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Myeloid STAT3 inhibits T-cell–mediated hepatitis by regulating T helper 1 cytokine and interleukin-17 production

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Abstract

Background & Aims—T-cell–mediated hepatitis is a leading cause of acute liver failure; there is no effective treatment and the mechanisms underlying its pathogenesis are obscure. The aim of this study was to investigate the immune-cell signaling pathways involved—specifically the role of signal transducer and activator of transcription 3 (STAT3)—in T-cell–mediated hepatitis in mice.

Methods—T-cell–mediated hepatitis was induced in mice by injection of concanavalin A (Con A). Mice with myeloid cell-specific and T-cell–specific deletion of STAT3 were generated.

Results—STAT3 was activated in myeloid and T cells following Con A injection. Deletion of STAT3 specifically from myeloid cells exacerbated T-cell hepatitis and induced STAT1-dependent production of a Th1 cytokine (IFN- γ), and to a lesser extent of Th17 cytokines (IL-17 and IL-22), in a STAT1-independent manner. In contrast, deletion of STAT3 in T cells reduced T-cell mediated hepatitis and IL-17 production. Furthermore, deletion of IFN- γ completely abolished Con A-induced T-cell hepatitis whereas deletion of IL-17 slightly but significantly reduced such injury. *In vitro* experiments indicated that IL-17 promoted liver inflammation but inhibited hepatocyte apoptosis.

Conclusion—Myeloid STAT3 activation inhibits T-cell–mediated hepatitis via suppression of a Th1 cytokine (IFN- γ) in a STAT1-dependent manner whereas STAT3 activation in T cells promotes T-cell hepatitis to a lesser extent, via induction of IL-17. Therefore, activation of STAT3 in myeloid cells could be a novel therapeutic strategy for patients with T-cell hepatitis.

Keywords

STAT3; liver; T-cell hepatitis; myeloid cells; IL-17

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Introduction

Viral hepatitis, which affects half a billion people worldwide, is a major cause of chronic liver injury, leading to fibrosis, cirrhosis, and hepatocellular carcinoma. Although hepatitis viruses themselves are not cytopathogenic and do not kill hepatocytes, T cell-mediated immune responses play central roles in inducing hepatocellular injury during viral infection.^{1, 2} Moreover, T-cell-mediated liver injury also contributes to the pathogenesis of autoimmune hepatitis,³ primary biliary cirrhosis,⁴ alcoholic liver disease,⁵ hepatic ischemia/reperfusion injury⁶ and allograft rejection.⁷ In the past two decades, major progress has been made in the understanding of the molecular and cellular mechanisms underlying T-cell-mediated liver injury through use of a murine model of T-cell hepatitis induced by concanavalin A (Con A).⁸ Evidence suggests that Con A-induced T-cell hepatitis is initiated and tightly controlled by interactions between multiple cell types, a variety of cytokines, and their downstream signaling pathways.⁹ Immune cells involved in Con A-induced hepatitis include CD4⁺ T cells, natural killer T cells,¹⁰ Kupffer cells/macrophages,¹¹ neutrophils,¹² and eosinophils.¹³ Natural killer cells do not play a role in this model.¹⁰ Of the cytokines, both IFN- γ (Th1 cytokine) and IL-4 (Th2 cytokine) have been shown to play a central role in Con A-induced hepatitis,^{14, 15} while the role of IL-17 (Th17 cytokine) has been controversial.^{16, 17} The inflammatory cytokine, TNF- α , has also been shown to play an essential role in T-cell hepatitis.¹⁸ In contrast, research findings show that IL-6, IL-22, and IL-10 protect against Con A-induced liver injury.^{15, 16, 19, 20} The effects of many cytokines mentioned above on Con A-induced hepatocellular injury are mediated, at least in part, by directly targeting activation of various signaling pathways in hepatocytes that control hepatocyte survival and proliferation. For example, activation of signal transducer and activator of transcription 1 (STAT1) by IFN- γ induces hepatocyte apoptosis and cell cycle arrest,^{15, 21, 22} while activation of STAT3 by IL-6 and IL-22 induces anti-apoptotic and anti-oxidative stress proteins and consequently promotes hepatocyte survival and proliferation.^{15, 16, 19, 23} Additionally, activation of I kappa B kinases or STAT3 in hepatocytes protects against hepatocyte apoptosis,²⁴⁻²⁸ while JNK activation in hepatocytes has no effect on Con A-induced hepatitis.²⁹

