitutively elevated compared to wild-type and STAT3^{Hep^{-/-}} mice. Weak pSTAT3 activation was also detected in the liver after sham operation in wild-type and STAT3^{Mye^{-/-}} mice but not in STAT3^{Hep^{-/-}} and STAT3^{Mye^{-/-}Hep^{-/-}} mice (Fig. S4a). Interestingly, in the spleen STAT3 was activated similarly after PHx in all four lines of mice, while pSTAT1 activation was not detected in any of them (Fig. S4b).

Expression of cyclin D was induced in wild-type mice 24 and 40 hrs post PHx. Such induction was higher in STAT3^{Mye^{-/-}} mice but lower in STAT3^{Hep^{-/-}} and STAT3^{Mye^{-/-}Hep^{-/-}} mice (Fig. 4 and Fig. S5), which is consistent with the grade of liver regeneration in these mice, as illustrated in Fig. 2. In addition, SOCS3 but not SOCS1 was induced after PHx in wild-type mice, consistent with earlier findings.¹¹ Similar induction of SOCS3 was also observed in STAT3^{Mye^{-/-}} mice. Interestingly, SOCS1 but not SOCS3 was significantly induced after PHx in both STAT3^{Hep^{-/-}} and STAT3^{Mye^{-/-}Hep^{-/-}} mice.

pSTAT3 and pSTAT1 activation were also examined in liver leukocytes after sham operation or PHx. As shown in Fig. 4B, pSTAT3 was detected post-PHx in the liver leukocytes from wild-type and STAT3^{Hep^{-/-}} mice but not from STAT3^{Mye^{-/-}} and STAT3^{Mye^{-/-}Hep^{-/-}} mice. Constitutive activation of pSTAT1 was detected in the liver leukocytes of STAT3^{Mye^{-/-}} mice before or after sham or PHx, in agreement with previous reports.¹⁷ pSTAT1 was detected in the liver leukocytes 3 hrs post PHx in all groups with the highest levels in STAT3^{Mye^{-/-}Hep^{-/-}} mice.

Deletion of STAT1 restores liver regeneration in STAT3^{Hep^{-/-}} mice, reduces inflammatory responses in STAT3^{Mye^{-/-}} mice, and rescues STAT3^{Mye^{-/-}Hep^{-/-}} mice from post-PHx liver failure

The above data (Fig. 4) indicate increased activation of pSTAT1 in the inflammatory cells of STAT3^{Mye^{-/-}} mice and in the liver of STAT3^{Hep^{-/-}} mice, respectively, and increased activation in both the inflammatory cells and the liver in STAT3^{Mye^{-/-}Hep^{-/-}} mice. Since STAT1 plays a key role in the induction of inflammation, cell apoptosis and cell cycle arrest,²¹ it is possible that elevation of STAT1 in hepatocytes contributes to reduced liver regeneration in STAT3^{Hep^{-/-}} mice, elevation of STAT1 in inflammatory cells contributes to enhanced inflammation in STAT3^{Mye^{-/-}} mice, while the simultaneous elevation of pSTAT1 in both inflammatory cells and the liver contributes to liver failure and impaired liver regeneration in STAT3^{Mye^{-/-}Hep^{-/-}} mice. To test these possibilities, we generated STAT3^{Hep^{-/-}}STAT1^{-/-}, STAT3^{Mye^{-/-}}STAT1^{-/-}, and STAT3^{Mye^{-/-}Hep^{-/-}}STAT1^{-/-} mice.

As illustrated in Fig. 5A, expression of STAT1 protein in the liver was induced in STAT3^{Hep^{-/-}} mice but not in wild-type mice, which is consistent with previous findings.¹² Western blot analyses confirmed the absence of STAT1 and STAT3 protein expression in the liver of STAT3^{Hep^{-/-}}STAT1^{-/-} mice (Fig. 5B). All STAT3^{Hep^{-/-}}STAT1^{-/-} mice survived after PHx (Fig. 5C) and had a greater number of Brdu⁺ hepatocytes than STAT3^{Hep^{-/-}} mice after PHx (Fig. 5D), suggesting that deletion of STAT1 in STAT3^{Hep^{-/-}} mice restores the ability of the liver to regenerate. Fig. 5E illustrates that treatment with a low dose of IFN- γ induced stronger pSTAT1 activation in STAT3^{Hep^{-/-}} than in wild-type hepatocytes. As expected, no STAT1 or STAT3 proteins were detected in STAT3^{Hep^{-/-}}STAT1^{-/-} hepatocytes. Furthermore, STAT3^{Hep^{-/-}} hepatocytes were more susceptible to IFN- γ inhibition of cell proliferation, an effect that was abolished in STAT3^{Hep^{-/-}}STAT1^{-/-} hepatocytes.

Western blot analyses (Fig. 6A) confirmed that in STAT3^{Mye^{-/-}}STAT1^{-/-} mice STAT1 protein was undetectable and STAT3 protein was very low in liver leukocytes. All STAT3^{Mye^{-/-}}STAT1^{-/-} mice survived after PHx (data not shown) and had comparable liver regeneration as STAT3^{Mye^{-/-}} mice (Fig. 6B). Infiltration of neutrophils and macrophages was reduced in STAT3^{Mye^{-/-}}STAT1^{-/-} mice compared with STAT3^{Mye^{-/-}} mice (data not shown). Elevation of serum inflammatory cytokines was also diminished in the former relative to the latter group (Fig. 6C).

