

## Basic Study

## Liver infiltration of multiple immune cells during the process of acute liver injury and repair

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**Abstract****BACKGROUND**

Immune cells, including neutrophils, natural killer (NK) cells, T cells, NKT cells and macrophages, participate in the progression of acute liver injury and hepatic recovery. To date, there has been no systematic study on the quantitative changes in these different immune cells from initial injury to subsequent recovery.

**AIM**

To investigate the infiltration changes of various immune cells in acute liver

injury models over time, and to study the relationship between the changes in leukocyte cell-derived chemotaxin 2 (LECT2) and the infiltration of several immune cells.

### METHODS

Carbon tetrachloride- and concanavalin A-induced acute liver injury models were employed to mimic toxin-induced and autoimmune-mediated liver injury respectively. The quantitative changes in various immune cells were monitored at different time points. Serum samples were collected, and liver tissues were harvested. Ly6G, CD161, CD4, CD8 and F4/80 staining were used to indicate neutrophils, NK/NKT cells, CD4<sup>+</sup> T cells, CD8<sup>+</sup> T cells and macrophages, respectively. Lect2-KO mice were used to detect the function of LECT2.

### RESULTS

During the injury and repair process, different types of immune cells began to increase, reached their peaks and fell into decline at different time points. Furthermore, when the serum alanine transaminase (ALT) and aspartate transaminase (AST) indices reverted to normal levels 7 d after the injury, the infiltration of immune cells still existed even 14 d after the injury, showing an obvious lag effect. We found that the expression of LECT2 was upregulated in acute liver injury mouse models, and the liver injuries of Lect2-KO mice were less severe than those of wild-type mice. Compared with wild-type mice, Lect2-KO mice had different immune cell infiltration.

### CONCLUSION

The recovery time of immune cells was far behind that of serum ALT and AST during the process of liver repair. LECT2 could regulate monocyte/macrophage chemotaxis and might be used as a therapeutic target for acute liver injury.

**Key Words:** Immune cells; Liver injury; Liver repair; Leukocyte cell-derived chemotaxin 2

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**Core Tip:** In our study, we systematically described the infiltration changes of various immune cells during the process of acute liver injury and repair. We found that the recovery time of immune cells was far behind that of serum alanine transaminase and aspartate transaminase during the process of liver repair. Moreover, we found that leukocyte cell-derived chemotaxin 2 (LECT2) was upregulated in acute liver injury mouse models and the changes in LECT2 were related to the changes in several immune cells. LECT2 could regulate monocyte/macrophage chemotaxis and might be used as a therapeutic target for acute liver injury.

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## INTRODUCTION

Acute liver injury is induced by a variety of causes, including viral infection, autoimmune diseases, alcohol or drug abuse and toxin intake, which can lead to liver failure and even death[1,2]. Various immune cells, including neutrophils, natural killer (NK) cells, T cells, NKT cells and macrophages, are directed and recruited to damaged sites and inflammatory lesions following acute injury[3]. Accumulated studies have indicated that these immune cells may participate in the progression of liver injury[4-6] and hepatic recovery[7-9]. A complete description of the changes in the number of various immune cells during the process of liver injury and repair could indicate the potential roles of these immune cells and may also help to treat acute liver injury by regulating these different immune cells at different time points. To date, there has been no systematic study on the quantitative changes in these different immune cells from initial injury to subsequent recovery. Here, we employed carbon tetrachloride (CCl<sub>4</sub>)- and concanavalin A (ConA)-induced acute liver injury mouse models to mimic toxin-induced and autoimmune-mediated liver injury, respectively, and monitored the dynamic changes in various immune cells over time.

Leukocyte cell-derived chemotaxin 2 (LECT2) is a 16-kDa protein mainly produced by hepatocytes, first isolated in PHA-activated human T-cell leukemia SKW-3 cells in 1996[10]. Increasing evidence suggests that LECT2 is a pleiotropic protein, it not only functions as a cytokine to exhibit chemotactic properties but also plays multifunctional roles in some physiological conditions and pathological abnormalities[11], such as neuronal development[12], liver regeneration[13], home-ostasis of hematopoietic stem cells[14], liver fibrosis[15,16], insulin resistance[17] and renal amyloidosis[18]. We found that, during the repair of acute liver injury, the changes in LECT2 were related to the changes in several immune cells. Acute liver injury might be treated by inhibiting LECT2.

## MATERIALS AND METHODS

### Mice

C57BL6/J (wild-type) mice were purchased from Guangzhou University of Chinese Medicine. Lect2-KO mice were provided by Professor Jiong Chen from Ningbo University. The genomic DNA from the Lect2-KO mice was extracted for PCR amplification with the forward and reverse primers (Forward: 5'-CATAGCCAGGGGACTATGTTTAA-3'; Reverse: 5'-ATATAGTCATAGCTGCACACAGCA-3'). The PCR products were further used to confirm genotyping by Sanger sequencing. Mice were kept in a standard 12 h light-dark cycle under specific-pathogen-free conditions, and were allowed free access to water and food. All mice used for the study were male, healthy and immune-normal, weighing 22-25 g. Animal-related research protocols were consistent with the US Public Health Service Policy on Use of Laboratory Animals, and were approved by the Ethics Committee on Use and Care of Animals of Southern Medical University.

### Acute liver injury models

CCl<sub>4</sub> (Maclin, C805332) and ConA (Solarbio, C8110) were separately used for the induction of acute liver injury model. For the CCl<sub>4</sub>-induced acute liver injury model, mice were injected intraperitoneally with a CCl<sub>4</sub> mixture at a dose of 10 mL/kg body weight once (CCl<sub>4</sub> dissolved in olive oil at a volume ratio of 1:4). For the ConA-induced acute liver injury model, mice were injected intravenously with ConA solution at a dose of 10 mg/kg body weight once (ConA dissolved in PBS at a concentration of 2 mg/mL). Serum samples and liver tissues were collected and stored at -80 °C refrigerator for subsequent analyses. There were five mice per group.

### Immunohistochemical staining

Harvested liver tissues were fixed in 4% neutral buffered formalin, and then dehydrated and embedded in paraffin. Sections of 3.5-mm thickness were dewaxed, and incubated in antigen retrieval solution for 5 min at 120 °C, and then endogenous peroxidases were blocked with 3% H<sub>2</sub>O<sub>2</sub> for 15 min. The slides were incubated with goat serum for 40 min at 37 °C to block nonspecific binding sites, followed by incubation with the appropriate primary antibodies overnight at 4 °C, and then with HRP (horseradish peroxidase) anti-rabbit IgG for 30 min at 37 °C. Color was developed by incubation with a DAB substrate kit (Zsbio, ZLI-9017). After washing with PBS, sections were counterstained with hematoxylin. Major primary antibodies used in the study included anti-Ly6G antibody (1:100, CST, 87048), anti-CD161 antibody (1:200, CST, 39197), anti-CD4 antibody (1:500, Abcam, ab183685), anti-CD8 antibody (1:1000, Abcam, ab209775) and anti-F4/80 antibody (1:200, CST, 70076). Images from ten random fields were taken for each section, under a 200 × light microscope. Cells positive for Ly6G, CD161, CD4 and CD8 were counted manually. The percentages of F4/80 positive areas were calculated automatically by ImageJ software.

