Interleukin-17 mediates triptolide-induced liver injury in mice

Xinzhi Wang, Zhenzhou Jiang, Mengtao Xing, Jing Fu, Yuwen Su, Lixin Sun, Luyong Zhang

A R T I C L E   I N F O

Article info
Received 31 March 2014
Accepted 6 June 2014
Available online 17 June 2014

Keywords:
Triptolide
Drug-induced liver injury
IL-17
Neutrophils

A B S T R A C T

Triptolide (TP)-induced liver injury can be attributed to the Th17/Treg imbalance with the enhancement of the expansion of Th17 cells and suppression of the production of Tregs, especially the significant increase of interleukin (IL)-17 secreted by helper T (Th) 17 cells. To further investigate the involvement of IL-17-mediated immune response in the TP-induced hepatotoxicity, we examined the plasma transaminase, histopathological changes, hepatic frequencies of Th17 cells, hepatic expression of transcriptional factors and cytokines genes and plasma IL-17 levels after administration of TP (600 μg/kg) by oral gavage to female C57BL/6 mice. Mice treated with TP displayed acute liver injury with significantly increased hepatic frequencies of Th17 cells, mRNA expression of retinoid-related orphan receptor (ROR)-γt and plasma IL-17 level as well as the plasma ALT and AST. Neutralization study using anti-IL-17 antibody ameliorated TP-induced liver injury. In contrast, when challenged by coadministration of recombinant IL-17, hepatotoxicity was exacerbated in the triptolide-administered mice. In summary, this report was demonstrated for the first time that IL-17-mediated immune response is involved in the pathogenesis of TP-induced liver injury in mice, which may shed light on the mechanisms of TP-induced liver injury.

© 2014 Elsevier Ltd. All rights reserved.

1. Introduction

Triptolide (diterpenoid triepoxide, TP) is a principal active component isolated from the traditional Chinese medicine Tripterygium wilfordii Hook F (TWHF), which exhibits notable immune-regulative effects (Chen, 2001). Although the ethyl acetate and methanol/chloroform extracts of TWHF have been used in the treatment of systemic lupus erythematosus, rheumatoid arthritis and nephritis (Liu et al., 2005; Zhang et al., 2010), TWHF and TP have been noted to induce severe adverse reactions, especially hepatotoxicity (Wang et al., 2013; Zhang et al., 2012). However, the mechanisms of TP-induced liver injury have not been fully clarified.

Helper T (Th) cell-mediated immune responses contribute to the pathogenesis of drug-induced liver injury (DILI) (Heneghan and McFarlane, 2002). The effect of Th cells in the liver is exerted through the production of cytokines, which target on the hepatocytes and immune cells by stimulating multiple signaling cascades (Oo and Adams, 2010). Th cells can be subdivided into Th1, Th2, regulatory T cells (Treg) and Th17 subsets by their unique transcription factors and characteristic secretion of cytokines (Table 1) (Kidd, 2003; Steinman, 2007). Thus, it is pivotal to understand the critical immune cells and cytokines that mediate DILI.

IL-17 and Th17 cells influence various liver injuries, such as DILI (Kobayashi et al., 2009), non-alcoholic fatty liver disease (Tang et al., 2011) and hepatocellular carcinoma (Kuang et al., 2010). It is well known that IL-17 plays important roles in activating chemotaxis and granulopoiesis through the stimulation of endothelial and epithelial cells to secrete macrophage inflammatory protein-2, granulocyte-colony stimulating factor and keratinocyte cytokine (Kolls and Linden, 2004). In addition, IL-17 acts mainly as a pro-inflammatory mediator activating and recruiting neutrophils into the liver (Ouyang et al., 2008). Activated neutrophils in the liver...
have been reported to release protease that can directly cause liver injury and to act as effector cells through cytotoxicity leading to hepatocyte necrosis (Jaeschke et al., 1999).

