Review Article

DOI: http://dx.doi.org/10.18203/2349-2902.isj20170844

Mechanism of Kupffer cells in hepatitis infected by hepatitis B and C virus

Yi-qing Tan¹, Xi-wen Zhu², Xing Lai², Jian-ping Gong², Qing-gang Yin³*

Received: 14 December 2016 **Accepted:** 16 January 2017

*Correspondence: Dr. Qing-gang Yin,

E-mail: iam.cool4586@aliyun.com

Copyright: © the author(s), publisher and licensee Medip Academy. This is an open-access article distributed under the terms of the Creative Commons Attribution Non-Commercial License, which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

ABSTRACT

The infection of hepatitis B virus or hepatitis C virus (HBV / HCV) is the most common cause of the chronic liver disease. Kupffer cells (KCs), the largest number of viscera macrophages, are located in the sinusoid of the hepar and play an extremely significant role in hepatic chronic inflammation after HBV / HCV infection. KCs could affect the secretion of cytokines and the interaction among cells via a variety of signalling pathways and they could regulate the inflammatory response and immune activities as well. The activation of KCs could balance inflammation and anti-inflammation, maintaining the stability of internal environment in vivo. Studies of KCs have the significance of the understanding of pathogenic mechanisms and the access to the treatment of HBV/HCV infection. Meanwhile, such studies might help to delay the development of fibrosis, cirrhosis and even carcinoma of liver after HBV / HCV infection.

Keywords: Infection, Inflammation, Immunity, Kupffer cell

INTRODUCTION

Kupffer cells (KCs) are located in the liver, and play an extremely important role as a kind of macrophage. The access to obtaining KCs for studies is a progress, while there are still problems reminded to be solved. The lack of specific receptors and markers makes it difficult to do further study. Recent researches show that KCs could regulate the inflammatory response and immune activities. Besides secreting cytokines, KCs might activate non-specific immune cells and inflammatory response as well. Whereas, KCs are considered to be involved in the tolerance of immune activities. Persistent inflammatory activities could cause the continuous activation of KCs, which might contribute to cells damage, fibrosis, and ultimately hepatocellular carcinoma (HCC). Upon those, researches on the mechanism of KCs in hepatitis B virus and hepatitis C virus infection would conduce to the treatment of these hepatitis, as well as slowing the evolution of fibrosis, cirrhosis and HCC, which makes the research of the distinctive receptors and markers a crucial role in the progress of serial study. This paper will discuss the characters of KCs, the isolation of KCs, the function of KCs in hepatitis B and hepatitis C virus infection, and the contribution of KCs to the fibrosis, cirrhosis and HCC.

CHARACTERISTICS OF KUPFFER CELLS

Kupffer cells (KCs) are the largest number of viscera macrophages, almost 80% of monocyte-macrophages in the liver. KCs are located in the sinusoid of liver, where is near the portahepatis and veins. KCs are exposed to the pathogens earlier than any other innate immune cells,

¹Department of Endocrine Surgery, The First Affiliated Hospital of Chongqing Medical University, Chongqing, China ²Department of Hepatobiliary Surgery, The Second Affiliated Hospital of Chongqing Medical University, Chongqing, China

³Department of Hepatobiliary and Thoracic Surgery, Tongliang District People 's Hospital, Chongqing, China

which are considered as the first barrier of immunity, together with the sinusoidal cells to defense the pathogens from the gut refluxing through the venous portal blood. KCs play special roles in scavenging and phagocytosing, maintaining the homeostasis in vivo by the tolerance of immunity, which could avoid the excessive activation of immune system.²

Phenotypes of KCs

On the condition that KCs have sharing phenotypes of monocyte-macrophages and marrow cells. identification of KCs is rough via immunohistochemistry and flow cytometry, especially in the steady state condition. Researchers indicated that KCs could be identified by the antibodies directly by CD14, CD16, and CD68, which are not only found in KCs or macrophages but also in dendritic cells. All of them, KCs, macrophages as well as dendritic cells, are deemed as monocytederived macrophages.3 Therefore, phenotypes available currently are non-specific. Besides the specific markers, it could be speculated that KCs might be identified by the morphology and phagocytic ability.

