GASTROINTESTINAL, HEPATOBILIARY, AND PANCREATIC PATHOLOGY

Intestinal Epithelial Chemokine (C-C Motif) Ligand 7 Overexpression Enhances Acetaminophen-Induced Hepatotoxicity in Mice

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Acetaminophen (APAP) overdose—induced hepatotoxicity is the leading cause of drug-induced liver injury worldwide. The related injury pathogenesis is mainly focused on the liver. Here, the authors report that gut barrier disruption may also be involved in APAP hepatotoxicity. APAP administration led to gut leakiness and colonic epithelial chemokine (C-C motif) ligand 7 (CCL7) up-regulation. Intestinal epithelial cell (IEC)-specific CCL7 transgenic mice (CCL7^{tgIEC} mice) showed markedly increased myosin light chain kinase phosphorylation, and elevated gut permeability and bacterial translocation into the liver compared to wild-type mice. Global transcriptome analysis revealed that the expression of hepatic proinflammatory genes was enhanced in CCL7^{tgIEC} mice compared with wild-type animals. Moreover, CCL7 overexpression in intestinal epithelial cells significantly augmented APAP-induced acute liver injury. These data provide new evidence that dysfunction of CCL7-mediated gut barrier integrity may be an important contributor to APAP-induced hepatotoxicity. (*Am J Pathol* 2020, 190: 57–67; <https://doi.org/10.1016/j.ajpath.2019.09.009>)

Acetaminophen (APAP) is a common over-the-counter drug and is generally safe at recommended therapeutic doses. However, APAP overdose is the predominant cause of acute liver failure in the Western world.^{1,2} The pathogenesis of APAP hepatotoxicity is complex. Briefly, APAP is metabolized into *N*-acetyl-*p*-benzoquinone-imine (NAPQI) by CYP2E1 in the liver and is then conjugated and detoxified with glutathione (GSH). Upon exhausting hepatic GSH, excess NAPQI ultimately leads to mitochondrial damage and hepatocyte death.^{3,4} It is believed that APAP mainly targets the liver and has low side effects on the intestine.⁵ However, recent reports have shown that APAP was able to change the Caco-2 cell membrane properties and influence intestinal functional gene expression *in vitro*,^{6,7} thus indicating that gut barrier function may be involved in APAP toxicity. However, the details of the functional link between APAP and intestinal barrier integrity are poorly understood.

The gut barrier is predominately formed by intestinal epithelial cell (IEC) connections, and it effectively provides a

biochemical and physical barrier that limits bacterial toxins and antigens from passing through the mucosa.^{8,9} The gut barrier integrity is mainly regulated by the intestinal inflammatory microenvironment.¹⁰ Specifically, inflammatory factors released either from immune cells or epithelial cells trigger key intracellular kinase activation, such as myosin light chain kinase (MLCK); in turn, MLCK disrupts tight junction expression and finally causes elevated gut

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permeability.¹¹ Bacteria or their products could then penetrate into the circulatory system and access remote organs through the leaky gut and then activate immune cells to induce low-grade inflammation that sensitizes organs to toxin-induced damage, which mainly influences the liver.¹² In the present study, the authors explored the detailed association between APAP toxicity and gut leakiness in an animal model, and determined the key intestinal molecule that mediates APAP hepatotoxicity.

Materials and Methods

Animal Model

Six- to 8-week-old male specific-pathogen-free C57BL/6J mice were used. Intestinal epithelial CCL7-specific transgenic mice (carrying the human CCL7 gene, CCL7^{tgIEC} mice, C57BL/6J background) and their littermates used in this study were generated and purchased from GemPharmatech Co. (Nanjing, China). The mice (nonfasted) were injected intraperitoneally with 300 mg/kg APAP dissolved in phosphate-buffered saline (PBS) and were sacrificed 3 or 24 hours after APAP treatment. Mice were fed *ad libitum* and were housed in a temperature-controlled room on a 12 hour/12 hour light/dark cycle. All animal experimental protocols were in agreement with the NIH guidelines and were approved by the Animal Care and Use Committee of the Southern Medical University.