Our previous investigations have reported that TP can break the hepatic Th17/Treg equilibrium in TP-induced liver injury and Th17/Treg ratios positively correlated with serum levels of ALT and AST (Wang et al., 2014). Moreover, the hepatic frequencies of Th17 cells, the hepatic expression of IL-17 as well as the hepatic IL-17 level increased considerably, which was an interesting phenomenon to be further investigated. Clarifying the mechanisms of TP-induced liver injury helps attenuation of toxicity and provides theoretical guidance for its structural transformation. Based on these considerations, we investigated involvement of IL-17-mediated immune response in TP-induced liver injury in C57BL/6 mice following exposure to TP (600 μg/kg). Firstly, the hepatic expressions of transcription factors specific for Th1, Th2, Treg and Th17 cells were analyzed after TP administration to investigate the involvement of Th cells in the TP-induced liver injury. Secondly, neutralization and administration of recombinant IL-17 were performed, and the plasma IL-17 levels were measured to investigate the IL-17 involvement. The results indicated the liver injury induced by TP was partly mediated by activation of Th17 cells and secretion of IL-17, which has not been reported before and may represent a novel pathogenesis of TP-induced liver injury. In addition, neutralization of IL-17 suppressed hepatotoxicity whereas exogenous administration of recombinant IL-17 exacerbated hepatotoxicity suggesting that effective regulating strategy can be developed to control the progression of TP-induced liver injury.

2. Materials and methods

2.1. Chemicals

TP (purity, >98%) was a gift from the Dermatological Disease Research Institute of the Chinese Academy of Medical Sciences (Nanjing, China). Human/mouse myeloperoxidase (MPO) antibody, monoclonal anti-mouse IL-17A antibody (monoclonal rat IgG2a Clone#50104), rat IgG2a isotype and recombinant mouse IL-17A were from R&D Systems (Abingdon, UK).

2.2. Animals groups and TP administrations

Female C57BL/6 mice, 6–8 weeks of age, were purchased from the Vital River Experimental Animal Technology, Co., Ltd. (Beijing, China). All of the mice were housed under pathogen-free conditions and were provided with mouse chow and water ad libitum. The animals were raised at a controlled temperature (22 ± 2 °C) and photoperiod (12 h of light and 12 h of dark). The animals were acclimated to the laboratory for 1 week before the experiments. The animal experiments were conducted in compliance with standard ethical guidelines and with the approval of the faculty ethical committee. TP was reconstituted in propylene glycol and stored at −20 °C. TP was freshly diluted to the appropriate concentration with 0.2% carboxymethyl cellulose solution before use in the experiments. Female C57BL/6 mice were dosed by oral gavage with TP at a dose of 600 μg/kg per mouse, and the mice were sacrificed 6 h, 12 h, 24 h and 48 h after receiving the dose. The degree of liver injury was assessed by hematoxylin–eosin (H&E) staining, and the plasma aspartate aminotransferase (AST), alanine aminotransferase (ALT) was analyzed using an automatic clinical analyzer (7080, HITACHI Ltd., Tokyo, Japan). The neutrophil infiltration was assessed by immunostaining for MPO.

2.3. Isolation of mononuclear cell and intracellular cytokine and transcription factor labeling

Mononuclear cells were isolated and labeled as previously described (Guebre-Xabier et al., 2000). For intracellular cytokine labeling, mononuclear cells were incubated with phorbol 1, 2-myristate 1, 3-acetate (PMA, 50 ng/mL; Sigma-Aldrich, St Louis, MO, USA), ionomycin (500 ng/mL; Sigma–Aldrich) and BFA (1 mg/mL; Sigma–Aldrich). Next, the cells were labeled with anti-mouse CD4 antibodies (Becton Dickinson, San Diego, CA, USA) before permeabilization with Cytoperm/Cytofix (Becton Dickinson) according to the manufacturer’s instructions. After permeabilization, the cells were incubated with labeled antibodies which were specific to mouse IL-17A (Becton Dickinson). Next, the cells were centrifuged, and the pellets were washed to remove unbound antibodies. After surface and intracellular labeling, mononuclear cells were evaluated by flow cytometry (Calibrate; Becton Dickinson, Palo Alto, CA, USA) and the data were analyzed using CellQuest software (Becton Dickinson).

2.4. RNA extraction and real-time PCR

RNA was isolated from the liver sections with TRIzol reagent (Invitrogen Life Technologies, Carlsbad, CA, USA). cDNA synthesis was performed using the High-Capacity cDNA Reverse Transcription kit (Applied Biosystems, Foster city, CA, USA) according to the manufacturer’s instructions. Real-time PCR was performed in a 20 μL that contained 10 μL of 1 × SoFast EvaGreen Supermix (Bio-Rad, Hercules, CA, USA), 2 μL of cDNA, 6 μL of RNase/DNase-free water and 500 nM of each primer. The thermal cycle conditions included holds for 30 s at 95 °C, followed by 40 cycles of 5 s at 95 °C and 10 s at 60 °C. A melting curve analysis was performed for each reaction with a 65–95 °C ramp. The threshold cycle at which the fluorescent signal reached an arbitrarily set threshold near the middle of the log-linear phase of the amplification for each reaction was calculated, and the relative quantity of mRNA was determined. The mRNA levels were normalized against the mRNA levels of the housekeeping gene, GAPDH. The primer sequences for real-time PCR are shown in Table 2.