Although the roles of various signaling pathways in hepatocytes in Con A-induced hepatitis have been extensively investigated, their functions in immune cells in this model have just recently been revealed. For example, while deletion of JNK1/2 in hepatocytes does not yield any effects, deletion of JNK1/2 in hematopoietic cells protects against Con A-induced hepatitis by inhibiting TNF- α production.²⁹ Here, we demonstrated that STAT3 is activated in myeloid lineage cells and T cells during Con A-induced hepatitis. To clarify the role of STAT3 in immune cells during T-cell mediated hepatitis, we generated T-cell-specific STAT3 knockout (STAT3^{T-cell/-}) and myeloid cell-specific STAT3 knockout (STAT3^{Mye/-}) mice. Our findings suggest that STAT3 in myeloid cells and T cells plays an opposing role in controlling T-cell hepatitis via modulating differential expression of innate, Th1, and Th17 cytokines.

Materials and Methods

Animals

Eight- to 10-week old male mice were used in all studies performed. T-cell specific-STAT3 knockout (STAT3^{T-cell/-}), myeloid-specific-STAT3 knockout (STAT3^{Mye/-}), STAT3^{Mye/-}-STAT1^{-/-}, and STAT3^{Mye/-}-IL-17^{-/-} mice were described in the supporting materials. For each group, respective littermates were used as wild-type mice. IL-17A^{-/-} mice on a C57BL/6 background were provided generously by Dr. Iwakura of the University of Tokyo. IL-6^{-/-}, IL-10^{-/-}, and IFN- γ ^{-/-} mice on a C57BL/6 background were purchased from the Jackson Laboratory. All animals were housed in a pathogen-free environment and used in accordance with protocols approved by the Institutional Animal Care and Utilization Committee.

Statistical analysis

All data are expressed as mean $\pm$ SEM. Statistical analysis were performed with t test or the non-parametric Mann-Whitney U-test for significance using PRISM software (GraphPad). A *P*-value of less than 0.05 indicated a significant difference between groups.

All other materials and methods are described in the supporting document.

Results

Activation of STAT3 in myeloid cells and T cells during Con A-induced hepatitis

Our previous studies showed that STAT3 and STAT1 were activated in the liver during Con A-induced hepatitis.¹⁵ Here we showed by immunostaining that STAT3 activation occurs not only in hepatocytes, but also in virtually all other non-parenchymal cells including inflammatory cells (Fig. 1A). Next, we found that STAT3 and STAT1 were also activated in the spleen after Con A injection (Fig. 1B). Flow cytometry analyses further revealed that STAT3 and STAT1 phosphorylation occurred in CD3⁺ T cells and CD11b⁺ myeloid cells obtained from the spleen 2 hours after Con A administration (Fig. 1C), which is summarized in Fig. 1D showing that the absolute number of CD11b⁺ and CD3⁺ T with activated STAT3 and STAT1 significantly increased after Con A treatment.

Deletion of myeloid STAT3 enhances preferentially Th1 cytokine response, and Th17 cytokine response to a lesser extent, exacerbating liver injury in Con A-induced hepatitis

To assess the role of STAT3 activation in CD11b⁺ myeloid cells in Con A-induced hepatitis, we generated myeloid-cell specific STAT3 knockout mice (STAT3^{Mye-/-}). Serum ALT activity and liver histology analyses (Figs. 2A and 2B) revealed that Con A injection induced much greater liver injury in STAT3^{Mye-/-} mice than in wild-type mice. Hepatic expression of pSTAT3, pSTAT1, pNF- κ B was higher in STAT3^{Mye-/-} mice than in wild-type mice 3 hours post Con A injection (Fig. 2C). The enhanced hepatic injury observed in STAT3^{Mye-/-} mice was also associated with a marked increase in serum TNF- α , IL-6, MCP-1, and IL-27, IFN- γ , IL-10, IL-17, IL-22 (Fig. 2D). Among these cytokines, IFN- γ had the highest induction (about 35 fold), followed by IL-6, TNF- α , IL-17, MCP-1, and IL-12p70 (5-10 fold), IL-27 and IL-22 (about 2 fold) (Fig. 2E). Levels of Th2 cytokine IL-4 were comparable between STAT3^{Mye-/-} and wild-type mice. Immunohistochemical analyses of myeloperoxidase (MPO) showed that the number of neutrophils was much greater in the livers of STAT3^{Mye-/-} mice compared with wild-type mice post Con A injection (Fig. 2F).