Western blotting (Fig. 7A) confirmed the absence of STAT1 protein expression in hepatocytes and liver leukocytes, and the absence of STAT3 protein expression in hepatocytes and its very low level in liver leukocytes in STAT3^{Mye^{-/-}Hep^{-/-}}STAT1^{-/-} triple KO mice. All

STAT3^{Mye^{-/-}Hep^{-/-}}STAT1^{-/-} triple KO mice survived after PHx, in contrast to the 25% survival of STAT3^{Mye^{-/-}Hep^{-/-}} mice (Fig. 7B). Furthermore, as illustrated in Fig. 7C-D, the STAT3^{Mye^{-/-}Hep^{-/-}}STAT1^{-/-} mice had enhanced liver regeneration, reduced hepatocyte apoptosis, and reduced serum cytokines after PHx compared to STAT3^{Mye^{-/-}Hep^{-/-}} mice. These findings suggest that deletion of STAT1 rescues liver function and regeneration, and attenuates the innate inflammatory response as compared to STAT3^{Mye^{-/-}Hep^{-/-}} double KO mice.

Discussion

In this paper, we demonstrate for the first time that PHx results in STAT3 activation in immune cells, in addition to its activation in the liver, as reported previously.⁸ Additionally, our results indicate that activation of STAT3 in myeloid lineage cells and hepatocytes act in concert to effectively temper the systemic and hepatic inflammatory responses, ensuring normal liver regeneration, as summarized in a proposed model in Fig. 8. The rationale for this model is presented below.

STAT3 is activated in both the liver and myeloid cells after PHx (Fig. 1). Elevation of IL-6 are likely responsible for STAT3 activation in the liver because such activation is markedly diminished in IL-6 knockout mice.^{8, 11} At present, the mechanisms underlying PHx-induced STAT3 activation in myeloid cells are not clear. Both IL-6 and IL-10 are known to activate STAT3 in myeloid cells and to be elevated in the liver and serum after PHx.^{8, 22} Thus, both of these cytokines likely contribute to STAT3 activation in myeloid cells.

Deletion of STAT3 in myeloid cells resulted in increased infiltration of macrophages and neutrophils into the remnant liver following PHx. Production of the proinflammatory cytokines TNF- α and IL-6 by these cells leads to activation of STAT3 in the liver. This may be responsible for the enhanced liver regeneration observed in STAT3^{Mye^{-/-}} mice after PHx (Fig. 2), because all of these factors have been shown to promote liver regeneration.^{8, 23, 24} Deletion of STAT3 in myeloid cells also resulted in elevated serum levels of IFN- γ , a cytokine known to induce hepatocyte apoptosis and cell cycle arrest via activation of the STAT1 signaling pathway.²¹ However, despite the high serum levels of IFN- γ , activation of STAT1 was not detected in the liver of STAT3^{Mye^{-/-}} mice after PHx (Fig. 4A). This may be due to suppression of STAT1 signaling by hepatic STAT3 in STAT3^{Mye^{-/-}} mice. Indeed, deletion of hepatic STAT3 resulted in enhanced hepatic pSTAT1 in both STAT3^{Hep^{-/-}Mye^{-/-}} and STAT3^{Hep^{-/-}} mice. In addition, the strong inflammatory response in STAT3^{Mye^{-/-}} mice after PHx may be partly due to enhanced STAT1 activation in leukocytes (Fig. 4B), as deletion of STAT1 markedly reduced cytokine production (Fig. 6).

Disruption of STAT3 in hepatocytes resulted in decreased liver regeneration without mortality after PHx, consistent with previous reports.¹² Interestingly, we have previously shown that mortality rate was significantly higher in mice with STAT3 deficiency in hepatocytes and digestive tissues than wild-type controls.²⁵ These findings suggest that STAT3 in digestive tissues may play a hepatoprotective role while STAT3 in hepatocyte stimulates hepatocyte proliferation during liver regeneration. It is believed that the stimulatory effect of STAT3 on liver regeneration is mediated via induction of several immediate early genes.¹² Here we demonstrated that deletion of STAT1 restored liver regeneration in STAT3^{Hep^{-/-}} and STAT3^{Hep^{-/-}Mye^{-/-}} mice (Figs. 5 and 7), suggesting that inhibition of STAT1 signaling is one of the mechanisms through which STAT3 activation promotes liver regeneration. However, the mechanism by which STAT3 suppresses STAT1 signaling is not well understood. STAT signaling pathways can be negatively regulated by several mechanisms, including induction of SOCSs, tyrosine phosphatases, PIAS etc.²⁶ Fig. 4 shows that induction of SOCS3 and SOCS1 correlates with activation of STAT3 and STAT1, respectively, suggesting that STAT3

activation is responsible for SOCS3 induction while SOCS1 induction is dependent on STAT1 activation after PHx. It is probable that STAT3 inhibits STAT1 signaling via at least in part induction of SOCS3 expression because SOCS3 has been shown to inhibit STAT1 signaling.²⁷