Histologic Analysis

Ten percent buffered formalin was used to fix the harvested tissues. The sample was embedded in paraffin and sliced, and then hematoxylin and eosin staining was performed. Immunofluorescence and immunohistochemistry were performed using the primary antibodies for occludin (Invitrogen, Carlsbad, CA), claudin-4 (Invitrogen), and CD11b (Abcam, Cambridge, UK). For necrosis area quantification, 6 to 8 random fields per slide were examined with microscopy and analyzed by ImageJ software version 1.8.0 (NIH, Bethesda, MD; <https://imagej.nih.gov/ij>).

Western Blot Analysis

Total protein was isolated with lysis buffer (Thermo Fisher Scientific, Waltham, MA). Protein levels were analyzed by Western blot through primary antibodies targeting CYP2E1 (Proteintech, Wuhan, China), p-MLCK (Thermo Fisher Scientific), occludin (Invitrogen), claudin-4 (Invitrogen), Actin (Cell Signaling Technology, Danvers, MA), and GAPDH (Cell Signaling Technology).

Determination of APAP Adducts

APAP-protein adducts (APAP-AD) were determined as previously described.¹³ Liver samples were collected 2 hours

after APAP treatment and homogenized in radioimmunoprecipitation assay buffer with protease and phosphatase inhibitor cocktails. An equal protein amount was used for SDS-PAGE electrophoresis and transferred to nitrocellulose membrane. Membrane was incubated on a shaker with 5% milk in tris-buffered saline (TBS) with 0.05% Tween-20 (TBS-T) for 1 hour at room temperature. The blocking buffer was discarded, and the membrane was incubated on a shaker with polyclonal anti-serum for APAP-AD (1:2000 diluted in 5% bovine serum albumin-TBS) overnight at 8°C. Next, the membrane was transferred to a washing buffer (TBS-T) and kept on a shaker for 10 minutes at room temperature, and the washing step was repeated three times. The washing buffer was discarded, and the membrane was incubated on a shaker with anti-rabbit IgG-horseradish peroxidase (1:2000 diluted in 5% milk TBS-T) for 1 hour at room temperature. The

Table 1 PCR Primers Used in This Study

Gene	Primer pair
18s	Left 5'-CGATCCGAGGGCCTCACTA-3'
	Right 5'-AGTCCCTGCCCTTTGTACACA-3'
16s	Left 5'-GTGSTGCAYGGYTGTCGTCA-3'
	Right 5'-ACGTCRTCCMACCTTCCTC-3'
TNFA	Left 5'-CCACCAGCTCTTCTGTCTAC-3'
	Right 5'-AGGGTCTGGGCCATAGAACT-3'
IL6	Left 5'-TGATGCAC'TTGCAGAAAACA-3'
	Right 5'-ACCAGAGGAAATTTTCAATAGGC-3'
IL1B	Left 5'-TGTGAAATGCCACCTTTTGA-3'
	Right 5'-GGTCAAAGGTTTGAAGCAG-3'
CCL2	Left 5'-CCTGCTGTTTACAGTTGCC-3'
	Right 5'-ATTGGGATCATCTTGCTGGT-3'
CCL3	Left 5'-ACCATGACACTCTGCAACCA-3'
	Right 5'-GTGGAATCTCCGGCTGTAG-3'
CCL4	Left 5'-CATGAAGCTCTGCGTGTCTG-3'
	Right 5'-GAAACAGCAGGAAGTGGGAG-3'
CCL5	Left 5'-GTGCCCACGTCAGAGAGTAT-3'
	Right 5'-CCACTCTTCTCTGGGTGG-3'
CCL7(Mus)	Left 5'-CTGCTTTTACAGCATCCAAGTG-3'
	Right 5'-TTCTCTTGGGGATCTTTTG-3'
CCL7(Homo)	Left 5'-GAAGGACCACCAGTAGCCACTGTC-3'
	Right 5'-AGTCCTGGACCACCTCTGTGTG-3'
CCL8	Left 5'-TCTTTGCCCTGCTGCTCATAG-3'
	Right 5'-GAAGGGGATCTTCAGCTTT-3'
CXCL1	Left 5'-CCACACTCAAGATGGTCGC-3'
	Right 5'-TCTCCGTTACTTGGGGACAC-3'
CXCL2	Left 5'-CGGTCAAAAAGTTTGCCTTG-3'
	Right 5'-TCCAGGTCAAGTTAGCCTTGC-3'
CXCL10	Left 5'-CTCATCTGCTGGGTCTGAG-3'
	Right 5'-CCTATGGCCCTCATTCTCAC-3'
TLR2	Left 5'-AGGGGCTTCACTTCTCTGCT-3'
	Right 5'-CCAAAGAGCTCGTAGCATCC-3'
TLR4	Left 5'-GGACTCTGATCATGGCACTG-3'
	Right 5'-TGTCATCAGGGACTTTGCTG-3'
TLR9	Left 5'-GAATCCTCCATCTCCCAACA-3'
	Right 5'-CAGCTCACAGGGTAGGAAGG-3'
CYP2E1	Left 5'-GGCTGTCAAGAGGTGCTAC-3'
	Right 5'-CCTTCCATGTGGGTCCATTA-3'