### qRT-PCR

Total RNA from mouse livers was extracted using TRIzol reagent (AG, 21102), and reverse transcribed using Evo M-MLV RT Premix (AG, 11706). Quantitative qRT-PCR was performed on an ABI 7500 Fast real-time PCR system with SYBR Green Master PCR Mix (AG, 11701), Rox Reference Dye (AG, 11710) and primers. The primers applied in qRT-PCR were as follows: *Lect2* forward (mouse), 5'-GGACGTGTGACAGCTATGGC-3'; *Lect2* reverse (mouse), 5'-TCCCAGTGAATGGTGCATACA-3'; *GAPDH* forward (mouse), 5'-AGAAGGTGGTGAAGCAGGCATC-3', *GAPDH* reverse (mouse), 5'-CGAAGGTGGAA-GAGTGGGAGTTG-3'.

### ELISA

The serum levels of ALT, AST and TBil were detected by an Automated Chemical Analyzer (Mindray, BS-240VET) with standard diagnostic kits. The serum levels of LECT2 were tested by an ELISA kit (Cloud-Clone, SEF541Mu).

### RNA sequencing

The wild-type and Lect2-KO mice were injected with CCl<sub>4</sub> or ConA, and then livers from these mice

were harvested after 2 d. Livers were also obtained from wild-type mice without CCl<sub>4</sub> and ConA treatment as standard controls. Total RNA was extracted from the tissue using TRIzol reagent according to the manufacturer's instructions (Invitrogen) and genomic DNA was removed with DNaseI (TaKaRa). Then RNA quality was determined and only a high-quality RNA sample was used to construct a sequencing library. RNA-seq transcriptome libraries were prepared following the TruSeq™ RNA Sample Preparation Kit (Illumina, San Diego, CA, United States) using 1 µg of total RNA. Messenger RNA was isolated according to the poly (A) selection method by oligo (dT) beads and then fragmented by fragmentation buffer. Next, double-stranded cDNA was synthesized using a SuperScript double-stranded cDNA synthesis kit (Invitrogen, CA, United States) with random hexamer primers (Illumina). The synthesized cDNA was subjected to end-repair, phosphorylation and 'A' base addition according to Illumina's library construction protocol. Libraries were size selected for cDNA target fragments of 300 bp, followed by PCR amplification with Phusion DNA polymerase (NEB) for 15 PCR cycles. After quantification by TBS380, the paired-end RNA-seq sequencing library was sequenced with an Illumina NovaSeq 6000 sequencer. All steps of RNA sequencing were performed by Majorbio BioPharm Technology Corporation, Shanghai.

### Chemotaxis assay

Transwell inserts with 8-µm pores were used for the chemotaxis assay. THP-1 cells ( $2 \times 10^6$ /mL, 200 µL) were suspended in RPMI 1640 medium containing 10% FBS and 4000 nmol/L PMA (Bioss, D10289s), cells were seeded in each upper chamber. LECT2 (Abcam ab188467) and MCP-1 (Proteintech, Ag24085) were added to the lower chambers filled with 500 µl serum free medium containing 4000 nmol/L PMA. After incubation at 37 °C in 5% CO<sub>2</sub> for 2 h, the Transwell inserts were removed. Six consecutive pictures were taken with 200 × light microscope for the cells that migrated to the lower chambers. The number of cells was counted by ImageJ software for each picture.

### Statistical analysis

The results of the experiments are presented as the mean ± SD. The experimental data were analyzed by Student's *t* test. Statistical analysis and graph production were performed by GraphPad Prism software. A *P* value < 0.05 was considered to be statistically significant.