No mortality and no obvious hepatocyte apoptosis were observed in STAT3^{Mye^{-/-}} mice after PHx despite high levels of inflammatory cytokines such as TNF- α and IFN- γ . This is probably due to prolonged STAT3 activation in the liver that protects against hepatocyte death, STAT3 being a survival signal for hepatocytes.¹⁶ Indeed, deletion of hepatic STAT3 both in hepatocytes and myeloid cells caused massive apoptosis after PHx. Interestingly, deletion of the IL-6 signaling molecule gp130 in both hepatocytes and bone marrow cells did not result in liver failure after PHx.¹⁰ This suggests that the critical role of myeloid STAT3 activation in liver regeneration is mediated by a mediator other than IL-6. In addition, deletion of hepatic STAT3 also resulted in further increases in serum levels of TNF- α in STAT3^{Mye^{-/-}} mice after PHx (Fig. 3B). This may be due to increased hepatocyte apoptosis in STAT3^{Mye^{-/-}Hep^{-/-}} mice that can stimulate macrophages/Kupffer cells to produce inflammatory cytokines.²⁸ Such high levels of TNF- α may also contribute to the massive apoptosis and liver failure in STAT3^{Mye^{-/-}Hep^{-/-}} mice after PHx because abnormally high levels of TNF- α were shown to contribute to liver failure after PHx in Timp3^{-/-} mice.²⁹ Although STAT3 is a survival signal for hepatocytes, selective deletion of STAT3 in hepatocytes did not induce apoptosis and mortality. This may be due to maintained STAT3 activation in myeloid cells that limits inflammatory responses such as TNF- α and IFN- γ production. Deletion of STAT3 in both myeloid cells and hepatocytes in STAT3^{Hep^{-/-}Mye^{-/-}} mice resulted in high levels of serum TNF- α and IFN- γ and hepatic STAT1 activation, which resulted in massive apoptosis of hepatocytes and high mortality. It has been recently proposed that STAT3 inhibitors may be used in the treatment of hepatocellular carcinoma (HCC).³⁰ The present findings advocate caution with such an approach, because global inhibition of STAT3 may result in a strong innate inflammatory response and liver failure, especially in the remnant liver of HCC patients following liver resection. Indeed, liver failure after resection was often seen in HCC patients with elevated inflammatory responses due to sepsis.³¹ Additionally, elevated STAT1 expression and activation in the liver were found in patients with chronic liver disease,³² which may impair liver regeneration. Thus, a strategy to increase STAT3/STAT1 ratio in both hepatocytes and leukocytes may have a beneficial effect in preventing liver failure in HCC patients with elevated inflammatory responses after liver resection.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Abbreviations

KO	knockout
PHx	2/3 partial hepatectomy
STAT	signal transducer and activator of transcription
STAT3 ^{Hep^{-/-}}	hepatocyte-specific STAT3 knockout mice
STAT3 ^{Mye^{-/-}}	myeloid cell-specific STAT3 knockout mice

STAT3 ^{Hep^{-/-}Mye^{-/-}}	hepatocyte and myeloid cell-specific STAT3 double knockout mice
STAT3 ^{Hep^{-/-}Mye^{-/-}STAT1^{-/-}}	hepatocyte and myeloid cell-specific STAT3 and STAT1 triple knockout mice

References

1. Michalopoulos GK. Liver regeneration. *J Cell Physiol* 2007;213:286–300. [PubMed: 17559071]
2. Fausto N, Campbell JS, Riehle KJ. Liver regeneration. *Hepatology* 2006;43:S45–53. [PubMed: 16447274]
3. Taub R. Liver regeneration: from myth to mechanism. *Nat Rev Mol Cell Biol* 2004;5:836–847. [PubMed: 15459664]
4. Koniaris LG, McKillop IH, Schwartz SI, Zimmers TA. Liver regeneration. *J Am Coll Surg* 2003;197:634–659. [PubMed: 14522336]
5. Cornell RP. Gut-derived endotoxin elicits hepatotrophic factor secretion for liver regeneration. *Am J Physiol* 1985;249:R551–562. [PubMed: 2865902]
6. Campbell JS, Riehle KJ, Brooling JT, Bauer RL, Mitchell C, Fausto N. Proinflammatory cytokine production in liver regeneration is Myd88-dependent, but independent of Cd14, Tlr2, and Tlr4. *J Immunol* 2006;176:2522–2528. [PubMed: 16456013]
7. Seki E, Tsutsui H, Iimuro Y, Naka T, Son G, Akira S, et al. Contribution of Toll-like receptor/myeloid differentiation factor 88 signaling to murine liver regeneration. *Hepatology* 2005;41:443–450. [PubMed: 15723296]
8. Cressman DE, Greenbaum LE, DeAngelis RA, Ciliberto G, Furth EE, Poli V, et al. Liver failure and defective hepatocyte regeneration in interleukin-6-deficient mice. *Science* 1996;274:1379–1383. [PubMed: 8910279]
9. Blindenbacher A, Wang X, Langer I, Savino R, Terracciano L, Heim MH. Interleukin 6 is important for survival after partial hepatectomy in mice. *Hepatology* 2003;38:674–682. [PubMed: 12939594]
10. Wuestefeld T, Klein C, Streetz KL, Betz U, Lauber J, Buer J, et al. Interleukin-6/glycoprotein 130-dependent pathways are protective during liver regeneration. *J Biol Chem* 2003;278:11281–11288. [PubMed: 12509437]
11. Campbell JS, Prichard L, Schaper F, Schmitz J, Stephenson-Famy A, Rosenfeld ME, et al. Expression of suppressors of cytokine signaling during liver regeneration. *J Clin Invest* 2001;107:1285–1292. [PubMed: 11375418]
12. Li W, Liang X, Kellendonk C, Poli V, Taub R. STAT3 contributes to the mitogenic response of hepatocytes during liver regeneration. *J Biol Chem* 2002;277:28411–28417. [PubMed: 12032149]
13. Helling TS, Dhar A, Helling TS Jr, Moore BT, VanWay CW. Partial hepatectomy with or without endotoxin does not promote apoptosis in the rat liver. *J Surg Res* 2004;116:1–10. [PubMed: 14732342]
14. Desbarats J, Newell MK. Fas engagement accelerates liver regeneration after partial hepatectomy. *Nat Med* 2000;6:920–923. [PubMed: 10932231]
15. Bergheim I, Luyendyk JP, Steele C, Russell GK, Guo L, Roth RA, et al. Metformin prevents endotoxin-induced liver injury after partial hepatectomy. *J Pharmacol Exp Ther* 2006;316:1053–1061. [PubMed: 16322356]
16. Taub R. Hepatoprotection via the IL-6/Stat3 pathway. *J Clin Invest* 2003;112:978–980. [PubMed: 14523032]
17. Takeda K, Clausen BE, Kaisho T, Tsujimura T, Terada N, Forster I, et al. Enhanced Th1 activity and development of chronic enterocolitis in mice devoid of Stat3 in macrophages and neutrophils. *Immunity* 1999;10:39–49. [PubMed: 10023769]
18. Horiguchi N, Wang L, Mukhopadhyay P, Park O, Jeong WI, Lafdil F, et al. Cell type-dependent pro- and anti-inflammatory role of signal transducer and activator of transcription 3 in alcoholic liver injury. *Gastroenterology* 2008;134:1148–1158. [PubMed: 18395093]