Figure 2 Chemokine (C-C motif) ligand (CCL7) overexpressed in intestinal epitheliums enhances acetaminophen (APAP)-induced hepatotoxicity. **A** and **B**: Vector construction for CCL7^{tgIEC} mice generation. **C**: CCL7 mRNA levels in isolated intestinal epithelium cells. **D**: CCL7 protein levels in isolated intestinal epithelium cells. **E**: Plasma alanine aminotransferase (ALT) levels after 24 hours APAP treatment. **F**: Histopathological examination of hepatic hematoxylin and eosin staining (left panel, green dashed lines indicate necrotic areas) and quantification of the necrotic area after 24 hours of APAP treatment (right panel). Data are expressed as means $\pm$ SEM. $n = 8$ to 10. $*P < 0.05$. Original magnification, $\times 200$. DSI, distal small intestine; PSI, proximal small intestine; WT, wild-type.

Figure 3 Hepatic acetaminophen (APAP) metabolism is not affected in CCL7^{tgIEC} mice. **A:** Glutathione (GSH) levels in the liver homogenates after APAP treatment for 2 hours. **B:** APAP adducts levels in the livers after APAP treatment for 2 hours (the numbers inside the **bottom box** are the relative density of APAP adducts to GAPDH). **C:** CYP2E1 mRNA and protein levels in the liver. Data are expressed as means $\pm$ SEM. $n = 4$ to 6. * $P < 0.05$. WT, wild-type.

washing step was repeated before developing the signal on film with enhanced chemiluminescence reagents. For a loading control, the membrane used to determine APAP-AD was stripped with stripping buffer (BioLand Scientific, Paramount, CA), blocked with 5% milk-TBS-T, and incubated with anti-GAPDH-horseradish peroxidase (Sigma Aldrich, St. Louis, MO). Multiple difference exposure confirmed the linear range of signal.

Immunoblots were scanned and signal intensity was semiquantitated using ImageJ software. The full length of each lane in a single blot was selected to measure densitometry quantitation of APAP-AD. A single specific band of GAPDH was selected to measure densitometry. The densitometry ratio was calculated as APAP-AD/GAPDH.

Biochemical Analysis

The serum levels of alanine aminotransferase (ALT) activity were measured with commercial kits (Jiancheng Bioengineering, Nanjing, China) according to the manufacturer's instructions. Human and mouse CCL7 protein levels were detected by an enzyme-linked immunosorbent assay kit (CUSABIO, Wuhan, China).

Gene Expression Analysis

TRIzol reagent was used to isolate total RNA, and a reverse transcription enzyme (TOYOBO, Shanghai, China) was employed for reverse transcription. The real-time quantitative PCR primers are shown in Table 1. Transcriptome analysis has

been described previously.¹⁴ Briefly, 3 μg of total RNA of each sample was used to construct the Illumina sequencing libraries according to the manufacturer's instructions (Illumina, San Diego, CA). The libraries were sequenced using the Illumina NovaSeq 6000 platform to generate high-quality paired-end reads. The function and pathway enrichment for differentially expressed coding genes were referred by Gene Ontology (GO; <http://geneontology.org>) functional annotations and KEGG database (<https://www.genome.jp/kegg/pathway.html>). Raw sequencing data have been uploaded to the BioProject database (<https://www.ncbi.nlm.nih.gov/bioproject>; accession number PRJNA555333).