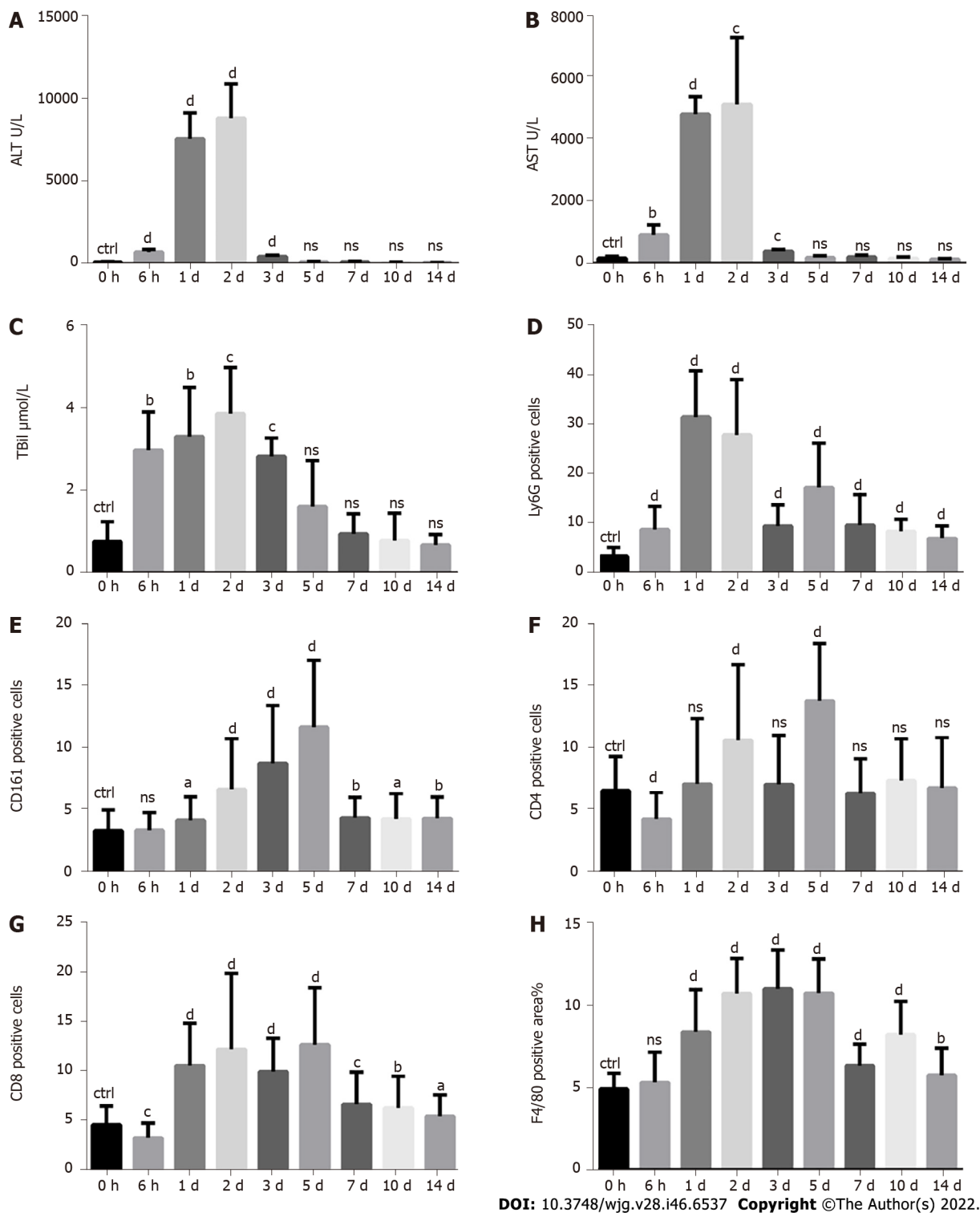
## RESULTS

### Immune cell infiltration during the process of CCl<sub>4</sub>-induced liver injury and repair

A single injection of CCl<sub>4</sub> at a dose of 10 mL/kg body weight was used to mimic toxin-induced acute liver injury in mice. The levels of serum alanine transaminase (ALT), aspartate transaminase (AST) and total bilirubin (TBil) were measured to evaluate the degree of liver injury from 0 h to 14 d after CCl<sub>4</sub> treatment. The levels of serum ALT, AST and TBil increased as early as 6 h, followed by a sharp rise and a peak on the 1<sup>st</sup> and 2<sup>nd</sup> days, respectively, and then decreased to normal on the 5<sup>th</sup> day (Figure 1A-C). Ly6G, CD161, CD4, CD8 and F4/80 staining were used to indicate neutrophils, NK/NKT cells, CD4<sup>+</sup> T cells, CD8<sup>+</sup> T cells and macrophages, respectively. Neutrophils increased as early as 6 h, followed by a sharp rise lasting for 2 d (Figure 1D and Supplementary Figure 1). NK/NKT cells increased as early as the 1<sup>st</sup> day, followed by a continuous rise with a peak on the 5<sup>th</sup> day (Figure 1E and Supplementary Figure 2). CD4<sup>+</sup> T cells increased as early as the 2<sup>nd</sup> day, and peaked on the 5<sup>th</sup> day (Figure 1F and Supplementary Figure 3). CD8<sup>+</sup> T cells increased as early as the 1<sup>st</sup> day, and peaked on the 5<sup>th</sup> day (Figure 1G and Supplementary Figure 4). Macrophages increased as early as the 1<sup>st</sup> day, followed by a peak lasting for approximately 4 d (Figure 1H and Supplementary Figure 5). It should be noted that the number of infiltrated neutrophils, NK/NKT cells, CD8<sup>+</sup> T cells and macrophages on the 14<sup>th</sup> day after CCl<sub>4</sub> treatment was still higher than that of the control, compared with the fact that the levels of serum ALT, AST and TBil decreased to normal on the 5<sup>th</sup> day after CCl<sub>4</sub> treatment.

### Immune cell infiltration during the process of ConA-induced liver injury and repair

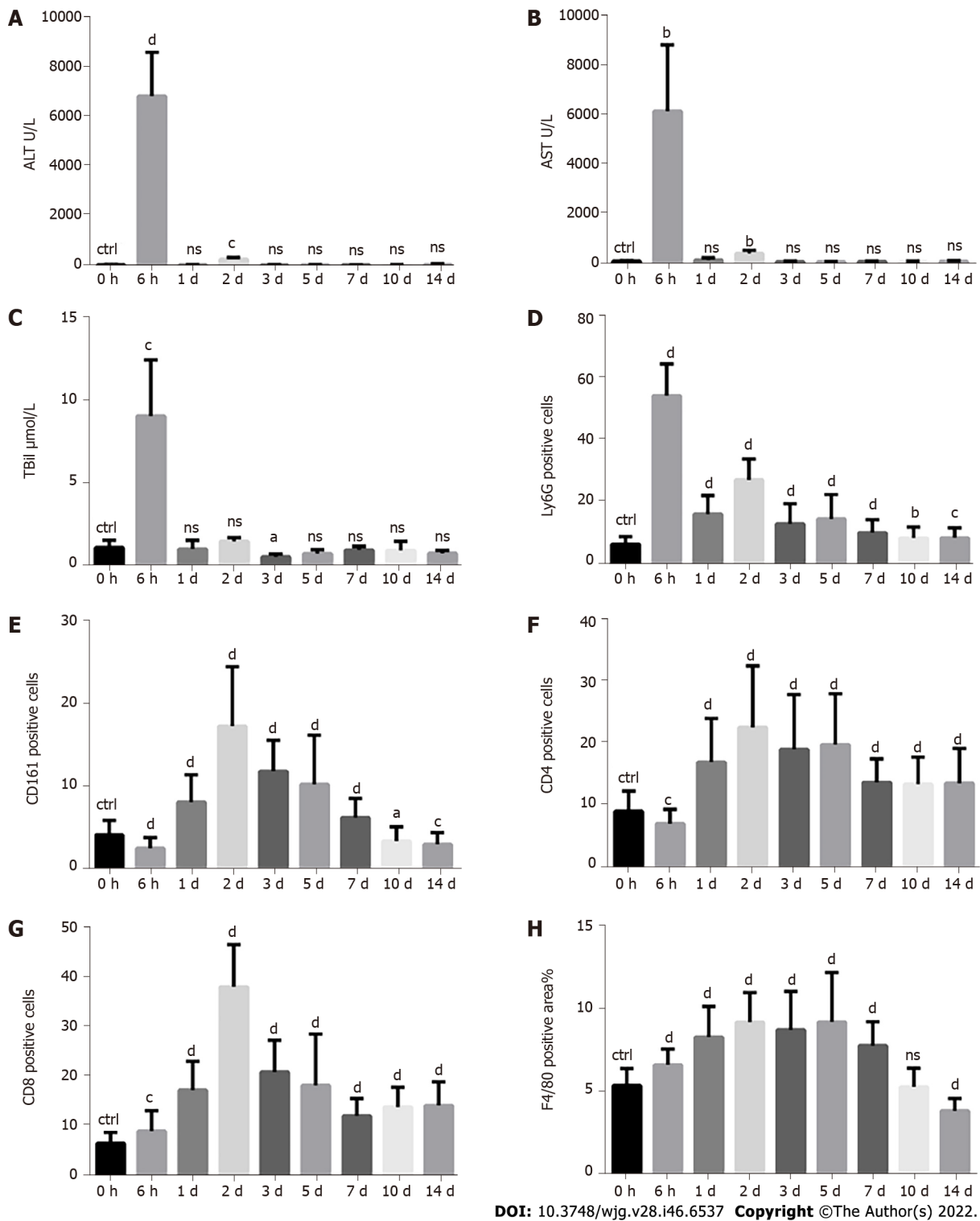
A single injection of ConA at a dose of 10 mg/kg body weight was used to mimic autoimmune-mediated acute liver injury in mice. In contrast to CCl<sub>4</sub>-treated mice, in ConA-treated mice, the levels of serum ALT, AST and TBil peaked as soon as 6 h, followed by a rapid decline on the 1<sup>st</sup> day, and then decreased to normal within 3 d (Figure 2A-C). In the ConA-induced acute injury model, neutrophils increased sharply and peaked as soon as 6 h (Figure 2D and Supplementary Figure 6). NK/NKT cells increased as early as the 1<sup>st</sup> day, and then peaked on the 2<sup>nd</sup> day (Figure 2E and Supplementary Figure 7). CD4<sup>+</sup> T cells increased as early as the 1<sup>st</sup> day, followed by a peak on the 2<sup>nd</sup> day (Figure 2F and Supplementary Figure 8). CD8<sup>+</sup> T cells increased gradually from 6 h to 2 d, and peaked on the 2<sup>nd</sup> day (Figure 2G and Supplementary Figure 9). Macrophages increased as early as 6 h, peaked on the 2<sup>nd</sup> day and lasted for approximately 4 d (Figure 2H and Supplementary Figure 10). Notably, the above immune cells could hardly revert to the normal count within 7 d.