19. Mitchell C, Willenbring H. A reproducible and well-tolerated method for 2/3 partial hepatectomy in mice. *Nat Protoc* 2008;3:1167–1170. [PubMed: 18600221]
20. Daley JM, Thomay AA, Connolly MD, Reichner JS, Albina JE. Use of Ly6G-specific monoclonal antibody to deplete neutrophils in mice. *J Leukoc Biol* 2008;83:64–70. [PubMed: 17884993]
21. Sun R, Park O, Horiguchi N, Kulkarni S, Jeong WI, Sun HY, et al. STAT1 contributes to dsRNA inhibition of liver regeneration after partial hepatectomy in mice. *Hepatology* 2006;44:955–966. [PubMed: 17006930]
22. Rai RM, Loffreda S, Karp CL, Yang SQ, Lin HZ, Diehl AM. Kupffer cell depletion abolishes induction of interleukin-10 and permits sustained overexpression of tumor necrosis factor alpha messenger RNA in the regenerating rat liver. *Hepatology* 1997;25:889–895. [PubMed: 9096593]
23. Selzner N, Selzner M, Odermatt B, Tian Y, Van Rooijen N, Clavien PA. ICAM-1 triggers liver regeneration through leukocyte recruitment and Kupffer cell-dependent release of TNF-alpha/IL-6 in mice. *Gastroenterology* 2003;124:692–700. [PubMed: 12612908]
24. Yamada Y, Kirillova I, Peschon JJ, Fausto N. Initiation of liver growth by tumor necrosis factor: deficient liver regeneration in mice lacking type I tumor necrosis factor receptor. *Proc Natl Acad Sci U S A* 1997;94:1441–1446. [PubMed: 9037072]
25. Moh A, Iwamoto Y, Chai GX, Zhang SS, Kano A, Yang DD, et al. Role of STAT3 in liver regeneration: survival, DNA synthesis, inflammatory reaction and liver mass recovery. *Lab Invest* 2007;87:1018–1028. [PubMed: 17660847]
26. Greenhalgh CJ, Hilton DJ. Negative regulation of cytokine signaling. *J Leukoc Biol* 2001;70:348–356. [PubMed: 11527983]
27. Song MM, Shuai K. The suppressor of cytokine signaling (SOCS) 1 and SOCS3 but not SOCS2 proteins inhibit interferon-mediated antiviral and antiproliferative activities. *J Biol Chem* 1998;273:35056–35062. [PubMed: 9857039]
28. Naugler WE, Sakurai T, Kim S, Maeda S, Kim K, Elsharkawy AM, et al. Gender disparity in liver cancer due to sex differences in MyD88-dependent IL-6 production. *Science* 2007;317:121–124. [PubMed: 17615358]
29. Mohammed FF, Smookler DS, Taylor SE, Fingleton B, Kassiri Z, Sanchez OH, et al. Abnormal TNF activity in Timp3^{-/-} mice leads to chronic hepatic inflammation and failure of liver regeneration. *Nat Genet* 2004;36:969–977. [PubMed: 15322543]
30. Lin L, Amin R, Gallicano GI, Glasgow E, Jogunoori W, Jessup JM, et al. The STAT3 inhibitor NSC 74859 is effective in hepatocellular cancers with disrupted TGF-beta signaling. *Oncogene* 2009;28:961–972. [PubMed: 19137011]
31. Capussotti L, Vigano L, Giuliani F, Ferrero A, Giovannini I, Nuzzo G. Liver dysfunction and sepsis determine operative mortality after liver resection. *Br J Surg* 2009;96:88–94. [PubMed: 19109799]
32. Duong FH, Filipowicz M, Tripodi M, La Monica N, Heim MH. Hepatitis C virus inhibits interferon signaling through up-regulation of protein phosphatase 2A. *Gastroenterology* 2004;126:263–277. [PubMed: 14699505]