FD-4 Permeability Experiment

In vivo gut leakiness was assessed by fluorescein isothiocyanate-dextran 4 kDa (FD-4) (Sigma, Shanghai, China) penetration. Mice were intragastrically administered with FD-4 (1 mg/kg) and 3 hours later, plasma FD-4 level was determined spectrofluorometrically with a 485-nm excitation wavelength and 530-nm emission wavelength in a microplate fluorescence reader.

Fecal Albumin Measurement

Fecal samples collected before sacrifice were immediately frozen and stored at -80°C until analysis. Fecal albumin concentration was determined by enzyme-linked immunosorbent assay (Bethyl Laboratories, Montgomery, AL) according to the manufacturer's instructions.

Intestinal Epithelial Cell Isolation

The mouse intestines were rinsed in ice-cold PBS, opened longitudinally, and cut into 1-cm-long pieces. These pieces were shaken in ice-cold RPMI1640 medium (Gibco; Thermo Fisher Scientific) with 5% fetal calf serum and dithiothreitol (1 mmol/L). The supernatant was discarded, and the tissue was incubated in RPMI1640 with 5% fetal calf serum and 1 mmol/L EDTA at 37°C with shaking for 20 minutes. To completely isolate the epithelial cells, this step was repeated.

Glutathione Assay

Glutathione (reduced) was measured in liver homogenate using reduced GSH assay kit (Jiancheng Bioengineering) according to the manufacturer's instructions. Briefly, GSH in the samples reacted with DNTB [5,5'-dithiobis(2-nitrobenzoic acid)] and generated a yellow compound. After the chromogenic reaction, the optical density at 405 nm was recorded, and the concentration was calculated.

Statistical Analysis

The results are expressed as means $\pm$ SEM. A two-tailed *t*-test was used for statistical analysis. Statistical differences between groups were analyzed; *P* < 0.05 was considered significant.

Results

APAP Induces Gut Permeability and Colonic Epithelial CCL7 Overexpression

The mouse APAP-induced liver injury model was first established, and the effects of APAP on the intestine were examined (Figure 1A). The gut permeability was evaluated by circulatory FD-4 and fecal albumin levels. The FD-4 concentration in the plasma of the APAP group was significantly higher than that in the PBS group at both 3 hours and 24 hours after APAP (Figure 1B). Furthermore, fecal albumin was prominently increased in the APAP group at both 3 hours and 24 hours after APAP (Figure 1C). Because gut barrier dysfunction is accompanied with bacterial translocation, the 16s/18s ratio was higher in liver of APAP-treated mice (Figure 1D). Additionally, APAP treatment markedly decreased tight junction occludin and claudin-4 protein levels (Figure 1E), and immunofluorescence also showed a significantly disrupted colonic epithelium barrier (Figure 1F).

Because a leaky gut is predominantly induced by intestinal inflammation, the main cytokines and chemokines produced throughout the entire colonic tissue and colonic enterocytes were detected. Although there were no obvious changes in these cytokines and chemokines throughout the colon, CCL7 was observed to exhibit the greatest elevation among all inflammatory factors in the colonic epithelial cells (Figure 1G). Furthermore, CCL7 expression in colonic lamina propria was comparable between the PBS and APAP groups (Supplemental Figure S1A). These data indicated that colonic epithelial cells, but not immune cells, may be the primary cells expressing CCL7 in APAP-overdosed mice. In addition, the CCL7 relative expression is higher in liver after APAP treatment (Supplemental Figure S1B), which resulted from the toxic and proinflammatory effects of APAP. These results revealed that APAP treatment caused development of mucosal damage, including an increase in intestinal permeability and increased expression of CCL7 in the colonic epithelium.