**Figure 1** Immune cell infiltration during the process of carbon tetrachloride-induced liver injury and repair. A-C: The levels of serum alanine transaminase, aspartate transaminase and total bilirubin in carbon tetrachloride (CCl<sub>4</sub>)-treated wild-type mice; D-H: Quantitative changes in the infiltration of various immune cells in the livers of CCl<sub>4</sub>-treated wild-type mice. The data represent the mean  $\pm$  SD. \* $P < 0.05$ ; <sup>b</sup> $P < 0.01$ ; <sup>c</sup> $P < 0.001$ ; <sup>d</sup> $P < 0.0001$ . ctrl: Control; ns: Not significant; ALT: Alanine transaminase; AST: Aspartate transaminase; TBil: Total bilirubin.

### The expression of LECT2 was increased in acute liver injury models

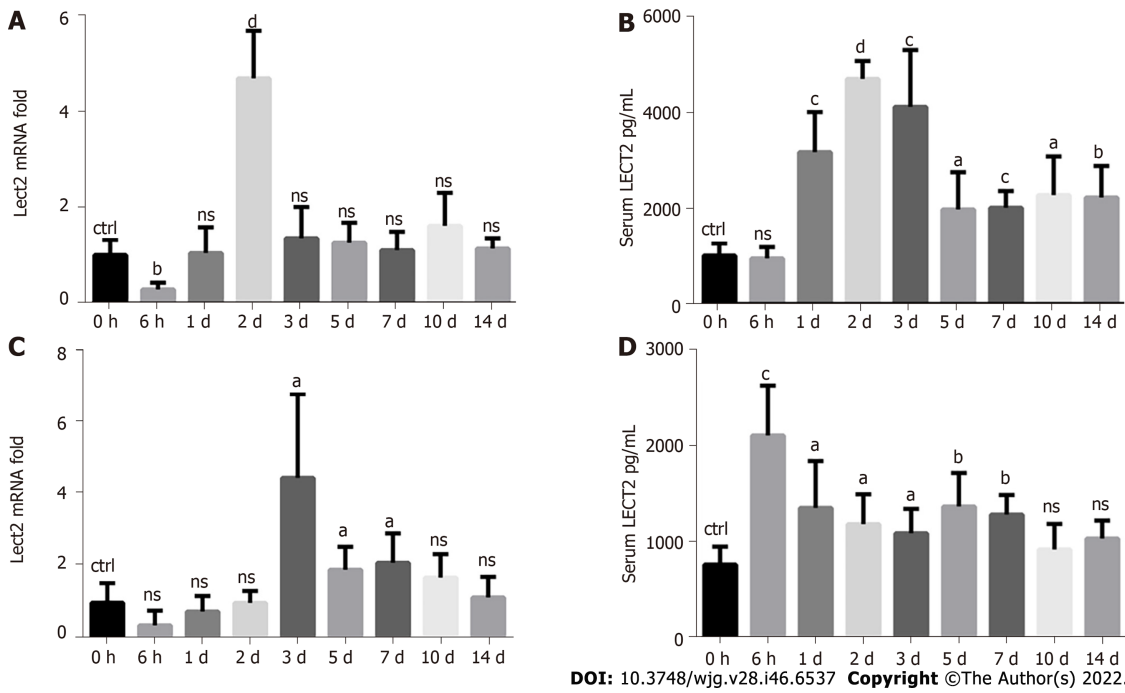
We detected the dynamic changes in LECT2 expression in liver tissues and serum from 0 h to 14 d after CCl<sub>4</sub> and ConA treatment. We found that the expression of Lect2 mRNA was significantly increased on the 2<sup>nd</sup> day after CCl<sub>4</sub> treatment (Figure 3A), while increased levels of serum LECT2 could be observed on the 1<sup>st</sup> day and lasted for 2 wk, with a peak on the 2<sup>nd</sup> day (Figure 3B). In the ConA-induced acute injury model, the expression of Lect2 mRNA was significantly increased on the 3<sup>rd</sup>, 5<sup>th</sup> and 7<sup>th</sup> days (Figure 3C), while increased levels of serum LECT2 could be observed as early as 6 h and lasted for 1 wk, with a peak at 6 h (Figure 3D).



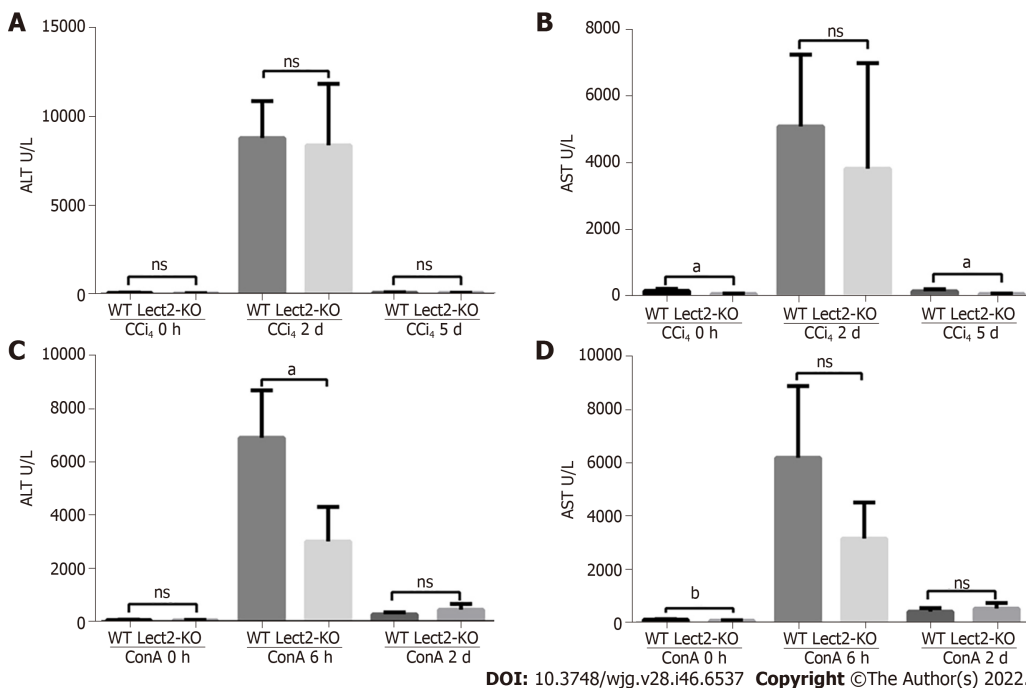
**Figure 2** Immune cell infiltration during the process of concanavalin A-induced liver injury and repair. A-C: The levels of serum alanine transaminase, aspartate transaminase and total bilirubin in concanavalin A (ConA)-treated wild-type mice; D-H: Quantitative changes in the infiltration of various immune cells in the livers of ConA-treated wild-type mice. The data represent the mean  $\pm$  SD. <sup>a</sup>*P* < 0.05; <sup>b</sup>*P* < 0.01; <sup>c</sup>*P* < 0.001; <sup>d</sup>*P* < 0.0001. ctrl: Control; ns: Not significant; ALT: Alanine transaminase; AST: Aspartate transaminase; TBil: Total bilirubin.

### Lect2-KO mice showed less severe liver injuries

The levels of AST were significantly decreased in CCl<sub>4</sub>-treated Lect2-KO mice on the 5<sup>th</sup> day compared with their WT (wild-type) counterparts (Figure 4A and B). The levels of ALT were significantly decreased in ConA-treated Lect2-KO mice at 6 h compared with their WT counterparts (Figure 4C and D).