2.5. Administration of anti-mouse IL-17 antibody or recombinant mouse IL-17

Mice were administered anti-mouse IL-17A antibody by intraperitoneal injection (100 μg antibody in 0.5 mL of sterile PBS) at 9 h after TP administration. Rat IgG2a was intraperitoneally administered (100 μg of rat IgG2a in 0.5 mL of sterile PBS) as a control. Recombinant mouse IL-17A was administered by intraperitoneal injection (1 μg of recombinant IL-17A in 0.2 mL of sterile PBS containing 0.5% BSA) immediately after TP administration.

2.6. Detection of plasma IL-17 level by enzyme linked immunosorbent assay (ELISA)

The plasma IL-17 level was measured using enzyme linked immunosorbent assay (ELISA) (eBioscience, San Diego, CA, USA), according to the manufacturers’ protocol. All of the assays were performed in triplicate.

2.7. Statistical analysis

The data were expressed as the mean ± standard deviation (SD). The groups were evaluated using student’s t-test between two groups and a one-way analysis of variance (ANOVA) and Dunnett’s t-test among groups that is more than two. P < 0.05 was considered to be statistically significant.

3. Results

3.1. Time-dependent liver injury induced by TP in mice

The time-dependent hepatotoxicity of TP was investigated at a dose of 600 μg/kg (Fig. 1A and B). Plasma ALT and AST showed tendencies to increase at 6 and 12 h, peaked at 24 h after TP challenge,
and then decreased after 24 h. H&E staining demonstrated hepatocyte ballooning, focal necrosis and inflammatory cell infiltration in the mouse livers at 24 h after TP administration (Fig. 1C). The neutrophil infiltration was assessed by immune-staining for MPO. In the anti-MPO antibody staining, infiltration of neutrophil into the hepatocytes was observed at 24 h after the TP administration (Fig. 1D).

3.2. Time-dependent changes of transcriptional factors and IL-17 gene expression, plasma IL-17 levels and frequencies of Th17 cells in TP-administered mice

The hepatic expressions of transcriptional factors for each Th cells were measured by real-time RT-PCR to investigate the involvement of Th cells in the TP-induced liver injury (Fig. 2). The hepatic expressions of T-bet and GATA-3 in TP-administered mice were not changed compared with the control mice (Fig. 2A and B). The hepatic mRNA expression level of FoxP3 in TP-administered mice decreased significantly at 12 h and 24 h compared with that of control mice (Fig. 2C) whereas the hepatic expression of RORγt in TP-administered mice increased significantly at 12 h and 24 h compared with that of control mice (Fig. 2D). STAT3 is also required for the development of Th17 cells, whose hepatic mRNA expression level also significantly increased at 12 h and 24 h after TP administration (Fig. 3A). IL-17 produced by Th17 cells plays an important role in neutrophils infiltration and activation. Therefore, we measured hepatic and plasma IL-17 levels. The mRNA level of IL-17 in TP-treated livers gradually increased and peaked at 24 h after TP challenge (Fig. 3B). Importantly, the concentration of IL-17 in the plasma was much higher at 24 h than that in control livers (Fig. 3C). Hepatic Th17 cells were measured by flow cytometry. Mice treated with TP had significantly higher frequencies of hepatic CD4⁺Th17 than the control group at 24 h (Fig. 3D and E). Moreover, the above changes induced by TP were attenuated after 24 h.