Myeloid-cell specific STAT3 deficiency promotes T-cell STAT3 activation

The inhibitory effect of STAT3 in myeloid cells (such as macrophages) on inflammatory and Th1 cytokines has been well documented;³⁰ however how myeloid STAT3 controls IL-17 response in T-cell hepatitis remains unknown. As STAT3 is required for IL-17 expression in T-cells,^{31, 32} we analyzed its activation on isolated liver CD3⁺ T cells and CD11b⁺ myeloid cells 2 hours after Con A administration. As expected, the number of pSTAT3⁺CD11b⁺ myeloid cells increased in the livers of wild-type mice, but not in STAT3^{Mye-/-} mice, while pSTAT3⁺CD3⁺ T cell accumulation was greater in the livers of STAT3^{Mye-/-} mice than in wild-type mice (Fig. 3A). Isolated spleen CD4⁺ T cells from STAT3^{Mye-/-} mice showed higher levels of *Rora* and *Rorc* mRNA (Fig. 3B), encoding for two transcription factors indispensable for IL-17 synthesis.^{31, 32} Induction of these 2 transcription factors was associated with 70-fold induction of *Il17a* and 2 fold induction of *Il17f* mRNA expression in splenic CD4⁺ T cells from STAT3^{Mye-/-} mice vs. wild-type mice post Con A injection (Fig. 3B). Induction of *Il17a* and *Il17f* mRNA expression was also more profound in the livers of STAT3^{Mye-/-} mice compared with wild-type mice (Fig. 3C). In addition, induction of IL-17 and IL-22 was reduced in

IL-6^{-/-} mice, but was enhanced in IL-10^{-/-} mice (Figs. 3D, 3E), suggesting that IL-6 promotes, while IL-10 inhibits, Th17 cytokine production.

Activation of STAT3 in T cells contributes to the Th17 cytokine IL-17, but not Th1/Th2 cytokine production, and promotes Con A-induced hepatitis

To further define whether STAT3 activation in T cells is responsible for IL-17 production in T-cell hepatitis, we generated T cell specific STAT3 knockout mice (STAT3^{T-cell/-}). STAT3 depletion in T cells was confirmed by flow cytometry analysis (Fig. 4A). As expected, STAT3 phosphorylation was enhanced in CD3⁺ T cells from wild-type liver lymphocytes and splenocytes, but was completely abolished in STAT3^{T-cell/-} mice. Induction of serum IL-17 after Con A injection was markedly diminished (reduced 8-fold) in STAT3^{T-cell/-} mice, while induction of many other cytokines were comparable between wild-type and STAT3^{T-cell/-} mice (Fig. 4B). Induction of IL-6 and MCP-1 was slightly reduced in STAT3^{T-cell/-} mice compared with wild-type mice (Fig. 4B). Finally, serum ALT activity, liver necrosis, and neutrophil infiltration were lower in STAT3^{T-cell/-} mice than in wild-type animals 12 hours after Con A administration (Figs. 4C-E). These results suggest that STAT3 activation in T cells promotes Con A-mediated hepatitis.

IFN-γ plays an essential role, while IL-17 plays a minor but significant role in T-cell-mediated liver injury

Since both IFN-γ and IL-17 have been shown to play an important role in inducing inflammation,^{15, 32, 33} we hypothesized that higher levels of IFN-γ and IL-17 may contribute to enhanced liver injury in STAT3^{Mye/-} mice. To test this hypothesis, we first defined the role of IL-17 and IFN-γ in Con A-induced liver injury by using IL-17^{-/-} and IFN-γ^{-/-} mice, respectively. Histological and biochemical analyses (Figs. 5A-B) revealed a marked reduction (90%) of serum ALT levels in IFN-γ^{-/-} mice compared with wild-type mice, in agreement with earlier findings.¹⁵ Unlike the diminished Con A-induced liver injury found in IFN-γ^{-/-} mice, which was very significant and repeatable, the difference in Con A-induced liver injury between wild-type and IL-17^{-/-} mice varied from experiment to experiment. In order to see the true effect, we performed 3 independent experiments (total 12 control mice and 13 IL-17^{-/-} mice), which showed that serum ALT levels were slightly but significantly reduced in IL-17^{-/-} mice post Con A injection compared with wild-type animals (Fig. 5A). Similarly, inflammatory cytokine release was reduced in both IL-17^{-/-} and IFN-γ^{-/-} mice with a stronger inhibition observed in the latter especially for TNF-α and IL-12 (Fig. 5C). These data suggest that both IL-17 and IFN-γ are involved in Con A-induced hepatitis where IFN-γ plays a dominant role in inducing hepatocellular injury.