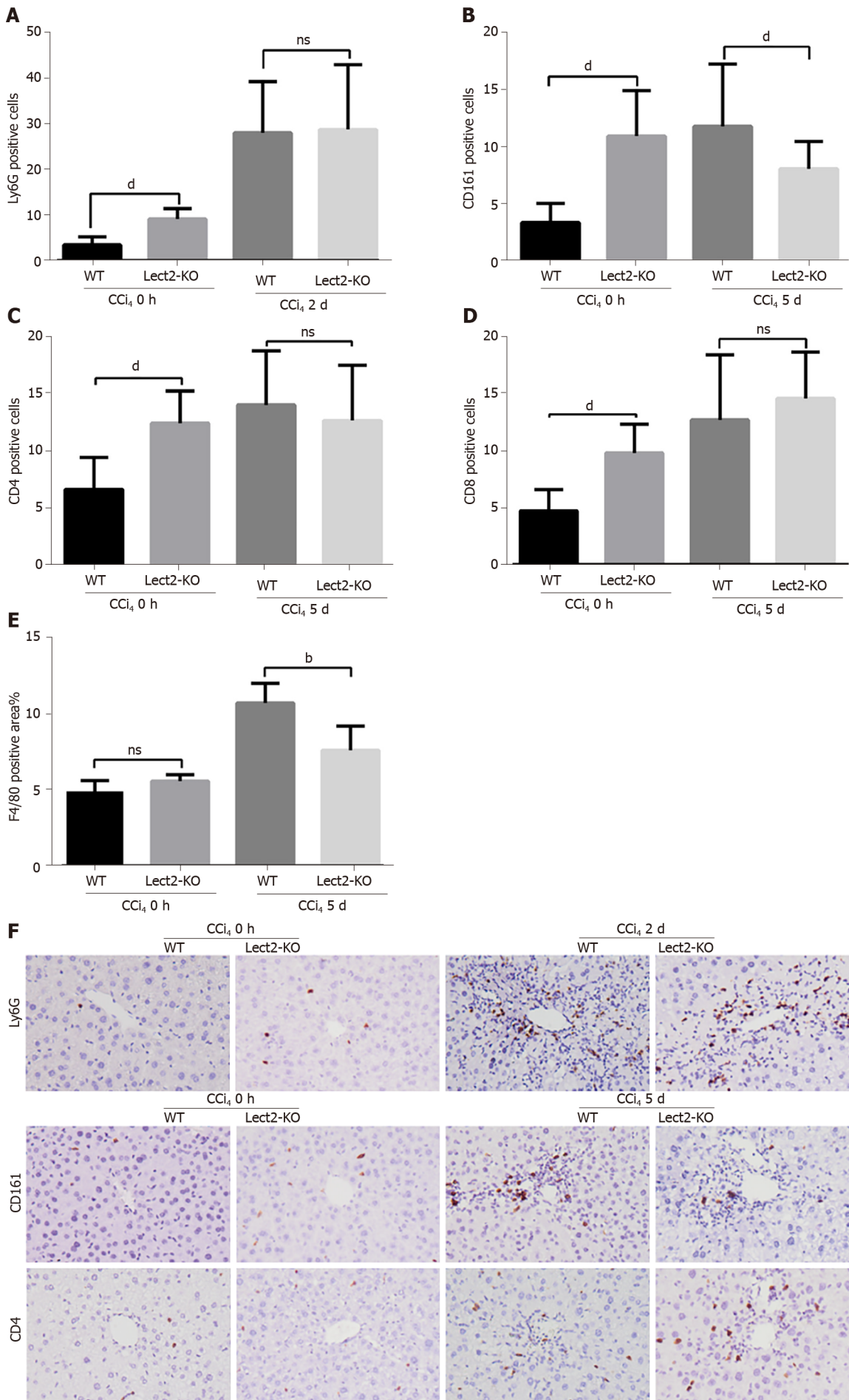
**Figure 3** The expression of leukocyte cell-derived chemotaxin 2 was increased in acute liver injury models. A: The relative expression of Lect2 mRNA in the liver tissues of carbon tetrachloride (CCl<sub>4</sub>)-treated wild-type mice; B: The levels of serum leukocyte cell-derived chemotaxin 2 (LECT2) in CCl<sub>4</sub>-treated wild-type mice; C: The relative expression of Lect2 mRNA in the liver tissues of concanavalin A (ConA)-treated wild-type mice; D: The levels of serum LECT2 in ConA-treated wild-type mice. The data represent the mean ± SD. ctrl: Control; ns: Not significant; <sup>a</sup>P < 0.05; <sup>b</sup>P < 0.01; <sup>c</sup>P < 0.001; <sup>d</sup>P < 0.0001. ctrl: Control; ns: Not significant; LECT2: Leukocyte cell-derived chemotaxin 2.



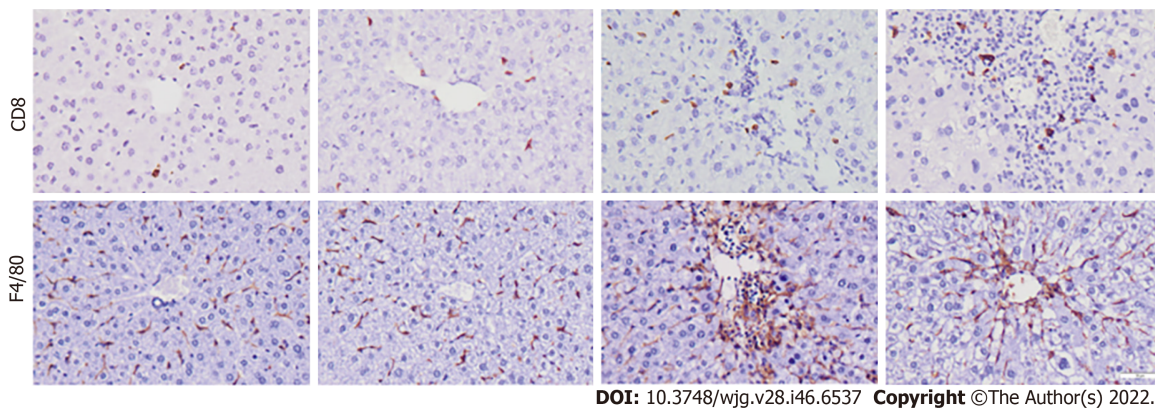
**Figure 4** Lect2-KO mice showed less severe liver injuries. A and B: The levels of serum alanine transaminase (ALT) and aspartate transaminase (AST) in the wild-type and Lect2-KO mice, with or without carbon tetrachloride treatment; C and D: The levels of serum ALT and AST in the wild-type and Lect2-KO mice, with or without concanavalin A treatment. The data represent the mean ± SD. <sup>a</sup>P < 0.05; <sup>b</sup>P < 0.01. ns: Not significant; ALT: Alanine transaminase; AST: Aspartate transaminase.