IEC Overexpression of CCL7 Exacerbates APAP Hepatotoxicity

To further define the role of CCL7 from enterocytes in mediating the susceptibility of APAP hepatotoxicity in mice, transgenic mice that express constitutively active CCL7 specifically within the intestinal epithelia were chosen (CCL7^{tgIEC} mice). Vector construction is shown in Figure 2, A and B. It was confirmed that CCL7 expression is much higher in CCL7^{tgIEC} mice than wild-type (WT) mice at both mRNA and protein levels in isolated intestinal epithelial cells (Figure 2, C and D). The CCL7 protein expression was not altered in the liver of CCL7^{tgIEC} mice (Supplemental Figure S2A). CCL7^{tgIEC} mice displayed more severe liver damage upon APAP treatment, as revealed by significantly increased plasma ALT (Figure 2E), markedly increased necrotic area compared with WT mice (Figure 2F), and more hepatic CD11b-positive cells after APAP in CCL7^{tgIEC} mice (Supplemental Figure S2B). Taken together, APAP-induced hepatotoxicity is more prominent in mice with CCL7 overexpressed in intestinal epithelium cells.

Hepatic APAP Metabolism Is Not Altered in CCL7^{tgIEC} Mice

GSH levels and APAP protein adduct (APAP-AD) formation are key modulators in the development of APAP hepatotoxicity.^{15,16} To preclude the possibility that CCL7 overexpressed on IECs increased APAP-induced hepatotoxicity as a

Figure 4 CCL7 overexpression in intestinal epithelial cells (IECs) leads to gut barrier dysfunction and promotes bacterial translocation into the liver. **A:** Fluorescein isothiocyanate–dextran 4 kDa (FD-4) level in the plasma. **B:** Fecal albumin (Alb) content. **C:** Occludin and claudin-4 protein levels in the colon. **D:** Representative immunofluorescence staining for occludin and claudin-4 in the colon. **E:** Electron microscopy of mice colon. **Arrows** indicate the connection between two epithelia cells. **F:** Western blotting for phosphorylated myosin light chain kinase (p-MLCK) in the colon. **G:** Plasma endotoxin level and relative 16s/18s ratio in the liver. **H:** mRNA levels of toll-like receptors (TLRs) in the liver. **I:** mRNA levels of key cytokines and chemokines in the liver. Data are expressed as means $\pm$ SEM. *n* = 4 to 6. **P* < 0.05. Original magnification: $\times 400$ (**D**); $\times 40,000$ (**E**). WT, wild-type.

Figure 5 Liver transcriptome analysis. **A:** Principal component analysis displaying the overall gene expression profiles across all samples between WT and CCL7^{tgIEC} mice. **B:** The heat map depicting the gene expression alteration of the differentially expressed genes. **C:** The top 20 enrichment GO pathways of differentially expressed genes. **D:** The key KEGG pathways with $P < 0.05$ related to inflammation activation. **E:** Up-regulated and down-regulated gene counts for the inflammation pathways in CCL7^{tgIEC} mice. $n = 5$. WT, wild-type.

result of altered GSH and CYP2E1 levels, the depletion of GSH and APAP-AD formation 2 hours after APAP administration was examined in the liver. No differences were observed between CCL7^{tgIEC} mice and WT mice (Figure 3, A and B). Additionally, the gene expression levels and protein levels of CYP2E1, the main metabolic enzyme of APAP, were measured. No changes were observed in the baseline characteristics (Figure 3C). These data indicated that APAP-induced augmented hepatotoxicity in CCL7^{tgIEC} mice was not dependent on the alteration of metabolism process.

