80338_Auto_Edited-check.docx

WORD COUNT	4563	TIME SUBMITTED	14-NOV-2022 10:29PM
		PAPER ID	92631828

Name of Journal: *World Journal of Gastroenterology*

Manuscript NO: 80338

Manuscript Type: ORIGINAL ARTICLE

Basic Study

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

Xie Y *et al*. Immune cell infiltration in acute liver injury

Yuan Xie, Ke-Bo Zhong, Yang Hu, Yong-Lun Xi, Shi-Xing Guan, Meng Xu, Yuan Lin, Feng-Yong Liu, Wei-Jie Zhou, Yi Gao

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, $CD4^+$ T cells, $CD8^+$ 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

Xie Y, Zhong KB, Hu Y, Xi YL, Guan SX, Xu M, Lin Y, Liu FY, Zhou WJ, Gao Y. Liver infiltration of multiple immune cells during the process of acute liver injury and repair. *World J Gastroenterol* 2022; In press

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.

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_4)- 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], homeostasis 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′-CATAGCCAGGGGACTATGTTTTA-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_4 (Maclin, C805332) and ConA (Solarbio, C8110) were separately used for the induction of acute liver injury model. For the CCl_4-induced acute liver injury model, mice were injected intraperitoneally with a CCl_4 mixture at a dose of 10 mL/kg body weight once (CCl_4 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 ℃, and then endogenous peroxidases were blocked with 3% H_2O_2 for 15 min. The slides were incubated with goat serum for 40 min at 37 ℃to block nonspecific binding sites, followed by incubation with the appropriate primary antibodies overnight at 4 ℃ and then with HRP (horseradish peroxidase) anti-rabbit IgG for 30 min at 37 ℃ 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′-CGAAGGTGGAAGAGTGGGAGTTG-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_4 or ConA, and then livers from these mice were harvested after 2 d. Livers were also obtained from wild-type mice without CCl_4 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) 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) 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 (2×10^6/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_2 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_4-induced liver injury and repair

A single injection of CCl_4 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_4 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 1st and 2nd days, respectively, and then decreased to normal on the 5th day (Figure 1A-C). Ly6G, CD161, CD4, CD8 and F4/80 staining were used to indicate neutrophils, NK/NKT cells, CD4$^+$ T cells, CD8$^+$ 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 1st day, followed by a continuous rise with a peak on the 5th day (Figure 1E and Supplementary Figure 2). CD4$^+$ T cells increased as early as the 2nd day, and peaked on the 5th day (Figure 1F and Supplementary Figure 3). CD8$^+$ T cells increased as early as the 1st day, and peaked on the 5th day (Figure 1G and Supplementary Figure 4). Macrophages increased as early as the 1st 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, $CD8^+$ T cells and macrophages on the 14th day after CCl_4 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 5th day after CCl_4 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_4-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 1st 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 1st day, and then peaked on the 2nd day (Figure 2E and Supplementary Figure 7). $CD4^+$ T cells increased as early as the 1st day, followed by a peak on the 2nd day (Figure 2F and Supplementary Figure 8). $CD8^+$ T cells increased gradually from 6 h to 2 d, and peaked on the 2nd day (Figure 2G and Supplementary Figure 9). Macrophages increased as early as 6 h, peaked on the 2nd 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.

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_4 and ConA treatment. We found that the expression of Lect2 mRNA was significantly increased on the 2nd day after CCl_4 treatment (Figure 3A), while increased levels of serum LECT2 could be observed on the 1st day and lasted for 2 wk, with a peak on the 2nd day (Figure 3B). In the ConA-induced acute injury model, the expression of Lect2 mRNA was significantly increased on the 3rd, 5th and 7th days (Figure 3C), while increased levels of serum LECT2 could be observed at as early as 6 h and lasted for 1 wk, with a peak at 6 h (Figure 3D).

Lect2-KO mice showed less severe liver injuries

The levels of AST were significantly decreased in CCl_4-treated Lect2-KO mice on the 5th 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).