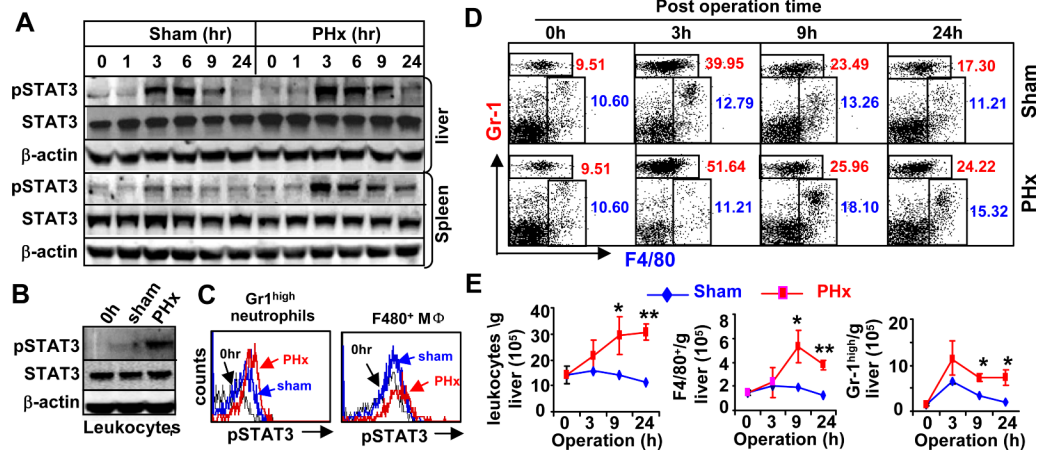


Fig. 1. STAT3 activation in liver neutrophils and macrophages after PHx

A. Western blot analyses of mouse liver and spleen tissues post sham and PHx. **B.** Western blot analyses of liver leukocytes 3 hrs post sham and PHx. **C.** STAT3 activation in liver neutrophils and macrophages 3 hrs post PHx as determined by FACS analyses. **D.** Percentage of Gr1^{high} neutrophils and F4/80⁺ macrophages analyzed by flow cytometric analyses post sham and PHx. **E.** Total number of F4/80⁺ macrophages and Gr1^{high} neutrophils increased after PHx. Values represent means ± SD (n=6). *P<0.05, **P<0.01 in comparison with corresponding sham group.

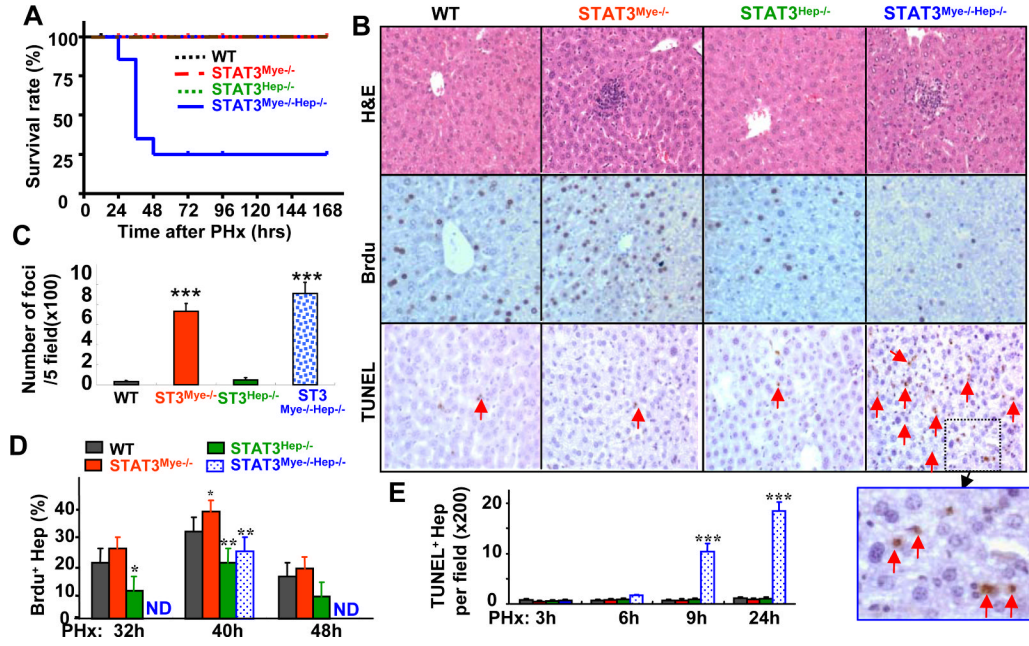


Fig. 2. Increased liver regeneration in STAT3^{Mye-/-} mice, reduced liver regeneration in STAT3^{Hep-/-} mice, and liver failure in STAT3^{Mye-/-Hep-/-} mice after PHx. **A.** Survival rate post PHx. **B.** Representative photographs of H&E, Brdu, and TUNEL staining of livers 9, 40, and 24 hrs post PHx, respectively. Red arrows indicate TUNEL⁺ apoptotic hepatocytes. **C.** The number of inflammatory foci was quantified 9 hrs post PHx. **D, E.** The percentage of BrdU⁺ and TUNEL⁺ hepatocytes were quantified. Values represent means ± SD (n=5-10 mice). ND: not done in STAT3^{Mye-/-Hep-/-} group. *P<0.05, **P<0.01, ***P<0.0001 in comparison with corresponding wild-type littermate controls (WT).