Function of KCs

Since KCs are settled in the hepatic sinusoid, they are deemed as the first barrier, and are exposed easily to pathogens from the gut. When hepatic portal vein recruits blood from mesenteric vein and liver, KCs could be activated by lipopolysaccharide (LPS) through the toll like receptor (TLR).⁴ To avoid damaging the liver on the condition of excessive inflammatory response, KCs are able to restrict the self-activation, playing a role in the immune tolerance with the secretion of Interleukin-10 (IL-10).⁵

Some studies suggested that KCs obtained from the liver tissue could release low level of TNF and IL-12p40 upon the stimulation with the agonist for TLR4 ex-vivo.^{4,5} However, KCs turned to secrete more cytokines like IL-12, and tumor necrosis factor (TNF) with the ligating of TLR4 and some other TLRs.⁶ The weak ability of KCs to produce cytokines might be related to the immunity tolerance in the homeostasis *in-vivo*.

KCs are also considered to play a role in the expansion and differentiation of liver progenitor cells (LPC) during chronic liver injury. LPCs can be activated and then differentiated into hepatocytes and cholangiocytes, leading to thegeneration of regenerative nodules and functional restoration, which are found usually in chronic and severe injury. But the mechanism of it haven't been stated clearly.

ISOLATION OF KCs

The source of liver materials is important for the analysis of KCs. The material is always obtained from the liver graft perfusate or the liver tissue. The liver graft perfusate

has its advantage of preservation over the liver tissue. Tissue-derived KCs are extracted by the collagenase, which causes much cellular debris and might change the function and phenotypes of KCs. There is an upgraded method to obtain KCs. The procedure includes the Collagenase perfusion in vivo, discontinuous density gradient centrifugation, and selective wall-pasting, which is proved to could obtain KCs with high biological-activity and the operation is easy at the same time.⁹

MECHANISM OF KCs IN HBV/HCV INFECTION

HBV is a double-stranded DNA virus replicating via RNA intermediates, while HCV is a positive-strand RNA retrovirus. ¹⁰ The infection of HBV or HCV is restricted by the macrophages with activation of the receptors inside or outside cells. KCs probably control the infection by the restricting the infection, secreting cytokines or maintaining the interaction among inflammatory cells. ^{10,11}

KCs regulate the infection of HBV and HCV

CD14+ is identified as the costimulatory molecules. Research suggested that HBsAg could interact with monocytes via CD14+-dependent receptors, and with dendritic cells via the mannose receptors. Receptors mentioned are both expressed on KCs.⁴ Meanwhile, HCV-E2 is able to bind to KC on the dependence of CD81 when KCs are incubated together with HCV-E2. The DC-SIG (dendritic cell-specific intercellular adhesion molecule- 3- grabbing non-intergrin), however, which can not be found in hepatocytes, is observed to promote HCV to bind on KCs.¹¹

HBV virus particles and HbsAg could induce nonparenchymal hepatocytes to produce some cytokines like TNF. And NF-kB are deemed to be involved in this procedure. Meanwhile, HCV core could simulate KCs and macrophages to produce pro-inflammatory mediators and the immunosuppressive factor. IL-10, Which is mentioned before as cytokines playing a role in immunity tolerance, is included. 11-13 Then replication of HBV in the hepatocytes is suppressed by TNF in a non-cytopathic method. IL-1\beta is a product of CD68 non-parenchymal hepatocytes. In the chronic HCV patients, IL-1β and **CD68** observed co-expression immunofluorescence. And the concentration of IL-1β in HCV petients is much higher than that in healthy people. 11,13,14 IL-1β is induced by inflammasome 3 (NLRP3) via Caspase-1. HBV could activate NLRP3 via Rac1, while T cell immunoglobulin and mucindomaincontaining molecule (TIM-4) inhibit the activation of NLRP3.4 As certain study shows, KCs with ED1+ expresse rather low level of IL-1β, IL-6 and TNF when exposed to HBV, but could produce imminoregulatory cyokines TGFβ instead.¹⁶ Meanwhile, NS3 could be recognized by KCs via TLR4 and the signaling is able to transmit into intracellular, contributing to the activation of NK-κB and promoting the release of TNF-α.¹⁷ However, TNF contributes to the greater permeability of hepatocytes. The fact that LPS could promote KCs to produce TNF could indirectly facilitates the HCV infenction of liver.⁶