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_4 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_4 and ConA, respectively, and livers were harvested on the 2nd and 5th days after CCl_4 treatment. For the mice treated with ConA, livers were harvested at the 6th hour and 2nd day. First, we compared the number of immune cells between Lect2-KO mice and WT controls at 0 h without CCl_4 and ConA treatment. We found that more neutrophils, NK/NKT cells, $CD4^+$ and $CD8^+$ 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_4 or ConA. In the CCl_4-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, $CD4^+$ T cells or $CD8^+$ T cells (Figure 5A-F). In the ConA-induced acute injury model, the livers of Lect2-KO mice had more infiltrated neutrophils, $CD4^+$ T cells and $CD8^+$ 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 injuried liver samples derived from CCl_4 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_4-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_4- 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_4- 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.

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 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_4)- 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.

ACKNOWLEDGEMENTS

We thank Central Laboratory, Southern Medical University for providing facilities and technical support.

REFERENCES

1 **Khoury T**, Rmeileh AA, Yosha L, Benson AA, Daher S, Mizrahi M. Drug Induced Liver Injury: Review with a Focus on Genetic Factors, Tissue Diagnosis, and Treatment Options. *J Clin Transl Hepatol* 2015; **3**: 99-108 [PMID: 26356634 DOI: 10.14218/JCTH.2015.00007]

2 **Bernal W**, Auzinger G, Dhawan A, Wendon J. Acute liver failure. *Lancet* 2010; **376**: 190-201 [PMID: 20638564 DOI: 10.1016/S0140-6736(10)60274-7]

3 **Karlmark KR**, Wasmuth HE, Trautwein C, Tacke F. Chemokine-directed immune cell infiltration in acute and chronic liver disease. *Expert Rev Gastroenterol Hepatol* 2008; **2**: 233-242 [PMID: 19072358 DOI: 10.1586/17474124.2.2.233]

4 **Ayata CK**, Ganal SC, Hockenjos B, Willim K, Vieira RP, Grimm M, Robaye B, Boeynaems JM, Di Virgilio F, Pellegatti P, Diefenbach A, Idzko M, Hasselblatt P. Purinergic $P2Y_2$ receptors promote neutrophil infiltration and hepatocyte death in mice with acute liver injury. *Gastroenterology* 2012; **143**: 1620-1629.e4 [PMID: 22974709 DOI: 10.1053/j.gastro.2012.08.049]

5 **Bi Y**, Li J, Yang Y, Wang Q, Wang Q, Zhang X, Dong G, Wang Y, Duan Z, Shu Z, Liu T, Chen Y, Zhang K, Hong F. Human liver stem cells attenuate concanavalin A-induced acute liver injury by modulating myeloid-derived suppressor cells and $CD4^+$ T cells in mice. *Stem Cell Res Ther* 2019; **10**: 22 [PMID: 30635035 DOI: 10.1186/s13287-018-1128-2]

6 **Geng J**, Wang X, Wei H, Sun R, Tian Z. Efficient attenuation of NK cell-mediated liver injury through genetically manipulating multiple immunogenes by using a liver-directed vector. *J Immunol* 2013; **190**: 4821-4829 [PMID: 23554312 DOI: 10.4049/jimmunol.1203129]

7 **Yang W**, Zhao X, Tao Y, Wu Y, He F, Tang L. Proteomic analysis reveals a protective role of specific macrophage subsets in liver repair. *Sci Rep* 2019; **9**: 2953 [PMID: 30814596 DOI: 10.1038/s41598-019-39007-6]

8 **Miura A**, Hosono T, Seki T. Macrophage potentiates the recovery of liver zonation and metabolic function after acute liver injury. *Sci Rep* 2021; **11**: 9730 [PMID: 33958644 DOI: 10.1038/s41598-021-88989-9]

9 **Nguyen NT**, Umbaugh DS, Sanchez-Guerrero G, Ramachandran A, Jaeschke H. Kupffer cells regulate liver recovery through induction of chemokine receptor CXCR2 on hepatocytes after acetaminophen overdose in mice. *Arch Toxicol* 2022; **96**: 305-320 [PMID: 34724096 DOI: 10.1007/s00204-021-03183-0]