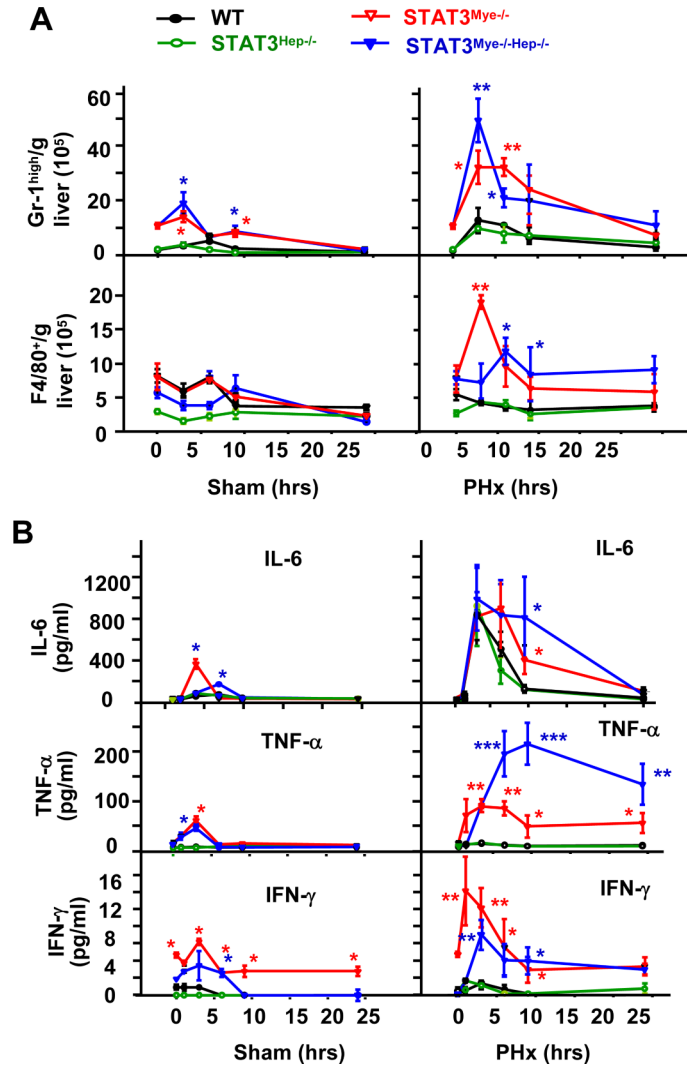


Fig. 3. Depletion of STAT3 in myeloid cells enhances inflammation after PHX. **A**, Total number of Gr1^{high} neutrophils and F4/80⁺ macrophages after sham and PHX. **B**, Serum cytokines after sham and PHX. Values represent means \pm SD (n=5-10). * P <0.05, ** P <0.01; *** P <0.001 in comparison with corresponding wild-type littermate controls.

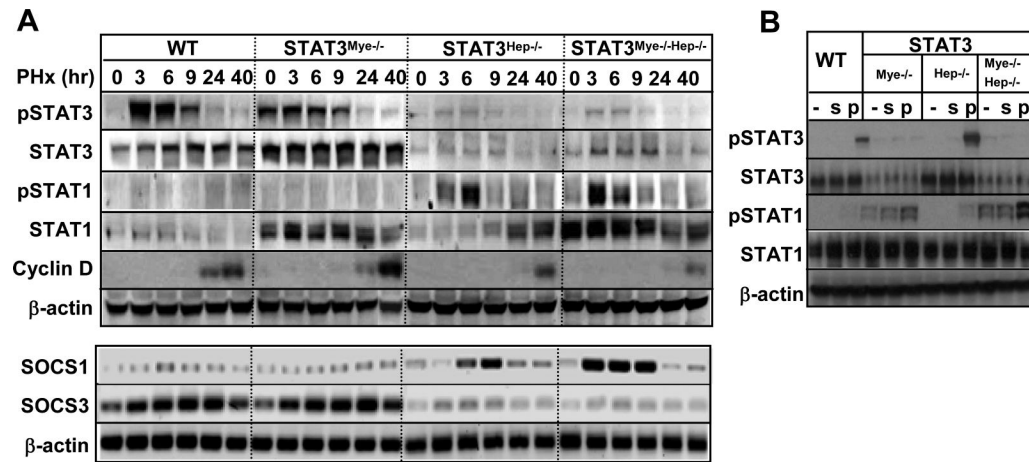


Fig. 4. STAT1 activation is enhanced the liver and leukocytes in STAT3^{Hep-/-} and STAT3^{Mye-/-} mice, respectively, and is enhanced in both the liver and leukocytes in STAT3^{Mye-/-Hep-/-} mice after PHx. **A**, Western blot (upper panel) and RT-PCR (low panel) analyses of liver tissues post PHx. **B**, Western blot analyses of liver leukocytes 3 hrs post sham (s) and PHx (p). Representative Western blot photography was shown from 2 independent experiments with similar results. “-” represents control group without operation. WT: represent littermate controls.

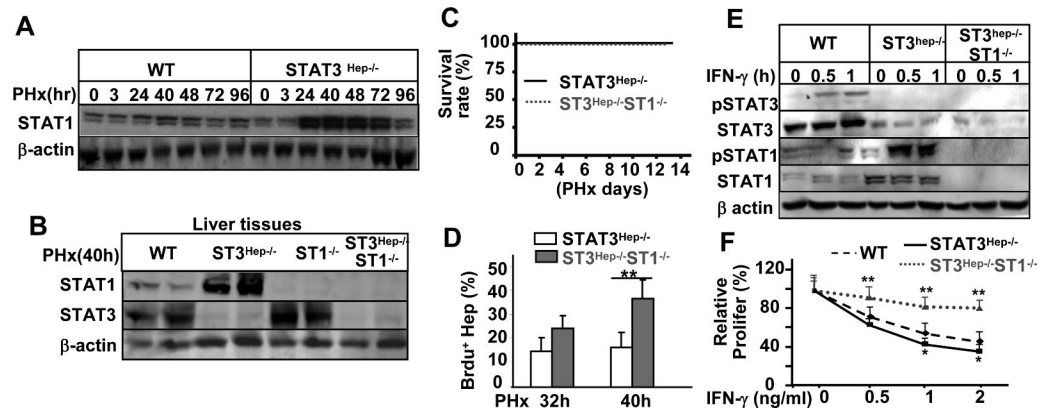


Fig. 5. Deletion of STAT1 restores liver regeneration in $STAT3^{Hep-/-}$ mice and abolishes $IFN-\gamma$ inhibition of hepatocyte proliferation