KCs could kill the infected cells in various ways. KCs would activate the natural killer (NK) and natural killer T (NKT) by secreting cytokines. NK and NKT would produce cytokines like TNF, IFN-γ, chemotactic factors, even some kind of cytotoxins. The chemotactic factors and cytokines could affect the immune response of liver by infiltrating leukocytes which were recruited and activated already.² Research also indicated that KCs of the mouse could present antigens to CD4+ and CD8+ T cells, contributing to the produce of IFN-γ.¹⁸

Besides that, KCs, which are known as non-specific innate immune cells, are able to express cytotoxin molecules such as Fas-ligand, perforin, granzyme B, and ROS, facilitating the lysis of infected hepatocytes, even non-infected hepatocytes, which damages the liver furtherly. Besides the protective impact, the cytotoxins even damage more on the liver although it is originally produced to prevent the harm. ^{19,20} KCs do play an inverse role in the HBV / HCV infection. ²⁰

KCs regulate the immune in HBV / HCV infection

It is reported that HBV/HCV, which interferes TRL pathway, RIG-I signaling pathway and inflammatory reaction in hepatocytes and other immune cells, is able to inhibit the function of KCs. For instance, as for patients infected with HBV and found HBeAg-positive, TLR2 is much lower than it is in the HBeAg-negative individuals. IL-6, TNF, and IL-12, which could be induced by TLR2, were inhibited as well in a study that incubated human monocytes together with HBeAg or HBsAg. 20-22 IFN-y, TRAIL, and TLR2 all could be produced by KCs. 10,21 In the liver tissue obtained from chronic HCV patients, the cytokines produced by the macrophagocytes and the TLR induced by monocytes are suppressed by the HCV core protein. Moreover, HCV core protein could suppressed IFN-γ and TNF-related apoptosis inducing ligand(TRAIL) via TLR pathways. 20,21 Likewise, TLR2 signaling pathway is involved in the inhibitory action of HBeAg. The expression of TLR3 is much lower in the chronic HBV patients than the controlled-infection patients. After the antiviral theraby, the level of TLR3 can be promoted and almost restored.²³

HBV/HCV may influence the produce of immune-regulation cells and improve the tolerance of immune activities. In some study of mouse, it's suggested that the HCV core protein would cuase the higher product of IL-10, and IL-10 could help with immunity tolerance. Moreover, IL-10 is observed to be markedly higher not only in chronic HCV patients but also in chronic HBV patients. IL-10 could down-regulate the Major Histocompatibility Complex, MHC- II and co-stimulatory molecular, suppressing interaction between KC-NK and the presentation of antigen. In the HBV-infected mouse

model, KCs and IL-10 all play roles in the immune tolerance of antigen-specific towards HBsAg vaccination. In addition, some study claimed that HBV could make KCs produce TGF- β instead of proinflammatory factors, and TGF- β was reported to maintain the immune tolerance of autologous antigen. Is

In chronic HBV/HCV infection, programmed death 1(PD-1), cytotoxic T lymphocyte-associated antigen-4(CTLA-4), TIM-3, CD244 and other markers which could testify the differentiation grade of T cells, such as CCR7, can be identified on the virus specificity CD4+T cells. The level of PD-L1, PD-L2 and PD-1 are all higher in the chronic HBV / HCV infected patients in comparison with healthy individuals, and are considered to have relations to the severity of the infection.[28] And the continuous presentation of inhibitors such as PD-1 and TIM-3 might involve with the function and exhaustion of CD+8 T and CD+4 T.29 Nevertheless, the up-regulation of inhibitory receptors, which are based on the macrophages of the liver and the inflammatory activities involved with macrophages caused directly by the infection of HBV/HCV or by the mechanism of negative feedback after ongoing inflammation, still remains uncertain.