The proinflammatory effect of IFN-γ in T-cell hepatitis is probably due to activation of STAT1 in various cell types including hepatocytes, Kupffer cells, and endothelial cells.³⁴ However, how IL-17 modulates T-cell hepatitis remains unknown. Here we examined the pro-inflammatory effect of IL-17 in liver Kupffer cells. As shown in Figs. 5D and 5E, treatment of liver Kupffer cells with IL-17 induced NF-κB activation and production of TNF-α and IL-6 without affecting IFN-γ and IL-10.

As NF-κB activation in hepatocytes is known as an important key signal in hepatocyte survival,^{25, 35} we next asked whether in addition to its pro-inflammatory function on macrophages, IL-17 can also modulate IFN-γ-induced hepatocyte apoptosis. As shown in Fig. 5F, IL-17 treatment reduced IFN-γ-induced lactate dehydrogenase release and caspase-3 activity in primary hepatocytes. Taken together, these data showed that IL-17 may play a double edged-sword role, by promoting liver inflammation through activation of macrophages, and by protecting hepatocytes from IFN-γ-induced apoptosis.

Deletion of IL-17 in STAT3^{Mye-/-} mice does not reduce Con A-induced hepatitis

To further determine the role of IL-17 in liver inflammation and injury in STAT3^{Mye-/-} mice, we generated myeloid-specific STAT3 and IL-17 double knockout mice (STAT3^{Mye-/-}IL-17^{-/-}). Surprisingly, the deletion of IL-17 in STAT3^{Mye-/-} mice did not protect them from Con A-induced liver injury, as shown in Figs. 6A-B by similar levels of serum ALT and liver necrosis compared to STAT3^{Mye-/-} mice. In addition, the high level of the pro-inflammatory cytokine release observed in STAT3^{Mye-/-} mice was not affected in STAT3^{Mye-/-}IL-17^{-/-} double knockout animals (Fig. 6C).

Deletion of STAT1 reduces innate and Th1 cytokines, but not Th2/Th17 cytokine production, and ameliorates liver injury in STAT3^{Mye-/-} mice during T-cell hepatitis

Compared to wild-type mice, STAT3^{Mye-/-} mice had enhanced pSTAT1 activation in the liver after Con A injection (Fig. 2C). Moreover, enhanced pSTAT1 activation was also observed in the splenocytes of STAT3^{Mye-/-} mice compared with cells from wild-type mice (Fig. 7A). To define whether the enhanced STAT1 is responsible for the elevated Con A-induced liver injury in STAT3^{Mye-/-} mice, we generated STAT3^{Mye-/-}STAT1^{-/-} double knockout mice in which the STAT3 gene was disrupted in myeloid cells and the STAT1 gene was disrupted globally. Figs. 7B and 7C show serum cytokine levels from four lines of mice, including wild-type, STAT1^{-/-}, STAT3^{Mye-/-}, and double knockout mice. Serum levels of many inflammatory cytokines post Con A injection were comparable between STAT1^{-/-} and wild-type mice, except for IL-27, IL-12, and IL-17. Both IL-12 and IL-27 were downregulated, while IL-17 was upregulated in STAT1^{-/-} mice. Compared with wild-type mice, STAT3^{Mye-/-} mice had markedly higher levels of many inflammatory cytokines, except IL-4, which was consistent with the findings in Fig. 2. The pro-inflammatory cytokines, TNF- α , IL-6, and IL-27 and the chemokine MCP-1, as well as Th1 cytokines, were found at a significantly lower level in STAT3^{Mye-/-}STAT1^{-/-} mice compared with STAT3^{Mye-/-} mice (Figs. 7B-C). In contrast, serum levels of the Th2 cytokine, IL-4, and the Th17 cytokines, IL-17 and IL-22, were not repressed when STAT1 was additionally deleted in STAT3^{Mye-/-} mice (STAT3^{Mye-/-}STAT1^{-/-} mice) compared with STAT3^{Mye-/-} mice (Fig. 7C). IL-10 production was also reduced in STAT3^{Mye-/-}STAT1^{-/-} mice compared with STAT3^{Mye-/-} mice. Finally, deletion of STAT1 completely prevented Con A-induced liver injury in STAT3^{Mye-/-} mice (STAT3^{Mye-/-} vs. STAT3^{Mye-/-}STAT1^{-/-}) (Fig. 7D). Compared with wild-type mice, STAT1^{-/-} mice were resistant to Con A-induced liver injury, in agreement with earlier findings.^{15, 22}