**The effect of LECT2 on immune cell infiltration in acute liver injury models**

We selected the peak time for different types of immune cells in the CCl<sub>4</sub> or ConA-induced acute injury model to observe the effect of LECT2 on the liver infiltration of various immune cells. WT and Lect2-KO mice were treated with CCl<sub>4</sub> and ConA, respectively, and livers were harvested on the 2<sup>nd</sup> and 5<sup>th</sup> days







**Figure 5** The effect of leukocyte cell-derived chemotaxin 2 on immune cell infiltration in the carbon tetrachloride-induced acute liver injury model. A-E: Quantitative changes in the infiltration of various immune cells in the livers of wild-type and Lect2-KO mice, with or without carbon tetrachloride (CCl<sub>4</sub>) treatment; F: Representative images of infiltrated immune cells in CCl<sub>4</sub>-treated wild-type and Lect2-KO mice, with or without CCl<sub>4</sub> treatment. The data represent the mean ± SD. ns: Not significant; <sup>a</sup>*P* < 0.05; <sup>b</sup>*P* < 0.01; <sup>c</sup>*P* < 0.001; <sup>d</sup>*P* < 0.0001. Scale bar: 50 μm.

after CCl<sub>4</sub> treatment. For the mice treated with ConA, livers were harvested at the 6<sup>th</sup> hour and 2<sup>nd</sup> day. First, we compared the number of immune cells between Lect2-KO mice and WT controls at 0 h without CCl<sub>4</sub> and ConA treatment. We found that more neutrophils, NK/NKT cells, CD4<sup>+</sup> and CD8<sup>+</sup> T cells emerged in Lect2-KO mice (Figure 5 and Figure 6). Next, we compared the number of immune cells between Lect2-KO mice and WT controls when treated with CCl<sub>4</sub> or ConA. In the CCl<sub>4</sub>-induced acute injury model, we found that the livers of Lect2-KO mice had less infiltration of NK/NKT cells and macrophages than those of WT mice. There was no difference in the number of neutrophils, CD4<sup>+</sup> T cells or CD8<sup>+</sup> T cells (Figure 5A-F). In the ConA-induced acute injury model, the livers of Lect2-KO mice had more infiltrated neutrophils, CD4<sup>+</sup> T cells and CD8<sup>+</sup> T cells but fewer macrophages than those of WT mice. There was no difference in the number of NK/NKT cells (Figure 6A-F).

#### Chemokine-related genes changed in Lect2-KO mice

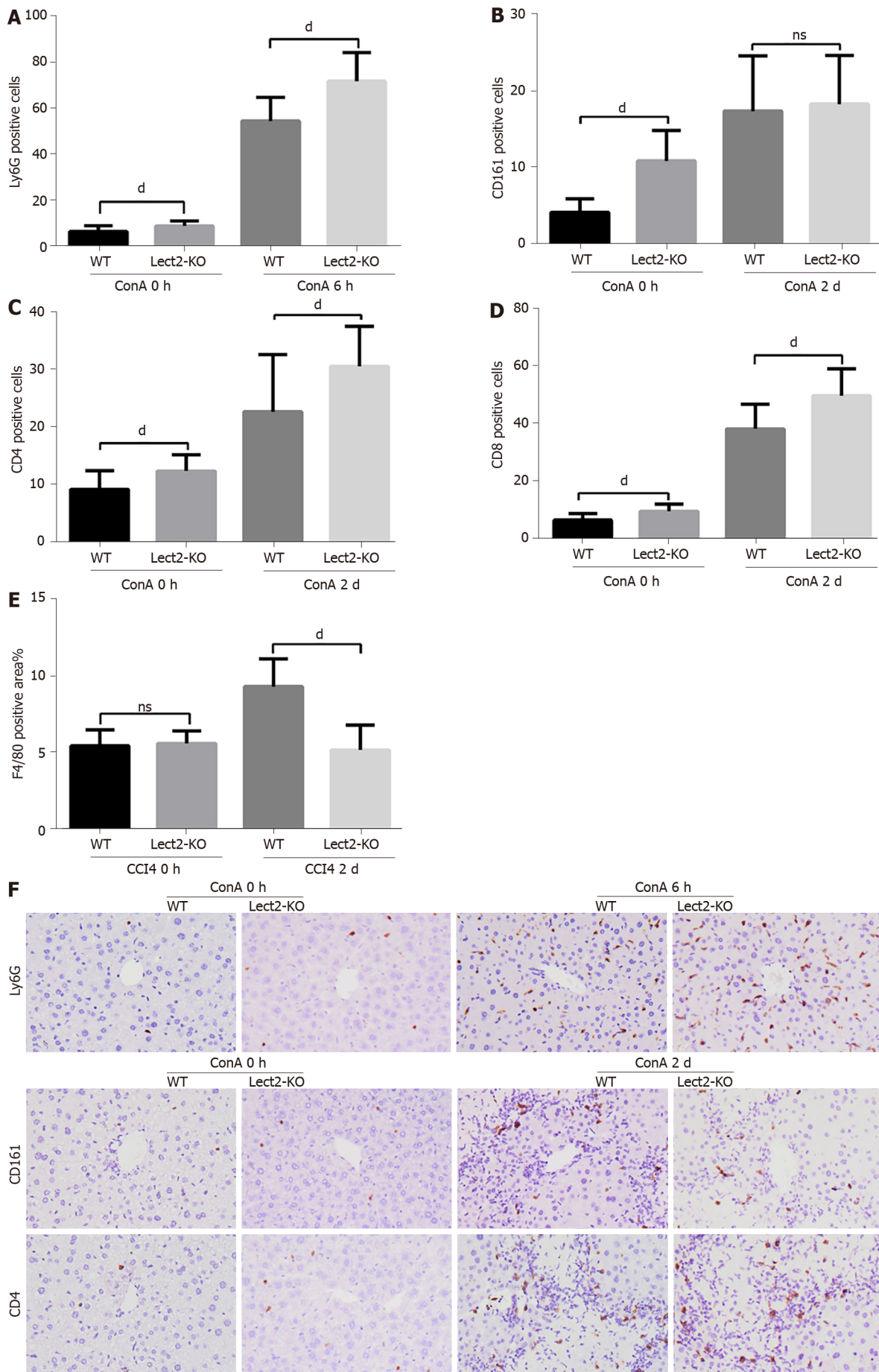
Chemokines are chemotactic cytokines that are capable of recruiting immune cells to sites of injury and inflammation. Next, we explored whether knockout of the Lect2 gene regulated the expression of chemokines in acute liver injury. RNA sequencing analysis was performed on injured liver samples derived from CCl<sub>4</sub> or ConA-treated Lect2-KO and WT mice. Differentially expressed genes were identified with a *p*-adjust value < 0.05 and fold change > 2. By Kyoto Encyclopedia of Genes and Genomes pathway enrichment analysis, we found that these changed genes could be partly categorized into chemokine signaling pathways (Figure 7A) and cytokine-cytokine receptor interactions (Figure 7B). Knockout of the Lect2 gene upregulated the expression of CXCL9, CXCL14, CCL6 and CCL22, and downregulated the expression of CCL19 in the CCl<sub>4</sub>-treated mice (Figure 7C). In the ConA-treated mice, knockout of the Lect2 gene led to upregulated expression of CXCL2, CXCL3, CXCL5, CCL2 and CCL6 and downregulated expression of CCL21a, CCL21b and CCL27a (Figure 7D).