When the NLRP3 is activated by virus, there are various pathways to suppress both the activities of KCs and the inflammatory response via inhibition of the NLRP3 activity. The following is the main mechanism of the inhibition by virus: interference with the function of ASC and the inhibition of Caspase-1 activation, preventing IL-1 β via the suppression of NF- κ B activation and prevent IL-1 β directly.³⁰

The increasing expression of FasL and the growing level of ALT were reported to be observed in the chronic HBV infected patients. In addition, the granzyme B and perforin are all expressed in the chronic HBV/HCV patients. The product mentioned does harm to both infected cells and normal cells, making extra damage to the liver. As it is known that HBV/HCV cannot cause death of hepatocytes directly. The death of hepatocytes is the outcome of antivirus activities in-vivo.³¹

THE ROLE OF KCs IN THE FIBROSIS AND HCC

Hepatocellular carcinoma is the most common carcinoma of the liver, which is always secondary to the chronic inflammation and cirrhosis. KCs are considered as the promotor of fibrosis, cirrhosis and even the carcinoma. The persistent inflammation would damage liver via the apoptosis and necrosis of hepatocytes, which could continuously activate KCs and promote the produce of downstream cellular molecular consequently.

TNF-α, for insurance, would cause extra damages on the tissue. The increasing cellular molecular secreted by KCs, the recruited monocyte as well as neutrophil could lead to the compensatory proliferation of hepatocytes.¹⁸

KCs accelerate fibrosis

Abundant of fiber is produced to the tissue-repair. The degree and the duration of the damage would determine the scope of the fibrosis. As some study reported, KCs could produce various pro-fibrinogenic factors, like ROS, and some cytokines such as IL-6, TNF, IL-1, PDGF and TGF- β . In adittion, certain enzymes are produced by KCs, covering collagenase and metalloenzyme, which could accelerate the fibrosis via the disturbance of steady state in-vivo. Nevertheless, enzymes abovementioned are non-specific of KCs.

Function of KCs in different phase of carcinoma

As mentioned, the persistent inflammation would cause degeneration and necrosis of hepatocytes, activating cells nearby including KCs. ^{18,31} So the cytokines such as IL-6 and TNF, which are secreted by KCs, remain to be at a high level and cause the excessive inflammation as well as the compensative proliferation of residual hepatocytes to maintain the form and function of the liver. The cyclic process of hepatocytes death and multiplication could increase the chance of DNA spontaneity mutations and damages, which is considered as the highly risk factors of hepatocellular carcinoma. ³²

At the telophase of HCC process, a large number of new capillaries are found in the tumor, which is the consequence of vascular endothelial growth factor (VEGF) released by KCs. The growth of tumor would slow down on the condition of the macrophages consumption, along with the decrease of the new capillaries as well as the tumor metastasis to liver and peritoneum. That is considered as the indirect evidence that KCs expedite the process of carcinoma.³² In intravital fluorescence microscopy, KCs are attracted by the tumor cell, and phagocytize the tumor cells.

In addition, the NO produced by KCs has impact on the tumor together with KCs. A study in which KCs were depleted by gadolinium chloride showed that tumor burden would increase during the exponential growth phase, and decrease at the telophase of tumor growth, which indicates that KCs play an inhibitory role at the early phase and has a stimulatory affect at the later phase on the contrary.³³ But the mechanism is still uncertain.

SUMMARY

KCs play a crucial role in viral hepatitis. KCs could affect the liver in opposite ways. Besides the role as the scavenger to prevent the liver from damage, it does damage on the liver to some degree, which would promote the development of fibrosis and cirrhosis. The lack of specific markers, the overlapping characteristics and sharing functions with macrophages make it difficult to expound the mechanism, and are considered as obstacles to do further research and cure HBV/HCV infection. Controlling the activities of KCs is probably

the crux to control the infection of HBV/HCV. Upon the above, discovery of the specific receptors or markers is able to help with further studies of KCs.