Discussion

T cell activation in antigen-dependent (e.g. HCV and HBV) as well as in antigen-independent (e.g. drug intoxication, alcoholic liver diseases) mediated hepatitis has been shown to play a critical role in the pathogenesis of liver diseases.¹⁻⁷ For example, in chronic HCV infection, while the virus itself has a non-cytolytic function, activation of CD8⁺ T cells kills viral infected hepatocytes via releasing perforin and granzyme, while activation of CD4⁺ T cells produces inflammatory cytokines and controls CD8⁺ T cell cytotoxicity, contributing to the progression of liver disease.^{1, 2} It has been well documented that T-cell hepatitis is controlled by the interplay between multiple signaling pathways induced by a wide variety of inflammatory, Th1 and Th2 cytokines.⁹ Data from the current study suggest that activation of STAT3 signaling pathway in myeloid cells and T cells play opposing roles in controlling Con A-induced hepatitis through differential regulation of these cytokines. The proposed interaction and effect of these cytokines are summarized in the model shown in Fig. 8.

Deletion of STAT3 in myeloid cells enhanced preferentially the Th1 cytokine response during Con A-induced hepatitis (Fig. 2). Based on our findings, it is plausible to speculate that deletion of STAT3 in myeloid cells results in enhanced STAT1 activation, which stimulates myeloid

cells to produce inflammatory cytokines such as IL-6, TNF- α , MCP-1, IL-27, and IL-12. The latter 2 cytokines stimulate Th1 cells to produce IFN- γ , which subsequently contributes to liver injury. This cascade is supported by several lines of evidence. First, myeloid lineage cells, including macrophages and dendritic cells, are the major producers of IL-12 and IL-27.³⁶ Deletion of STAT3 in myeloid cells promoted IL-12 and IL-27 production during Con A induced liver injury, while deletion of STAT1 diminished this effect (Fig. 7), suggesting that STAT3 inhibits while STAT1 promotes IL-12 and IL-27 production in myeloid cells. Second, both IL-12 and IL-27 have been shown to be the major cytokines to stimulate Th1 cells to produce IFN- γ .³⁶ Deletion of IL-27 abrogated IFN- γ production during T-cell hepatitis,³⁷ suggesting that IL-27 plays an important role in induction of IFN- γ in this model. Third, IL-27 can activate both STAT1 and STAT3 in T cells.³⁸ Since deletion of STAT1 abolished (Fig. 7), but deletion of STAT3 in T cells had no effect on the production of IFN- γ (Fig. 4), it is likely that IL-27 induces IFN- γ production in T cells via activation of STAT1 during T-cell hepatitis. Lastly, the critical roles of this cascade have been clearly demonstrated in mice with ablation of the genes encoding STAT1 (Fig. 7),^{15, 22} IL-27,³⁷ or IFN- γ .¹⁵ The latter 2 cytokines can activate STAT1 in hepatocytes, leading to hepatocellular injury.^{21, 39}