3.3. TP alters the expression of cytokines and chemokines in a time-dependent manner

IL-17 has been reported to induce chemotaxis and granulopoiesis through the stimulation of endothelial and epithelial cells to

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Primers for real-time RT-PCR.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gene</td>
<td>Forward primer</td>
</tr>
<tr>
<td>GAPDH</td>
<td>5'-CTTGGCCATTGAGAGGGCTT-3'</td>
</tr>
<tr>
<td>T-bet</td>
<td>5'-TTTGTGGATGTTGTCCTTTG-3'</td>
</tr>
<tr>
<td>GATA-3</td>
<td>5'-GTCCTCAGTCTTCACCTCCC-3'</td>
</tr>
<tr>
<td>FoxP3</td>
<td>5'-CACCCAGGAGAAGAAGCAG-3'</td>
</tr>
<tr>
<td>RORγt</td>
<td>5'-TCCATATTGACTTTTCCACCT-3'</td>
</tr>
<tr>
<td>IL-17(A)</td>
<td>5'-TCACGGTCCTAAACACACTG-3'</td>
</tr>
<tr>
<td>MIP-2</td>
<td>5'-CCACAAACACAGGCTCAGG-3'</td>
</tr>
<tr>
<td>TNFa</td>
<td>5'-TCTACTGAACTTCGGGGTAT-3'</td>
</tr>
<tr>
<td>CXCL-1</td>
<td>5'-ACCAAAACAGACATCAGAC-3'</td>
</tr>
<tr>
<td>IL-1β</td>
<td>5'-TAAAGAACGCGGCAACCCAC-3'</td>
</tr>
<tr>
<td>Eotaxin-1</td>
<td>5'-CCACCTCTCCGTGCTTTATCA-3'</td>
</tr>
<tr>
<td>FasL</td>
<td>5'-CAGGTTCCTTGCTTGCATTT-3'</td>
</tr>
</tbody>
</table>

FoxP3, Forkhead box P3; RORγt, retinoid orphan nuclear receptor γt; STAT3, Signal Transducers and Activators of Transcription 3; IL-17(A), interleukin-17(A); MIP-2, macrophage inflammatory protein-2; TNFa, tumor necrosis factor α; CXCL-1, C–X–C chemokine ligand-1.
produce cytokines and chemokines (Kolls and Linden, 2004). Previous studies confirmed that the expression levels of mRNA and protein were similar in chemokines and ILs (Kobayashi et al., 2009). The hepatic MIP-2, CXCL-1 and Eotaxin-1 mRNA expression levels considerably increased at 24 h after TP administration compared with the control mice. The hepatic TNFα, IL-1β and FasL mRNA expression levels gradually increased at 12 h or 6 h and peaked at 24 h after TP administration (Fig. 4). The enhancement of expressions of cytokines and chemokines decreased beyond 24 h into the recovery phase.
3.4. Anti-IL-17 antibody administration alleviates TP-induced liver injury

The IL-17 neutralization study was conducted to inhibit the function of IL-17. The anti-mouse IL-17 antibody was intraperitoneally administered at 9 h after TP administration resulted in significant lower plasma AST and ALT levels at 24 h after TP administration (Fig. 5A and B). Less hepatocyte damage was observed in the histopathological examination and the number of MPO positive cells decreased in the IL-17 neutralization group (Fig. 5C and D). Furthermore, the administration of rat IgG2a did not display these effects. The percentage of Th17 cells in the murine liver reduced to almost baseline levels (Fig. 6A and B) in anti-IL-17-treated mice. The plasma IL-17 level in anti-IL-17 antibody treated mice significantly decreased compared with the mice administered TP alone (Fig. 6C). Accordingly, the hepatic mRNA expressions of MIP-2, 

![Graphs showing mRNA expression levels of various cytokines and chemokines over time](image)

Fig. 4. Effects of TP administration on hepatic mRNA expression of pro-inflammatory cytokines and chemokines in a time-dependent manner. The mice were sacrificed at 6, 12, 18, 24, and 48 h after the oral administration of TP (600 μg/kg) or equal volumes of carboxymethylcellulose as a control. The relative expression levels of macrophage inflammatory protein-2 (MIP-2, A), tumor necrosis factor α (TNFα, B), C-X-C motif ligand 1 (CXCL-1, C), Interleukin-1β (IL-1β, D), Eotaxin-1 (E) and FasL (F) mRNA were measured using real-time RT-PCR and normalized to GAPDH mRNA. The data are shown as the mean ± SD of 6 mice. *P < 0.05, **P < 0.01, ***P < 0.005.