ACKNOWLEDGEMENTS

Authors would like to thank the Second Affiliated Hospital of Chongqing Medical University for their support.

Funding: National Natural Science Foundation of China Conflict of interest: None declared Ethical approval: Not required

REFERENCES

- 1. Crispe IN. The liver as a lymphoid organ. Annu Rev Immunol. 2009;27:147-63.
- 2. Ningappa M, Kumar AC, Higgs BW, Sun Q, Jaffe R, Mazariegos G, et al. Immune surveillance by the liver. Nat Immunol. 2013;14:996-1006.
- Gregori S, Tomasoni D, Pacciani V, Scirpoli M, Battaglia M, Magnani CF, et al. Differentiation of type 1 T regulatory cells (Tr1) by tolerogenic DC-10 requires the IL-10-dependent ILT4/HLA-G pathway. Blood. 2010;116:935-44.
- Kinoshita M, Uchida T, Sato A, Nakashima M, Nakashima H, Shono S, et al. Characterization of two F4/80-positive Kupffer cell subsets by their function and phenotype in mice. J Hepatol. 2010;53:903-10.
- 5. Movita D, Kreefft K, Biesta P, Oudenaren A, Leenen PJ, Janssen HL, et al. Kupffer cells express a unique combination of phenotypic and functional characteristics compared with splenic and peritoneal macrophages. J Leukoc Biol. 2012;92:723-33.
- Fletcher NF, Sutaria R, Jo J, Barnes A, Blahova M, Meredith LW, et al. Activated macrophages promote hepatitis C virus entry in a tumor necrosis factor-dependent manner. Hepatology. 2013;59:1320-30.
- 7. Shang H, Wang Z, Song Y. Liver progenitor cells-mediated liver regeneration in liver cirrhosis. Hepatol Int. 2016;10(3):440-7.
- 8. Van HN, Lanthier N, Español SR, Quinones AJ, Rooijen VN, Leclercq I. Kupffer cells influence parenchymal invasion and phenotypic orientation, but not the proliferation, of liver progenitor cells in a murine model of liver injury. Am J Pathol. 2011;179(4):1839-50.
- 9. Li PZ, Li JZ, Li M, Gong JP, He K. An efficient method to isolate and culture mouse Kupffer cells. Immunol Lett. 2014;158(1):52-6.
- 10. Heydtmann M. Macrophages in hepatitis B and hepatitis C virus infections. J Virol. 2009;83:2796-802.
- 11. Tu Z, Pierce RH, Kurtis J, Kuroki Y, Crispe IN, Orloff MS. Hepatitis C virus core protein subverts the antiviral activities of human Kupffer cells. Gastroenterology. 2010;138:305-14.