A, Expression of STAT1 in the liver is enhanced in $STAT3^{Hep-/-}$ mice after PHx. **B**, Western blot analyses of liver tissues to confirm $STAT3^{Hep-/-}STAT1^{-/-}$ mice. **C**, $STAT3^{Hep-/-}STAT1^{-/-}$ mice survived after PHx. **D**, $STAT3^{Hep-/-}STAT1^{-/-}$ mice showed increased liver regeneration compared to $STAT3^{Hep-/-}$ mice. **E**, Western blotting of primary mouse hepatocytes treated with $IFN-\gamma$. **F**, Primary hepatocytes were treated with $IFN-\gamma$ for 48 hrs, cell proliferation was then determined by measuring [3H] thymidine uptake. Values represent means \pm SD (n=6-8). In panel D, ** P <0.01 in comparison with $STAT3^{Hep-/-}$ mice. In panel F, * P <0.05, ** P <0.01, in comparison with corresponding wild-type littermate controls.

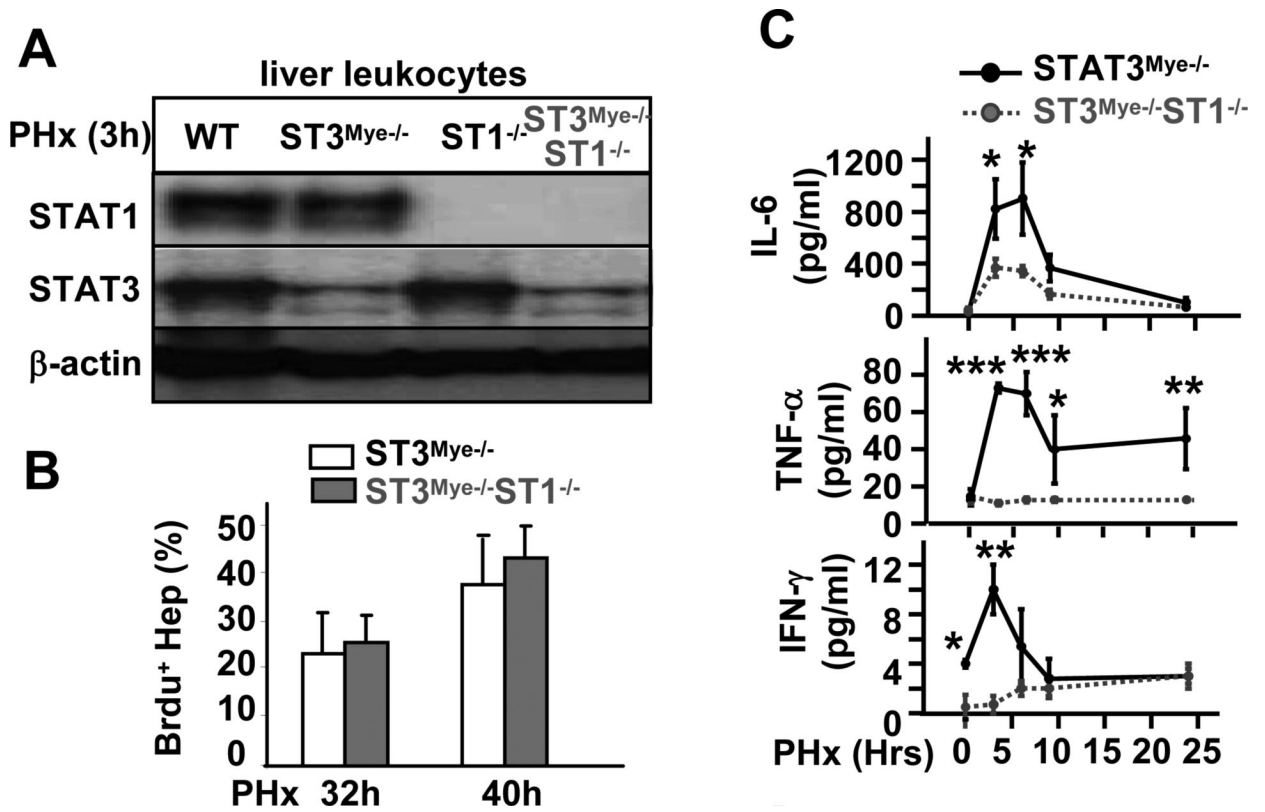


Fig. 6. Deletion of STAT1 diminishes inflammatory responses in STAT3^{Mye-/-} mice after PHx
A. Western blot analyses of liver leukocytes to confirm STAT3^{Mye-/-} STAT1^{-/-} mice. Liver leukocytes were isolated 3 hrs post PHx. **B.** STAT3^{Mye-/-} STAT1^{-/-} mice had comparable liver regeneration as STAT3^{Mye-/-} mice after PHx. **C.** STAT3^{Mye-/-} STAT1^{-/-} mice showed reduced serum cytokines after PHx compared to STAT3^{Mye-/-} mice. Values represent means \pm SD (n=5-9). * P <0.05, ** P <0.01, *** P <0.001 in comparison with STAT3^{Mye-/-} mice.