Deletion of STAT3 in myeloid cells also enhanced Th17 cytokine IL-17 in Con A-induced hepatitis. Several lines of evidence suggest that enhanced IL-17 production in STAT3^{Mye-/-} mice after Con A injection is due to elevated IL-6 and T cell STAT3 activation. First, it has been well documented that IL-6 is required for Th17 cell differentiation and IL-17 production.^{31, 32} Production of IL-6 was higher in STAT3^{Mye-/-} mice after injection of Con A (Fig. 2) and deletion of IL-6 markedly diminished IL-17 production (Fig. 3). Second, T cells from STAT3^{Mye-/-} mice had enhanced activation of STAT3 (Fig. 3A), a signal that is essential for Th17 cell differentiation and IL-17 production.^{31, 32} Third, deletion of STAT3 in T cells markedly diminished IL-17 production during Con A-induced hepatitis. These findings suggest that myeloid and T cell STAT3 inhibits and promotes IL-17 production during T-cell hepatitis, respectively. Interestingly, IL-17 production showed an increasing, but not significant, trend in STAT1^{-/-} mice compared with wild-type mice (Fig. 7C). This increase may be due to downregulation of IL-27 in STAT1^{-/-} mice (Fig. 7B) as IL-27 is an important inhibitor for IL-17 production.⁴⁰ In contrast, additional global deletion of STAT1 did not affect IL-17 production in STAT3^{Mye-/-} mice (Fig. 7C). This may be because deletion of STAT1 not only abolished IL-27 production but also reduced IL-6 synthesis, an IL-17 stimulatory cytokine (Fig. 7B), leading to a minimal effect on IL-17 production.

Production of IL-22, another Th17 cytokine, was also higher in STAT3^{Mye-/-} mice after Con A injection compared with wild-type mice (Fig. 2). This production was not affected after deletion of STAT3 in T cells (Fig. 4) or global deletion of STAT1 (Fig. 7), but was reduced in IL-6^{-/-} mice (Fig. 3). This suggests that IL-6 may promote IL-22 production in a T cell STAT3-independent manner via activation of STAT3 in other cell types rather than T cells, as IL-22 can be produced by NK cells in addition to T cells.⁴¹ In contrast to STAT3, STAT1 had no effect on IL-22 production because global deletion of STAT1 did not modulate IL-22 production (Fig. 7).

We and others have previously demonstrated that the Th2 cytokine, IL-4, plays an essential role in inducing Con A-induced hepatitis via activation of STAT6.¹⁴ Here we demonstrated that deletion of STAT3 in myeloid cells or T cells, or global deletion of STAT1 had no effect on Th2 cytokine IL-4 production during T-cell hepatitis. This suggests that myeloid and T cell STAT3 and STAT1 do not affect IL-4 production during Con A-induced hepatitis, which is consistent with earlier findings that IL-4 is controlled by STAT6/GATA3.⁴²

The extensive liver damage observed in STAT3^{Mye-/-} mice was associated with an increase in innate and Th1 inflammatory cytokine production. Enhanced elevation of TNF- α , IFN- γ ,

IL-12, and IL-27 likely contributes to the exacerbated liver injury observed in STAT3^{Mye-/-} mice because all of these cytokines have been shown to play an important role in Con A-induced hepatitis,^{15, 37} while enhanced elevation of hepatoprotective cytokines IL-6¹⁵ and IL-22^{16, 19} and anti-inflammatory cytokine IL-10²⁰ may play a compensatory role in preventing hepatitis in STAT3^{Mye-/-} mice. Surprisingly, deletion of IL-17 did not reduce hepatitis in STAT3^{Mye-/-} mice, suggesting enhanced IL-17 levels did not contribute to the enhanced hepatitis in these mice. This is probably because IFN- γ plays a dominant role while IL-17 has a less important function in inducing liver injury in this model (see discussion below), and the fold of IFN- γ elevation (35 fold) was much higher than IL-17 induction (9 fold) in STAT3^{Mye-/-} mice after Con A injection compared to wild-type mice (Fig. 2F).