- 12. Hösel M, Quasdorff M, Wiegmann K, Webb D, Zedler U, Broxtermann M, et al. Not interferon, but interleukin-6 controls early gene expression in hepatitis B virus infection. Hepatology. 2009;50:1773-82.
- Negash AA, Ramos HJ, Crochet N, Lau DT, Doehle B, Papic N, et al. IL-1β production through the NLRP3 inflammasome by hepatic macrophages links hepatitis C virus infection with liver inflammation and disease. Plos Patho. 2013;9:1003330.
- Shrivastava S, Mukherjee A, Ray R, Ray RB. Hepatitis C virus induces IL-1beta/IL-18 in circulatory and resident liver macrophages. J Virol. 2013;87:12284-90.
- Eitel J, Meixenberger K, Laak C, Orlovski C, Hocke A. Racl regulates the NLRP3 inflammasome which mediates IL-lbeta production in chlamydophila pneumoniae infected human mononuclear cells. PLoS One. 2012;10:1371.
- 16. Li H, Zheng HW, Chen H, Xing ZZ, You H, Cong M, et al. Hepatitis B virus particles preferably induce Kupffer cells to produce TGF-beta1 over pro-inflammatory cytokines. Dig Liver Dis. 2012;44:328-33.
- 17. Luan X1, Liu Y, Li M. The role of CD14 and Toll-Like receptor 4 of Kupffer cells in hepatic ischemia-reperfusion injury in rats. Transplant Proc. 2012;44:937-41.
- 18. Ebrahimkhani MR, Mohar I, Crispe IN. Cross-presentation of antigen by diverse subsets of murine liver cells. Hepatology. 2011;54:1379-87.
- Smith JS, Xu Z, Tian J, Stevenson SC, Byrnes AP. Interaction of systemically delivered adenovirus vectors with Kupffer cells in mouse liver. Hum Gene Ther. 2008;19:547-54.
- 20. Woltman AM, Boonstra A, Janssen HL. Dendritic cells in chronic viral hepatitis B and C: victims or guardian angels? Gut. 2010;59:115-25.
- 21. Chung H, Watanabe T, Kudo M, Chiba T. Hepatitis C virus core protein induces homotolerance and cross-tolerance to toll-like receptor ligands by activation of toll-like receptor. J Infect Dis. 2010;202:853-61.
- 22. Wang S, Chen Z, Hu C, Qian F, Cheng Y, Wu M, et al. Hepatitis B virus surface antigen selectively inhibits TLR2 ligand-induced IL-12 production in monocytes/macrophages by interfering with JNK activation. J Immunol. 2013;190:5142-51.
- 23. Huang YW, Lin SC, Wei SC, Hu JT, Chang HY, Huang SH, et al. Reduced toll-like receptor 3

- expression in chronic hepatitis B patients and its restoration by interferon therapy. Antivir Ther. 2013;18:877-84.
- 24. Thomson AW, Knolle PA. Antigen-presenting cell function in the tolerogenic liver environment. Nat Rev Immunol. 2010;10:753-66.
- Liu BS, Groothuismink ZM, Janssen HL, Boonstra A. Role for IL-10 in inducing functional impairment of monocytes upon TLR4 ligation in patients with chronic HCV infections. J Leukoc Biol. 2011;89:981-8.
- 26. Lian ZR, Xu YF, Wang XB, Gong JP, Liu ZJ. Suppression of histone deacetylase 11 promotes expression of IL-10 in Kupffer cells and induces tolerance following orthotopic liver transplantation in rats. J Surg Res. 2012;174:359-68.
- 27. Xu L, Yin W, Sun R, Wei H, Tian Z. Liver type I regulatory T cells suppress germinal center formation in HBV-tolerant mice. Proc Natl Acad Sci. 2013;110:16993-8.
- 28. Chen J, Wang XM, Wu XJ, Wang Y, Zhao H, Shen B, et al. Intrahepatic levels of PD-1/PD-L correlate with liver inflammation in chronic hepatitis B. Inflamm Res. 2011;60:47-53.
- Raziorrouh B, Heeg M, Kurktschiev P, Schraut W, Zachoval R, Wendtner C, et al. Inhibitory phenotype of hbv-specific cd4+ T-cells is characterized by high PD-1 expression but absent coregulation of multiple inhibitory molecules. PLoS One. 2014;9(8):105703.
- 30. Taxman DJ, Huang MT, Ting JP. Inflammasome inhibition as a pathogenic stealth mechanism. Cell Host Microbe. 2010;8:7-11.
- 31. Wu J, Li J, Salcedo R, Mivechi NF, Trinchieri G, Horuzsko A. The proinflammatory myeloid cell receptor TREM-1 controls Kupffer cell activation and development of hepatocellular carcinoma. Cancer Res. 2012;72:3977-86.
- 32. Arzumanyan A, Reis HM, Feitelson MA. Pathogenic mechanisms in HBV and HCV-associated hepatocellular carcinoma. Nat Rev Cancer. 2013;13:123-35.
- 33. Wen SW, Ager EI, Christophi C. Bimodal role of Kupffer cells during colorectal cancer liver metastasis. Cancer Biol Ther. 2013;14(7):606-13.

Cite this article as: Tan YQ, Zhu XW, Lai X, Gong JP, Yin QG. Mechanism of Kupffer cells in hepatitis infected by hepatitis B and C virus. Int Surg J 2017;4:856-60.