![Graphs showing effects of anti-mouse IL-17 antibody on plasma AST, ALT and histopathological changes](image)

Fig. 5. Effect of anti-mouse IL-17 antibody on plasma AST, ALT and histopathological changes in the liver in TP-administered mice. Mice were administered by intraperitoneal injection of monoclonal anti-mouse IL-17 antibody (100 μg/mouse) or rat IgG2a (100 μg/mouse) at 9 h after the TP administration. Plasma AST and ALT was collected and analyzed at 24 h after TP administration (A and B). Liver specimens were excised and fixed in 4% paraformaldehyde to generate tissue sections stained with H & E (C) or immune-stained with anti-MPO antibody (D). Arrows indicated MPO-positive cells. The data are shown as the mean ± SD of 6 mice. *P < 0.05, **P < 0.01, ***P < 0.005.
IL-1β and FasL mRNA significantly decreased compared with mice administered TP alone (Fig. 7). Therefore, our data demonstrated that IL-17 was required during TP-induced liver injury, which revealed an interesting and novel phenomenon.

3.5. Recombinant IL-17 aggravates the hepatotoxicity of TP

The recombinant IL-17 study was performed to further confirm IL-17 involvement in the TP-induced hepatotoxicity. AST and ALT levels had no obvious changes in mice administered recombinant IL-17 alone (data not shown). The administration of recombinant IL-17 immediately after the TP administration induced considerable increases of the plasma AST and ALT levels at 24 h after TP administration (Fig. 8A and B). The histopathological changes indicated focal necrosis with inflammatory cell infiltration in hepatocytes, which were more severe in the recombinant IL-17 group compared with mice administered TP alone. Moreover, MPO-positive cells infiltrated heavily into the liver in the recombinant IL-17 and TP-treated mice (Fig. 8D). The percentage of Th17 cells in the murine liver considerably increased (Fig. 9A and B) in recombinant IL-17 and TP-administered mice. In the recombinant IL-17 group, the plasma IL-17 level significantly increased at 24 h after the TP administration (Fig. 9C).
administration compared with mice administered TP alone (Fig. 9C). The hepatic mRNA expressions of MIP-2, CXCL-1, IL-1β, Eotaxin-1 and FasL in recombinant IL-17 and TP-administered mice significantly increased compared with TP administration alone (Fig. 10). It can be concluded that recombinant IL-17 aggravated the hepatotoxicity of TP.

4. Discussion

The Chinese herbal medicine TWHF and TP have been widely used for the treatment of rheumatoid arthritis and leukemia (Liu et al., 2005; Zhang et al., 2010). However, severe hepatotoxicity limited their clinical applications (Wang et al., 2013; Zhang et al., 2012). In the present study, the hepatotoxicity of TP was evaluated after oral administration of the drug to female 6–8 week age C57BL/6 mice. Previous researches have showed that oral gavage of 400 μg/kg of TP induced liver injury in rats (Fu et al.). Acute toxicity study demonstrated that the LD₅₀ value of single p.o. administration of TP was 1280 μg/kg in C57BL/6 mice (Wang et al., 2008). Based on these considerations, we have chosed the dosage of 600 μg/kg of TP in the present study, reproducibly caused TP-induced acute liver injury in mice. A number of changes in serum biochemistry and histopathological examinations demonstrated that oral administration of 600 μg/kg of TP to C57BL/6 mice induced acute hepatotoxicity at 24 h (Fig. 1). The increase of ALT and AST were not significant at 6 h and 12 h after TP administration.

Our previous researches have investigated Th17/Treg imbalance in TP-induced liver injury, which was associated with the enhancement of the expansion of Th17 cells and suppression of the production of Tregs, especially the significant increase of IL-17.
secreted by Th17 cells (Wang et al., 2014). A key player in hepatic immune regulation that has recently discovered is the lymphocyte subpopulation known as Th17 cells which mainly produce IL-17 (Ye et al., 2011). IL-17 and Th17 cells are actively engaged in various kinds of liver disorders (Hammerich et al., 2011). It has been reported that IL-17 from Th17 cells regulates hepatic neutrophil recruitment and stimulates the activation of a variety of pro-inflammatory cytokines and chemokines (Zhu and Paul, 2008). To test the involvement of the IL-17 and Th17 cells in TP-induced liver injury, we investigated the expression of hepatic ROR-γt, STAT3, IL-17 as well as plasma IL-17 level and hepatic frequencies of Th17 cells (Fig. 2 and 3). At 24 h after the administration of TP the expression of hepatic ROR-γt, STAT3, IL-17 as well as the plasma IL-17, AST and ALT levels, hepatic frequencies of Th17 cells significantly increased. ROR-γt is the master regulator of Th17 cells (Steinman, 2007). STAT3 is an important determinant for the differentiation of Th17 cells and regulates transcription of inflammatory genes (Harris et al., 2007). In addition, neutralization of IL-17 tended to inhibit the increase of the plasma AST and ALT levels and down-regulated plasma IL-17 level and hepatic frequencies of Th17 cells significantly (Fig. 5 and 6). The administration of recombinant IL-17 caused a remarkable increase of the plasma AST, ALT, IL-17 levels and hepatic frequencies of Th17 cells resulting in exacerbation of the hepatotoxic effect. From these lines of evidence, IL-17-mediated factors were involved in TP-induced hepatotoxicity, which was a novel and interesting phenomenon (Figs. 8 and 9).