The critical roles of Th1 cytokine IFN- γ and Th2 cytokine IL-4 in T-cell hepatitis have been well documented;^{14, 15} while the findings regarding the role of IL-17 in this model have been controversial.^{16, 17} Zenewicz et al¹⁶ reported that IL-17 did not play a role in Con A-induced hepatitis, while Nagata et al¹⁷ showed that IL-17RA contributed to liver injury in this model. Our findings showed the difference in serum ALT between IL-17^{-/-} and wild-type mice is small but reached statistical difference, which is consistent with Nagata et al.¹⁷ The reason for the discrepancy between our findings and Zenewicz et al¹⁶ is not clear and could be attributed to the different environment of the animal facilities that may affect IL-17^{-/-} mice. In contrast to the small difference in serum ALT levels between IL-17^{-/-} and wild-type mice, the serum ALT levels were reduced by 90% in IFN- γ ^{-/-} mice compared to wild-type mice after Con A injection. These findings suggest that both IL-17 and IFN- γ participate to T-cell-mediated hepatitis, and that IL-17 plays a mild role as compared to the major contribution of IFN- γ . The essential role of IFN- γ in T-cell hepatitis is probably attributed to multiple detrimental functions of IFN- γ in the liver, including IFN- γ induction of hepatocyte apoptosis and cell cycle arrest,²¹ IFN- γ induction of expression of chemokines and their receptors on liver cells,³⁴ and IFN- γ activation of Kupffer cells/macrophages.⁴³ Although IL-17 also targets multiple cell types in the liver to induce expression of inflammatory cytokines and chemokines⁴⁴ (Fig. 5D), IL-17 protects against rather than potentiates IFN- γ -induced hepatocyte apoptosis (Fig. 5F). In addition, intraperitoneal injection of IL-17 caused recruitment of neutrophils into the peritoneum⁴⁵ and intratracheal instillation of IL-17 markedly induced recruitment of neutrophils into the lung.⁴⁶ However, neither intraperitoneal nor intrahepatic injection of IL-17 caused liver injury and neutrophil recruitment into the liver (Lafdil and Gao, unpublished observation). Taken together, these findings suggest that IL-17 appears to play a less important role in T-cell hepatitis than IFN- γ . Although serum levels IFN- γ were comparable between STAT3^{T cell-/-} and wild-type mice after Con A injection (Fig. 4B), liver injury (serum ALT) was reduced in STAT3^{T cell-/-} than wild-type mice. Such reduced liver injury in STAT3^{T cell-/-} mice may be attributed to a decrease in IL-17 in these mice as IL-17 has been shown to contribute to Con A-induced hepatitis (Fig. 5).

Taken together, our findings suggest that myeloid STAT3 inhibits T-cell hepatitis via downregulation of a variety of cytokines. In macrophage, STAT3 inhibits STAT1 signaling pathway, followed by attenuating IL-12, IL-27, and IFN- γ production. STAT3 may also inhibit NF- κ B signaling pathway,⁴⁷ leading to a decrease in IL-6, MCP-1, and TNF- α production. Finally, many of these cytokines can target hepatocytes, leading to enhanced activation of several signaling pathways including STAT1, STAT3, and NF- κ B (Fig. 2C) that either promote or prevent hepatocellular injury during T-cell hepatitis. The outcome and progression of liver injury are determined by the balance between these signaling pathways. Activation of STAT3 in myeloid cells or blocking IFN- γ could be novel therapeutic strategies to treat T-cell hepatitis in patients, while blockage of IL-17 may not be effective.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Abbreviations

STAT3	signal transducer and activator of transcription factor 3
STAT3^{T-cell/-mice}	T-cell-specific STAT3 knock out mice
STAT3^{Mye/-mice}	myeloid cell-specific STAT3 knock out mice
MPO	myeloperoxidase

Fig. 1. Activation of STAT3 and STAT1 in myeloid and T cells in Con A-induced hepatitis
 C57BL/6 mice were injected with vehicle (saline) or Con A for various time points. **A**, Phospho-STAT3 immunostaining on liver tissues from control and Con A-treated mice. Blue and red arrows depict pSTAT3 in hepatocytes and small cells in the sinusoids, respectively. **B**, Western blot analyses of the spleen tissues. **C**, Flow cytometry analyses of CD11b⁺ myeloid cells and CD3⁺ T cells from the spleen of mice treated with Con A for 2 hrs with pSTAT3 or pSTAT1 antibodies. **D**, Absolute number of CD11b⁺ and CD3⁺ cells stained with pSTAT3 or pSTAT1. * $P < 0.05$ and ** $P < 0.01$.

Fig. 2. Myeloid STAT3 depletion exacerbates Con A-induced hepatitis and promotes preferentially innate inflammatory, Th1 (IFN- γ) cytokines and Th17 cytokines to a lesser extent without affecting Th2 (IL-4) cytokine

A, Serum ALT levels. **B**, H & E staining of liver sections 12 hrs post Con A injection. Arrows indicate necrotic areas. **C**, Western blot analyses of liver protein extracts from mice 3 hrs post Con A injection. **D**, Serum pro-inflammatory cytokines. **E**, Relative induction of cytokines 2 hrs post Con A injection. The values from wild-type mice were set as 1. **F**, Myeloperoxidase immunostaining of liver tissues 12 hrs post Con A injection. * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.005$ ($n = 5-8$), in comparison with the corresponding WT groups. ND for Not Detected.