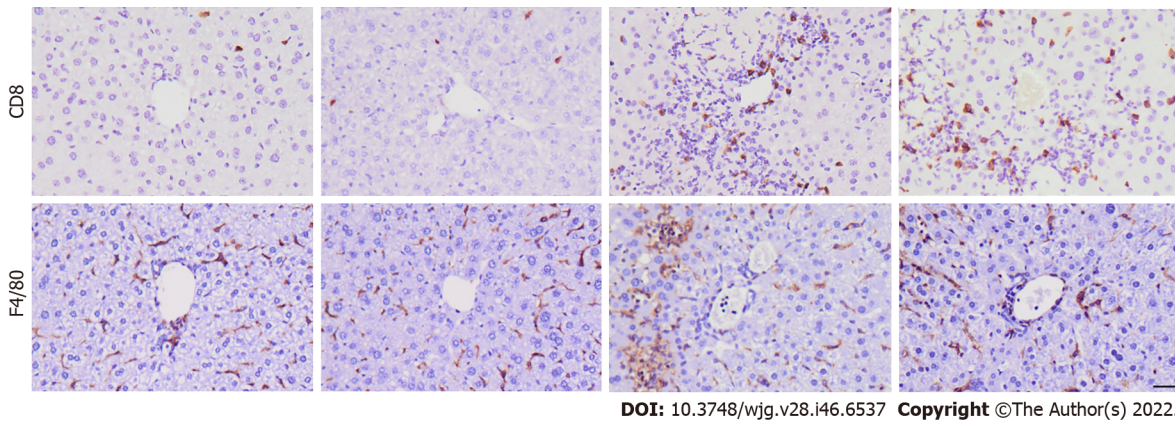
#### LECT2 promoted THP-1 monocyte chemotaxis

Since Lect2-KO mice displayed less macrophage infiltration than their WT counterparts in both CCl<sub>4</sub>- and ConA-induced acute injury models, we explored whether LECT2 promoted monocytes/macrophage chemotaxis. The transwell assay showed that LECT2 could promote THP-1 monocytes chemotaxis (Figure 8A), but its chemotaxis was weaker than that of MCP-1, which is a well-recognized monocyte chemokine (Figure 8B).

## DISCUSSION

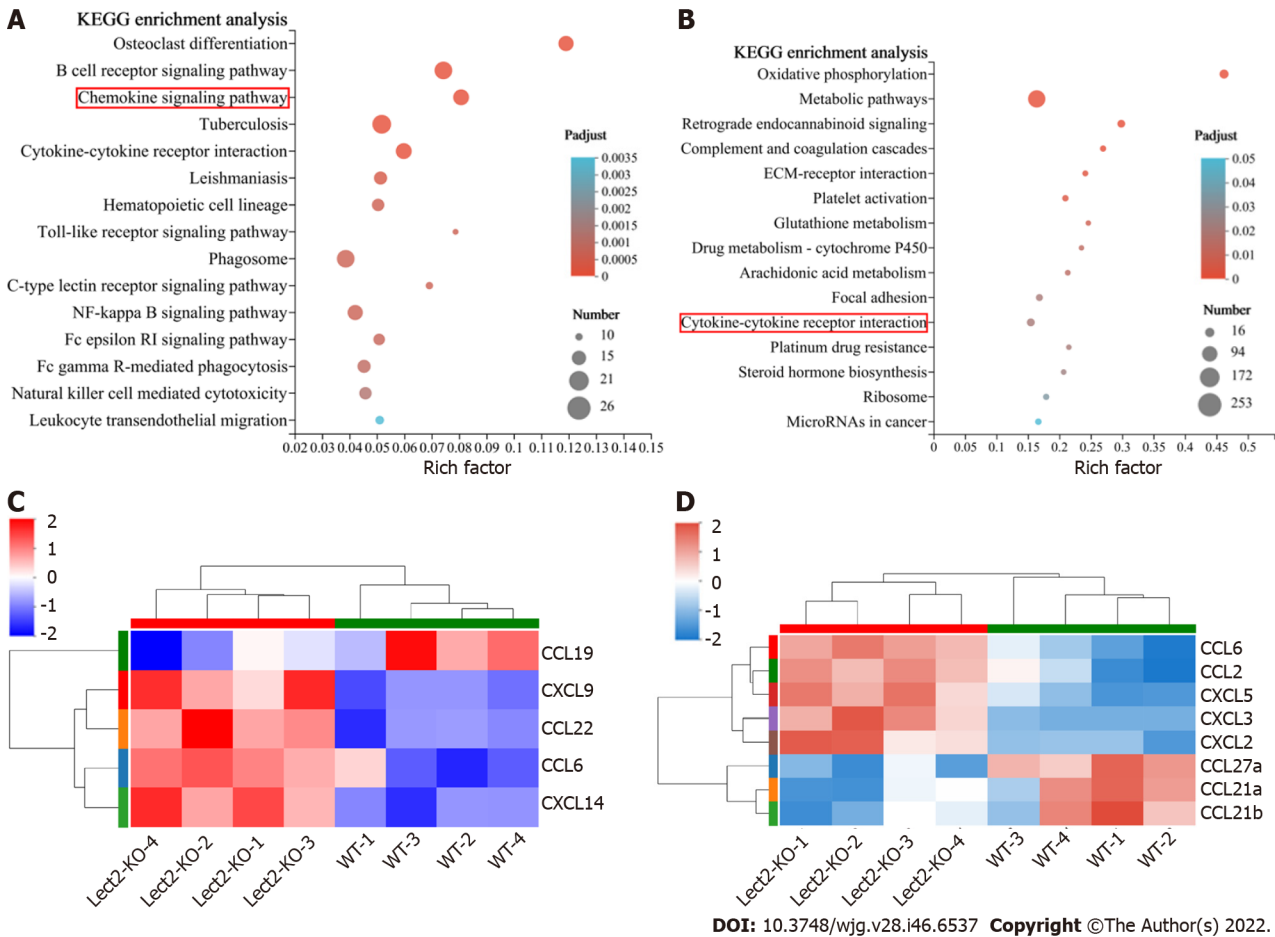
The liver infiltration of various immune cell populations, including neutrophils, NK/NKT cells, T cells and monocytes/macrophages, is a critical pathological feature following acute liver injury and plays important roles in liver injury or repair[1-9]. Although there are many studies on the function of immune cells during liver injury or repair, there is still no study on the quantitative changes in immune cell infiltration at different time points from the beginning of injury to the end of repair. For the first time, employing CCl<sub>4</sub>- and ConA-induced acute liver injury models, we monitored the infiltration of immune cells over time and found that different types of immune cells began to increase, reached their peaks and fell into decline at different time points, indicating that they played different roles in different injuries or repair stages. Therefore, appropriate regulation of immune cells at different time points may be a better strategy for the treatment of acute liver injury.