CCL7 Overexpression in IECs Induces Gut Barrier Defects and Bacterial Translocation

The proinflammatory mediators during inflammatory response have been shown to increase gut permeability,^{17–19} and gut barrier dysfunction may sensitize liver to toxin-induced injury responses. To decipher the potential mechanism by which intestinal epithelial CCL7 promotes APAP-induced liver damage, the integrity of the intestinal mucosa was studied by measuring the plasma FD-4 and fecal albumin levels in CCL7^{tgIEC} mice. As expected, the penetration of FD-4 and the levels of fecal albumin were higher in mice with the selective activation of CCL7 on intestinal epithelial cells than in controls (Figure 4, A and B). The expression of the key tight-junction proteins occludin and claudin-4 was assessed in the colon. CCL7^{tgIEC} mice exhibited significantly decreased levels of these proteins compared with WT mice (Figure 4, C and D). Electron microscopy also confirmed the disrupted colonic tight junctions (Figure 4E). Phosphorylation of MLCK, a key regulator of tight junction expression and gut barrier integrity,²⁰ was markedly enhanced in the colon of CCL7^{tgIEC} mice compared with WT animals (Figure 4F). These data clearly demonstrate that MLCK activation maybe involved in the CCL7-mediated alteration of tight junctions and gut barrier dysfunction.

The onset of increased intestinal permeability could lead to bacterial product translocation into the systemic circulation and remote organs.^{21,22} Plasma endotoxin levels and 16s bacterial DNA were measured to recognize bacterial translocation. The plasma endotoxin levels and hepatic 16s/18s ratio were higher in CCL7^{tgIEC} mice than WT, which is consistent with the increased gut permeability observed in transgenic mice (Figure 4G). In addition, increased hepatic toll-like receptor mRNA levels, particularly *TLR2*, were detected in CCL7^{tgIEC} mice (Figure 4H), which may induce a low-grade inflammation. Consequently, intrahepatic cytokines such as tumor necrosis factor- α (TNF- α), IL-1 β , and IL-6 or other key chemokines were all increased in CCL7^{tgIEC} mice compared with WT mice (Figure 4I). These data indicate that IECs with CCL7 overexpression exhibited disruption of the integrity of the intestinal barrier, leading to bacterial translocation and the initiation of an inflammatory response in the liver.

Hepatic Transcriptome Analysis Confirms Inflammatory Signaling Activation in CCL7^{tgIEC} Mice

Because bacterial translocation promotes liver inflammation, the hepatic transcriptomes of CCL7^{tgIEC} mice and WT littermates were profiled. The expression profile of the two groups could clearly be separated by principal component analysis (Figure 5A). The heat map shows the up-regulated and down-regulated genes in CCL7^{tgIEC} mice (Figure 5B). These results indicate that CCL7 overexpression in IECs induces prominent transcriptome alterations. Further, Gene Ontology (GO) enrichment analysis was employed on differentially expressed genes. The top 20 enrichment map highlighted that IEC CCL7 overexpression up-regulated several inflammatory pathways such as T-cell aggregation and activation, response to cytokines, and regulation of the immune system process (Figure 5C). Similar to GO analysis, the KEGG pathways indicate that hepatic inflammatory pathways including the TNF signaling pathway, cytokine–cytokine receptor interaction, and toll-like receptor signaling pathway were all altered between the two strains (Figure 5D). Importantly, among the genes associated with inflammation pathway, the up-regulated genes were much more abundant than down-regulated genes in CCL7^{tgIEC} mice compared with controls (Figure 5E). These data clearly reveal that the immune system was activated in the liver of CCL7^{tgIEC} mice, which indicates that the livers of these mice had low-grade inflammation at baseline.

Discussion

The present study demonstrates that APAP overdose increases gut permeability and induces CCL7 expression in colonic epithelial cells, and therefore provides novel insights into the role of CCL7 in mediating gut barrier dysfunction and determining susceptibility to APAP-induced liver damage. *In vitro*, APAP changes the topography of Caco-2 cells; even subtoxic doses of APAP can induce gene transcription alteration in the intestinal epithelium.^{6,7} In this study, APAP overdose strongly increased the CCL7 mRNA level in colonocytes and remarkably increased gut permeability. Although there were no obvious differences in CCL7 protein levels in colon epithelium between the PBS and APAP groups (Supplemental Figure S1C), it cannot be excluded that CCL7 could be transiently secreted from colonic epithelial cells upon APAP overdose. Once the gut barrier is disrupted, bacteria translocation into liver occurs and finally induces inflammatory responses. Although the inflammation may contribute to hepatocyte necrosis, it is also possible that the factors released from permeabilized gut could directly target the hepatocytes and sensitize the early events in APAP-induced necrosis.