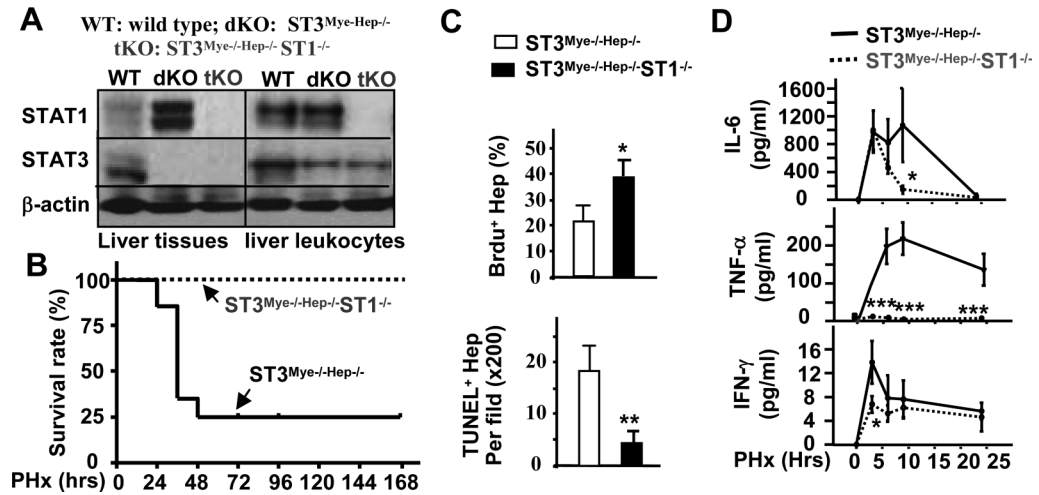


Fig. 7. Deletion of STAT1 rescues liver failure in $STAT3^{Mye-/-Hep-/-}$ mice after PHx

A. Western blot analyses of liver tissues and leukocytes to confirm the $STAT3^{Mye-/-Hep-/-} STAT1^{-/-}$ KO mice. **B-D.** Deletion of STAT1 increases the survival rate (**B**), restores liver regeneration and reduces apoptosis (**C**), and reduces the inflammatory response (**D**) in $STAT3^{Mye-/-Hep-/-}$ mice after PHx. Values represent means \pm SD (n=4-8). * P <0.05, ** P <0.01, *** P <0.001 in comparison with $STAT3^{Mye-/-Hep-/-}$ mice.

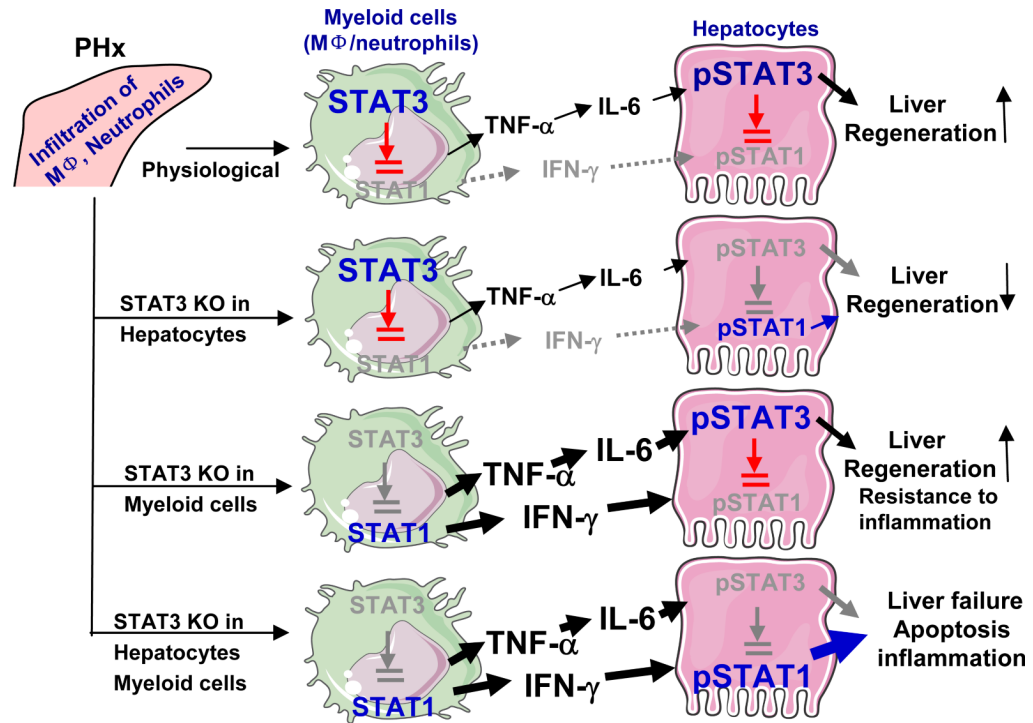


Fig. 8. An interplay of myeloid and hepatic STAT3 in preventing liver failure during liver regeneration via tempering innate immunity
 Myeloid STAT3 inhibits infiltration of innate immune cells, inhibits STAT1 activation in innate immune cells, and inhibits production of inflammatory cytokines such as TNF- α , IL-6, and IFN- γ ; while hepatic STAT3 inhibits inflammatory signaling STAT1 in the liver and promotes hepatocyte survival.