10 **Yamagoe S**, Yamakawa Y, Matsuo Y, Minowada J, Mizuno S, Suzuki K. Purification and primary amino acid sequence of a novel neutrophil chemotactic factor LECT2. *Immunol Lett* 1996; **52**: 9-13 [PMID: 8877413 DOI: 10.1016/0165-2478(96)02572-2]

11 **Xie Y**, Fan KW, Guan SX, Hu Y, Gao Y, Zhou WJ. LECT2: A pleiotropic and promising hepatokine, from bench to bedside. *J Cell Mol Med* 2022; **26**: 3598-3607 [PMID: 35656863 DOI: 10.1111/jcmm.17407]

12 **Koshimizu Y**, Ohtomi M. Regulation of neurite extension by expression of LECT2 and neurotrophins based on findings in LECT2-knockout mice. *Brain Res* 2010; **1311**: 1-11 [PMID: 19917270 DOI: 10.1016/j.brainres.2009.11.010]

13 **Ohtomi M**, Nagai H, Ohtake H, Uchida T, Suzuki K. Dynamic change in expression of LECT2 during liver regeneration after partial hepatectomy in mice. *Biomed Res* 2007; **28**: 247-253 [PMID: 18000337 DOI: 10.2220/biomedres.28.247]

14 **Lu XJ**, Chen Q, Rong YJ, Yang GJ, Li CH, Xu NY, Yu CH, Wang HY, Zhang S, Shi YH, Chen J. LECT2 drives haematopoietic stem cell expansion and mobilization *via* regulating the macrophages and osteolineage cells. *Nat Commun* 2016; **7**: 12719 [PMID: 27596364 DOI: 10.1038/ncomms12719]

15 **Xu M**, Xu HH, Lin Y, Sun X, Wang LJ, Fang ZP, Su XH, Liang XJ, Hu Y, Liu ZM, Cheng Y, Wei Y, Li J, Li L, Liu HJ, Cheng Z, Tang N, Peng C, Li T, Liu T, Qiao L, Wu D, Ding YQ, Zhou WJ. LECT2, a Ligand for Tie1, Plays a Crucial Role in Liver Fibrogenesis. *Cell* 2019; **178**: 1478-1492.e20 [PMID: 31474362 DOI: 10.1016/j.cell.2019.07.021]

16 **Lin Y**, Dong MQ, Liu ZM, Xu M, Huang ZH, Liu HJ, Gao Y, Zhou WJ. A strategy of vascular-targeted therapy for liver fibrosis. *Hepatology* 2022; **76**: 660-675 [PMID: 34940991 DOI: 10.1002/hep.32299]

17 **Lan F**, Misu H, Chikamoto K, Takayama H, Kikuchi A, Mohri K, Takata N, Hayashi H, Matsuzawa-Nagata N, Takeshita Y, Noda H, Matsumoto Y, Ota T, Nagano T, Nakagen M, Miyamoto K, Takatsuki K, Seo T, Iwayama K, Tokuyama K, Matsugo S, Tang H, Saito Y, Yamagoe S, Kaneko S, Takamura T. LECT2 functions as a hepatokine that links obesity to skeletal muscle insulin resistance. *Diabetes* 2014; **63**: 1649-1664 [PMID: 24478397 DOI: 10.2337/db13-0728]

18 **Kowalski A**, Cabrera J, Nasr S, Lerma E. Renal LECT2 amyloidosis: a newly described disorder gaining greater recognition. *Clin Nephrol* 2015; **84**: 236-240 [PMID: 26308079 DOI: 10.5414/CN108549]

80338_Auto_Edited-check.docx

ORIGINALITY REPORT

21%
SIMILARITY INDEX

PRIMARY SOURCES

1	coek.info Internet	136 words — 3%
2	www.frontiersin.org Internet	117 words — 3%
3	lib.smu.edu.cn Internet	83 words — 2%
4	www.nature.com Internet	76 words — 2%
5	www.ncbi.nlm.nih.gov Internet	67 words — 2%
6	www.spandidos-publications.com Internet	31 words — 1%
7	pure.rug.nl Internet	30 words — 1%
8	amb-express.springeropen.com Internet	29 words — 1%
9	Mingjia Xiao, Xiangjing Liang, Zhengming Yan, Jingyang Chen et al. "A DNA-Methylation-Driven Genes Based Prognostic Signature Reveals Immune	28 words — 1%