Intestinal homeostasis is maintained through the interplay of the intestinal mucosa, immune activation, and microbiota.²³ Variations in intestinal microbiota could influence

APAP hepatotoxicity.^{24,25} Within the gastrointestinal mucosa, chemokines, cytokines, and other small molecules play key roles in lymphocyte and leukocyte recirculation, and maintain the balance of mucosal immunity.²⁶ Chemokines can lead to increased immune cell infiltration into the lamina propria and to subsequent inflammation and tissue damage. Consistent with this, CD11b-positive immune cells were recruited into the colon lamina propria in CCL7^{tgIEC} during the early phase of APAP challenge (Supplemental Figure S2C). Cytokine-mediated barrier dysfunction contributes to the pathology of intestinal diseases, during which MLCK is activated and plays a key role in cytokine-induced barrier loss.²⁷ MLCK phosphorylation was associated with CCL7-induced tight junction dysregulation. However, it is unclear whether MLCK phosphorylation alone is sufficient to regulate CCL7-mediated tight junction permeability. A more detailed signaling pathway should be studied in the future.

Gut barrier dysfunction and bacterial translocation are common in liver diseases such as non-alcoholic fatty liver disease and cirrhosis.^{28,29} Gut-derived bacterial products or components such as microbe-associated molecular patterns induce a proinflammatory intrahepatic milieu.^{30,31} Blocking bacterially derived products such as lipopolysaccharide has been shown to ameliorate liver injury.³² Inflammatory mediators and toll-like receptors that were activated, particularly TLR2 in CCL7^{tgIEC} mice, were detected and showed a proinflammatory pattern of immune dysregulation associated with accelerated progression of APAP-induced liver injury (Figure 4). Thus, the disturbed intestinal barrier could be defined as inducing a proinflammatory response in the liver. The analysis of hepatic transcriptomes for CCL7^{tgIEC} mice and WT littermates at baseline suggests that the related gene alterations belong to several inflammatory pathways, thus the liver of the CCL7^{tgIEC} mice had low-grade inflammation at baseline. Upon APAP challenge, the immune reaction was further activated in the liver, and more injury occurred. However, the roles of the mucosal immune system in the effects of epithelial CCL7 overexpression on the gut barrier need further study. Furthermore, although baseline inflammation in the liver in CCL7^{tgIEC} mice was enhanced, it is unclear whether this hepatic inflammation or its enhancement after gut permeabilization directly influences the APAP-induced liver damage. Alternatively, the bacterial products or metabolites released from the gut may directly sensitize APAP toxicity. Greater permeabilization in the transgenic mice may then enhance the exposure of the liver to gut products after APAP.

Reportedly, the blockade of HMGB1 may present a novel therapy to prevent organ failure from gut bacterial translocation in APAP overdose.³³ However, the detailed mechanism of how bacterial pathogen-associated molecular patterns or other microbial products trigger APAP-induced liver injury requires further study. Pretreatment with D-glucose protects the liver against APAP

hepatotoxicity via the activation of the transporter SGLT-1 expressed by intestinal epithelial cells.³⁴ Therefore, targeting the intestine may provide a novel strategy to prohibit APAP hepatotoxicity.

In conclusion, using tissue-specific genetically modified mice, this study is the first to reveal that IEC CCL7 disrupts intestinal the epithelial barrier function and to highlight the necessity of a fully operational mucosal barrier against APAP-induced liver failure. This study also provides new evidence that the disruption of colonic integrity accompanies APAP hepatotoxicity. Maintaining intestinal integrity may serve as a novel strategy for combating APAP-induced liver damage. Further studies are required to understand the mechanism by which hepatic inflammation at baseline in CCL7^{tgIEC} mice sensitizes the liver to APAP toxicity.

Author Contributions

M.N., Z.L., S.G., and S.W. acquired and analyzed data; N.K., Y.J., and P.C. conceived and designed the study, wrote the manuscript, and supervised the study.

Supplemental Data

Supplemental material for this article can be found at <http://doi.org/10.1016/j.ajpath.2019.09.009>.

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