Fig. 3. Myeloid-cell specific STAT3 deficiency promotes T-cell STAT3 activation and IL-17 response

A, STAT3 activation (pSTAT3⁺ cells) in hepatic CD11b⁺ and CD3⁺ T cells 2 hrs post Con A treatment analyzed by flow cytometry. **B-C**, Real-time PCR analyses of mRNAs from spleen CD4⁺ T cells (**B**) and liver tissues (**C**) of mice treated with Con A. **D, E**, Serum levels of IL-17 and IL-22. **P* < 0.05, ***P* < 0.01, and ****P* < 0.005 (n=4-8), in comparison with the corresponding WT groups.

Fig. 4. Deletion of STAT3 in T cells reduces IL-17 production and liver injury in Con A-induced hepatitis

A, Phosphorylated STAT3 detection in CD3⁺ T cells in liver mononuclear cells (MNCs) and splenocytes from mice treated with Con A for 2 hrs by flow cytometry. **B**, Serum inflammatory cytokines. **C**, Serum ALT levels. **D**, Liver tissues stained by Hematoxylin and eosin 12 hrs post Con A injection. Arrows indicate necrotic areas. **E**, Myeloperoxidase immunostaining of liver tissues 12 hrs post-Con A injection. * $P < 0.05$ and ** $P < 0.01$ ($n = 6-12$). The number of mice used in the all groups in *panel B* was the same. ND for Not Detected.

Fig. 5. IFN- γ plays an essential role, while IL-17 plays a minor but significant role in T-cell-mediated liver injury: IL-17 stimulates Kupffer cells to produce cytokines but prevents IFN- γ -induced hepatocyte apoptosis

A, Serum ALT levels 12 hrs post Con A injection. **B**, H & E staining of liver sections 12 hrs post Con A injection. **C**, Serum inflammatory cytokines. **D**, Western blot analyses of phospho-P65 and phospho-STAT3 in IL-17-treated liver macrophages. **E**, Inflammatory cytokines from IL-17-treated liver macrophage culture medium 24 hrs later. **F**, Isolated hepatocytes were cultured for 3 days without or with IFN- γ (10ng/ml), IL-17 (10ng/ml), or both cytokines. Lactate dehydrogenase (LDH) activity and caspase 3 were measured. * P <0.05, ** P <0.01, *** P <0.001. ND for Not Detected.

Fig. 6. Deletion of IL-17 in STAT3^{Myc}^{-/-} mice does not reduce Con A-induced hepatitis
A, Serum ALT levels 12 hrs post Con A injection. **B**, H & E staining of liver sections 12 hrs post Con A injection. **C**, Serum inflammatory cytokines 12 hrs post Con A injection.

Fig. 7. Deletion of STAT1 in STAT3^{Mye-/-} mice ameliorates liver injury and abolishes innate immune and Th1 but not Th2/Th17 cytokine production during Con A-induced hepatitis
A, Activation of pSTAT1 in WT and STAT3^{Mye-/-} splenocytes 2 hrs post Con A injection analysed by Western blotting. **B-C**, Serum levels of cytokines. **D**, Serum ALT levels. * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.005$ (n=3-6). ND for Not Detected.

Fig. 8. A model depicting the hepatoprotection of myeloid cell STAT3 in T-cell hepatitis
During T-cell hepatitis, myeloid cell STAT3 inhibits STAT1 signaling in these cells, followed by preventing IL-12/IL-27 production and subsequently inhibiting IL12/IL-27 stimulation of IFN- γ production by Th1 cells. Myeloid cell STAT3 inhibits STAT1 and NF- κ B activation, followed by reducing production of inflammatory cytokines (IL-6 and TNF- α) and subsequently inhibiting IL-6 stimulation of IL-17 production by Th17 cells. IFN- γ plays an essential role in T-cell hepatitis via induction of inflammation and hepatocyte death, while IL-17 only stimulates weakly liver inflammation but prevents hepatocyte death, thereby playing a double-edged sword role in T-cell hepatitis. Myeloid cell STAT3 also inhibits IL-22 production via unknown mechanisms.