Microenvironment in Pancreatic Cancer", Frontiers in Immunology, 2022
Crossref

10 Yuan Lin, Meng - Qi Dong, Zhi - Min Liu, Meng Xu, Zhi - Hao Huang, Hong - Juan Liu, Yi Gao, Wei - Jie Zhou. "A New Strategy of Vascular - targeted Therapy for Liver Fibrosis", Hepatology, 2021 — 21 words — 1%
Crossref

11 immunenetwork.org — 20 words — < 1%
Internet

12 www.researchsquare.com — 19 words — < 1%
Internet

13 Meng Xu, Hong-Hai Xu, Yuan Lin, Xiangnan Sun et al. "LECT2, a Ligand for Tie1, Plays a Crucial Role in Liver Fibrogenesis", Cell, 2019 — 18 words — < 1%
Crossref

14 www.science.gov — 16 words — < 1%
Internet

15 id.nii.ac.jp — 15 words — < 1%
Internet

16 Muhammad I. Arshad. "NKT cells are required to induce high IL-33 expression in hepatocytes during ConA-induced acute hepatitis", European Journal of Immunology, 08/2011 — 14 words — < 1%
Crossref

17 Sungmi Jeon, Jeong Hyun Ha, Iljin Kim, Jiyoon Bae, Sang Wha Kim. "The Immunomodulatory Effect of Adipose-Derived Stem Cells in Xenograft Transplantation Model", Transplantation Proceedings, 2022 — 14 words — < 1%
Crossref

18	Md Sharif Reza, Md Jashimuddin, Jamiuddin Ahmed, Muhammad Abeer et al. "Pharmacological investigation of analgesic and antipyretic activities of methanol extract of the whole part of Aeginetia indica", Journal of Ethnopharmacology, 2021 Crossref	12 words — < 1%
19	Karlin Raja Karlmark, Hermann E Wasmuth, Christian Trautwein, Frank Tacke. "Chemokine-directed immune cell infiltration in acute and chronic liver disease", Expert Review of Gastroenterology & Hepatology, 2014 Crossref	11 words — < 1%
20	link.springer.com Internet	11 words — < 1%
21	"Posters (Abstracts 301-2389)", Hepatology, 2018 Crossref	10 words — < 1%
22	Akinori Okumura, Takeshi Saito, Isao Otani, Kazuo Kojima et al. "Suppressive role of leukocyte cell–derived chemotaxin 2 in mouse anti–type II collagen antibody–induced arthritis", Arthritis & Rheumatism, 2008 Crossref	10 words — < 1%
23	Chen, Y.. "Impaired function of hepatic natural killer cells from murine chronic HBsAg carriers", International Immunopharmacology, 200512 Crossref	10 words — < 1%
24	Mengxin Yang, Xinyue Zhang, Shuang Zhao, Ruyue Shao, Kerui Fan, Kai Hu, Li Zhang, Yongqiang Yang. "Protective effects of glutamine on lipopolysaccharide/D-galactosamine-induced fulminant hepatitis in mice", Experimental Biology and Medicine, 2022 Crossref	10 words — < 1%

25	Yanzhen Bi, Jiannan Li, Yonghong Yang, Quanyi Wang et al. "Human liver stem cells attenuate concanavalin A-induced acute liver injury by modulating myeloid-derived suppressor cells and CD4+ T cells in mice", Stem Cell Research & Therapy, 2019 Crossref	10 words — < 1%
26	aging-us.com Internet	10 words — < 1%
27	www.jstage.jst.go.jp Internet	10 words — < 1%

EXCLUDE QUOTES	ON	EXCLUDE SOURCES	< 10 WORDS
EXCLUDE BIBLIOGRAPHY	ON	EXCLUDE MATCHES	< 10 WORDS