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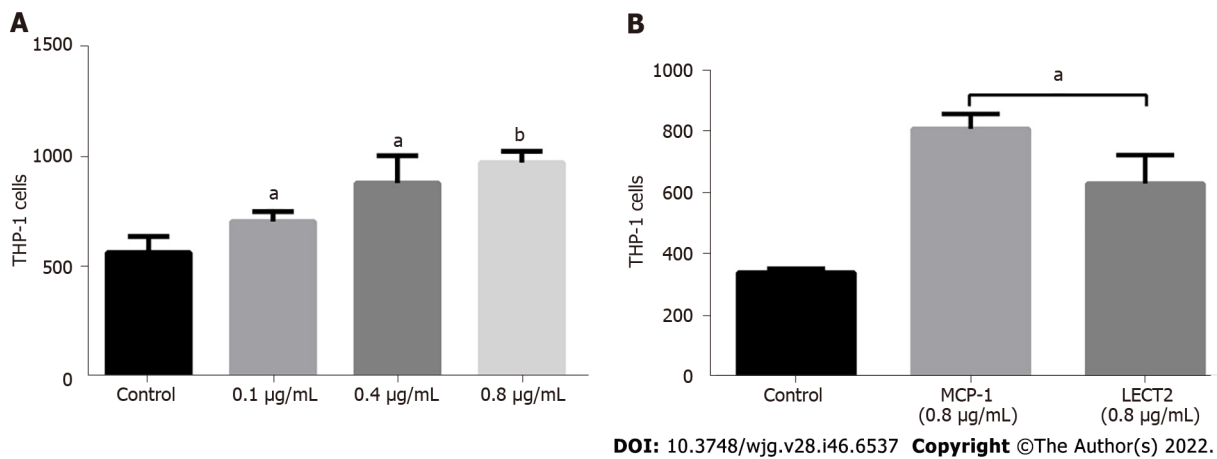
**Figure 6** The effect of leukocyte cell-derived chemotaxin 2 on immune cell infiltration in the concanavalin A-induced acute liver injury model. A-E: Quantitative changes in the infiltration of various immune cells in the livers of wild-type and Lect2-KO mice, with or without concanavalin A (ConA) treatment; F: Representative images of infiltrated immune cells in ConA-treated wild-type and Lect2-KO mice, with or without ConA treatment. The data represent the mean ± SD. <sup>a</sup>*P* < 0.05; <sup>b</sup>*P* < 0.01; <sup>c</sup>*P* < 0.001; <sup>d</sup>*P* < 0.0001. ns: Not significant; Scale bar: 50 μm.



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**Figure 7** Chemokine-related genes changed in Lect2-KO mice. A: Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis results for carbon tetrachloride (CCl<sub>4</sub>)-treated Lect2-KO and wild-type mice; B: KEGG analysis results for concanavalin A (ConA)-treated Lect2-KO and wild-type mice; C: Heatmap of the differentially expressed chemokine-related genes between the Lect2-KO and wild-type mice after CCl<sub>4</sub> treatment; D: Heatmap of the differentially expressed chemokine-related genes between the Lect2-KO and wild-type mice after ConA treatment.

We were surprised to find that during the repair process, when the serum ALT and AST indices had reverted to normal levels 7 d after the injury, the infiltration of immune cells still existed even 14 d after the injury, showing an obvious lag effect. This suggests that the liver does not completely recover to normal as quickly as we previously thought. The recovery of serum indicators (ALT, AST, *etc.*) does not mean that the liver has recovered to normal, and the corresponding treatment may need to be extended



**Figure 8 Leukocyte cell-derived chemotaxin 2 promoted THP-1 monocyte chemotaxis.** A: Chemotactic effects of Lect2 on THP-1 cells when treated with different doses; B: Comparison of chemotactic effects between Lect2 and MCP-1. The data represent the mean ± SD. <sup>a</sup> $P < 0.05$ ; <sup>b</sup> $P < 0.01$ . ctrl: Control.

accordingly. Therefore, in addition to the serological indices, we suggest immunological indices to measure the repair of liver injury, which may have important value for the clinical treatment and prognosis of liver diseases. These remaining immune cells may continue to regulate liver repair and play a role in the response of the liver to rechallenges.

We previously reported that LECT2 regulated chronic liver fibrogenesis[15,16], while its role in acute liver injury is unclear. Here, we found that the expression of LECT2 was upregulated in acute liver injury models, and the liver injuries of Lect2-KO mice were less severe than those of wild-type mice. Compared with wild-type mice, Lect2-KO mice had different immune cell infiltration, suggesting that LECT2 may regulate acute liver injury and repair by regulating the infiltration of various immune cells. We demonstrated that LECT2 could indeed regulate monocyte/macrophage chemotaxis in vitro. Blocking LECT2 might be able to treat acute liver injury.

The limitation of this study is that we need further functional tests to verify the roles that different immune cells play in liver injury. In particular, when the liver is rechallenged, the roles of retained immune cells in the last injury need to be further studied. When the liver is rechallenged, these retained immune cells may cause greater damage to the liver, or may make the liver more tolerant to the same challenge.

## CONCLUSION

In summary, we described the infiltration changes of various immune cells in acute liver injury models over time. We found that the recovery time of immune cells during the repair process was far behind that of serum ALT and AST. LECT2 could regulate monocyte/macrophage chemotaxis and might be used as a therapeutic target for acute liver injury.

## ARTICLE HIGHLIGHTS

### Research background

Immune cells, including neutrophils, natural killer (NK) cells, T cells, NKT cells and macrophages, participate in the progression of acute liver injury and hepatic recovery. leukocyte cell-derived chemotaxin 2 (LECT2) is a pleiotropic protein that not only functions as a cytokine to exhibit chemotactic properties but also plays multifunctional roles in some physiological conditions and pathological abnormalities.

### Research motivation

To date, there has been no systematic study on the quantitative changes in these different immune cells from initial injury to subsequent recovery. LECT2 regulates chronic liver fibrogenesis, but its role in acute liver injury is unclear.

### Research objectives

To investigate the infiltration changes of various immune cells in acute liver injury mouse models over time and to study the relationship between the changes in LECT2 and the infiltration of several immune

cells.

### Research methods

Carbon tetrachloride (CCl<sub>4</sub>)- and concanavalin A (ConA)-induced acute liver injury mouse models were employed to mimic toxin-induced and autoimmune-mediated liver injury, respectively. Immunohistochemical staining was used to indicate immune cells, and the quantitative changes in various immune cells were monitored at different time points. The levels of serum alanine transaminase (ALT), aspartate transaminase (AST) and total bilirubin (TBil) were measured by an automated chemical analyzer. The expression of LECT2 in liver tissues was detected by quantitative real-time polymerase chain reaction and the levels of LECT2 in serum were tested by enzyme-linked immunosorbent assay. Transwell assays were used to assess the chemotactic effect of LECT2 on THP-1 monocytes.

### Research results

During the injury and repair process, different types of immune cells began to increase, reached their peaks and fell into decline at different time points. Furthermore, when the serum ALT and AST indices reverted to normal levels 7 d after injury, the infiltration of immune cells still existed even 14 d after injury, showing an obvious lag effect. The expression of LECT2 was up-regulated in acute liver injury models, and the liver injuries of Lect2-KO mice were less severe than those of wild-type mice. Compared with wild-type mice, Lect2-KO mice had different immune cell infiltration.

### Research conclusions

The recovery time of immune cells was far behind that of serum ALT and AST during the process of liver repair. LECT2 could regulate monocyte/macrophage chemotaxis and might be used as a therapeutic target for acute liver injury.

### Research perspectives

Our study indicated the potential roles of these immune cells and proposed a therapeutic concept that acute liver injury might be treated by regulating different immune cells at different time points.

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## FOOTNOTES

**Author contributions:** Xie Y designed the experiments and wrote the manuscript; Zhong KB and Hu Y performed statistic and bioinformatic analyses; Xie Y, Zhong KB and Hu Y contributed equally to this work; Xi YL, Guan SX, Xu M, Lin Y helped to perform the experiments; Gao Y and Zhou WJ conceived the project; Liu FY supervised the schedule progress of the project; Gao Y, Zhou WJ and Liu FY were co-corresponding authors, and they contributed equally to this project; all authors have read and approved the final manuscript.

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