The immunostaining for MPO reflected neutrophil infiltration. A number of MPO positive cells had infiltrated in C57BL/6 mice liver at 24 h after the TP administration in the immune-histochemical analysis, suggesting that neutrophil infiltration occurred in the TP-administered C57BL/6 mice livers (Fig. 1D). IL-17 can induce a variety of immune responses, especially neutrophil activation and recruitment (Zhu and Paul, 2008). Neutrophils have been noted to induce liver injury including ischemia–reperfusion injury (Jaeschke et al., 1990) and acetaminophen–induced liver injury (Liu et al., 2006). It is well known that within the liver, activated neutrophils kill through intracellular oxidant stress (Jaeschke et al., 1990) and acetaminophen–induced liver injury (Jaeschke et al., 1990). Our previous researches have shown that enhanced oxidant stress and hepatocyte apoptosis are involved in the mechanisms of TP-induced liver injury (Fu et al., 2011; Yao et al., 2008). Interestingly, increased oxidant stress and apoptotic hepatocytes can also up-regulate the transcription of the pro-inflammatory cytokines (Agostini et al., 2004), which may formulate a positive-feedback loop during TP-induced liver injury.

IL-17 has been well known to stimulate the production of cytokines and chemokines (Zhu and Paul, 2008). Meanwhile, cytokine and chemokine in liver tissue could provide suitable environment for the generation and recruitment of Th17 cells, which may also formulate a positive-feedback loop during TP-induced liver injury. Cytokines and chemokines such as MIP-2, TNFα, CXCL-1, IL-1β, Eotaxin-1 and FasL significantly increased at 24 h in the TP-administered mice (Fig. 4). CXCL-1 and MIP-2 are potent chemokines for neutrophils extravasation and migration (Biedermann et al., 2000), which has been reported to be involved in liver injury (Uchida et al., 2009, 2010). Significantly lower MIP-2, IL-1β and FasL mRNA levels were noted in the mice that received anti-IL-17 antibody demonstrating that the increase of MIP-2, IL-1β and FasL expression in TP-administered C57BL/6 mice was mediated by IL-17. IL-1β acts mainly as a pro-inflammatory mediator activating and recruiting leukocytes into the liver (Bajt et al., 2001). TNFα is a pleiotropic pro-inflammatory cytokines produced by T cells and other cells (Tracey and Cerami, 1994). The two death receptor ligands, TNFα and FasL, bind to their receptors and induce apoptosis, suggesting that these mediators may be the cause of hepatocellular apoptosis in TP-induced liver injury. Eotaxin-1 is known to stimulate the migration of eosinophils via other chemokine
receptors (Rankin et al., 2000). Taking these lines of data into consideration, we can conclude that TP-induced liver injury could involve these immune mediators, which was elucidated for the first time.

5. Conclusion

In conclusion, IL-17 and IL-17-related immunological factors may play an important role in TP-induced liver injury in C57BL/6 mice. In addition, IL-17 might be involved in the pathogenesis of neutrophil infiltration and liver damage. This study provides new insights into the role of IL-17 during inflammation and sheds light on effective strategy that can be developed to control the progression of TP-induced liver injury.

Conflict of Interest

The authors declare that there are no conflicts of interest.

Transparency Document

The Transparency document associated with this article can be found in the online version.

Acknowledgments

We thank Professor Chen Yun for providing triptolide. The present study was supported by grants from the Fundamental Research Funds for the Central Universities (Z2014YW0032) and the National Natural Science Foundation of China (Nos. 81173651, 81001564, 81102887 and 81320108029). The study was partially supported by the 111 Project (No. 111-2-07) and the 2011 Program for Excellent Scientific and Technological Innovation Team of Jiangsu Higher Education.

References