response letter

Answering reviewers

We would like to thank the reviewers for their thorough and insightful comments. These comments have allowed us to improve the manuscript significantly. The following revisions have been incorporated in response to the reviewers' comments (underlined):

Reviewer comments:

Reviewer #1 (Comments for Author): The manuscript "Mononuclear phagocyte system in HCV infection" by Yu Yang et al. discusses the findings published in several articles on the interaction of HCV and the mononuclear phagocyte system. The review includes the three cell types: monocytes, dendritic cells and macrophages, and their role in HCV infection. Also, it describes the function and impairment of these cells after HCV infection. The paper is well written and includes a large number of references. The discussion of the papers is well conducted and argued. The authors have performed appropriate comments.

1 **Most of the articles discussed are in vitro studies, which are the most abundant in the literature. But there are also several in vivo published studies, with cells or tissues from infected HCV patients. The results obtained from these last papers are more convincing. Therefore, it would improve the review if more in vivo studies were included. For example, studies such as the following could be included: Journal of Medical Virology 88:843–851 (2016) by Aintzane Zabaleta et al; Molecular Therapy — Methods & Clinical Development (2015) 2, 15006 by Aintzane Zabaleta et al.**

Reply: Thanks for the reviewer's good suggestion. The reference "Journal of Medical Virology 88:843–851 (2016) by Aintzane Zabaleta et al" suggested by the reviewer was cited in appropriate site. However, the reference "Molecular Therapy — Methods & Clinical Development (2015) 2, 15006 by Aintzane Zabaleta et al" isn't closely related to the scope of our review and we failed to cite it in appropriate site.

2 There is a wrong underline on page 6 - Typographical error in pag. 10, Type I INF must be replaced by Type I IFN.

Reply: The error has been corrected

3 The end of paragraph "Regulatory function of monocytes following HCV infection"

(pag 13, reference 107) would need a little more description.

Reply: As the reviewer required, we added more description of reference 107.

4 The format of the bibliography must be carefully reviewed. There are several errors in the references. For example, in some the DOI is missing, the city of the journal should not appear, etc.

Reply: The bibliography has been revised.

5 Table 1 should fit the text of the manuscript

Reply: We changed the name of table 1 to fit the text of the manuscript

Reviewer #2 (Comments for Author): The review by Yu Yang et al summarizes and discusses the literature on the mononuclear phagocyte system in HCV infection. The review discusses the effect of HCV on monocytes, Kupffer cells and dendritic cells in patients in detail and provides a good overview of current knowledge. The review is interesting, well-written and concise.

1. **referentie 30 and 31 on page 6 are not correct. These should be references to the original paper and not a review. Please check all references.**

Reply: The reference 30 is really an original paper, whereas reference 31 is a review and is replaced by "Tomiyama, C., et al., *Suppressive role of hepatic dendritic cells in concanavalin A-induced hepatitis.* Clin Exp Immunol, 2011. **166**(2): p. 258-68."

2. **The part on infectability of the MPS by HCV is the opinion of the authors and does not reflect the general belief by all scientists in the field. The authors should stress that this is their personal opinion, and not pretend like this is commonly accepted. I would suggest to remove this part completely, since it does not add to the message of the review.**

Reply: Thanks for the reviewer' s valuable suggestion. I deleted this part as the reviewer suggested

3. **Page 10 "We hypothesize that HCVc induces hyporesponsiveness, leading to the evasion of immunity in the early period of infection, whereas IFN-γ–induced loss of tolerance may contribute to inflammation and subsequent liver damage in chronic infection" . It is unclear to me what this statement is based on, and why the authors hypothesize this.**

Reply: Thanks for the reviewer' s good question. In our review, we introduced that monocytes from HCV-infected patients show upregulated TNF-α in an IFN-γ-dependent manner, highlighting their roles in inflammation. Additionally, HCVc can downregulate IL-6 production after stimulation with TLR2 and TLR4 ligands. Monocytes produce large amount of IL-10 in the early time of infection, leading to chronicity. HCVc induced low IL-6 production is likely the consequences of IL-10. Inflammation and subsequent liver damage are the characteristics of chronic infection. So, it is reasonable to hypothesize that "HCVc induces hyporesponsiveness, leading to the evasion of immunity in the early period of infection, whereas IFN-γ–induced loss of tolerance may contribute to inflammation and subsequent liver damage in chronic infection" .

4. The sentence on page 10 "The expression of TLR2 is significantly correlated with serum TNF-α and alanine transaminase (ALT) levels [76], indicating that the inflammation associated with HCV infection is partially attributed to production of proinflammatory cytokines in a TLR2-dependent manner." is pure speculation, and this should be stressed. Throughout the review there are many more of these kind of statement that over-interpret a finding in a manuscript. All of these statements should be checked and rephrased. For instance "IL-10, an anti-inflammatory cytokine, is primarily produced by monocytes. " and "IL-10 preferentially targets TLR4 signaling" . But also words that certain mechanisms are "vital" or "critical role" . So, please double check all.

Reply: The statement in our review that "The expression of TLR2 is significantly correlated with serum TNF-α and alanine transaminase (ALT) levels [76], indicating that the inflammation associated with HCV infection is partially attributed to production of proinflammatory cytokines in a TLR2-dependent manner." is not a speculation. This statement can be supported by reference "Riordan SM, Skinner NA, Kurtovic J, Locarnini S, McIver CJ, Williams R, Visvanathan K. Toll-like receptor expression in chronic hepatitis C: correlation with pro-inflammatory cytokine levels and liver injury. Inflammation research : official journal of the European Histamine Research Society [et al] 2006; 55(7): 279-285

[PMID: 16955390 DOI: 10.1007/s00011-006-0082-0]." And the conclusion of this reference is: " Up-regulation of peripheral blood monocyte expression of TLR2 and TLR4 occurs in patients with chronic hepatitis C. Increased monocyte expression of TLR2, but not of TLR4, correlates significantly with both increased circulating TNF-alpha levels and hepatic necroinflammatory activity in this disorder." And so, it is reasonable to lead to our statement.

The statement that "IL-10, an anti-inflammatory cytokine, is primarily produced by monocytes. " is also not over-interpreting. We have read many references in this scope. Compared with DCs and macrophages, monocytes are indeed reported to secrete large amounts of IL-10. And thus we gave rise to the statement. However, this statement may be misleading, because we leaved other immune cells, such as T cells and B cells, out of account. Hence, we replace the statement by "IL-10, an anti-inflammatory cytokine, can be produced by monocytes" .

The statement "IL-10 preferentially targets TLR4 signaling" is based on fact and is not exaggerated. The reference "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. Journal of leukocyte biology 2011; 89(6): 981-988 [PMID: 21385948 DOI: 10.1189/jlb.1210680]" showed that the stimulation of CHC patient-derived monocytes by lipopolysaccharide (LPS) (a TLR4 ligand) rather than R848 (a TLR8 agonist) lead to lower TNF-α and IL-12 production. And thus the inhibitory role of IL-10 against the production of proinflammatory cytokines was preferentially mediated by TLR4 signaling.

5 Page 13 "IL-18 and IL-36 inhibitory proteins, which can reduce NK cell activation" . IL-18 does not reduce NK cell activation

Reply: "IL-18 and IL-36 inhibitory proteins" in our review means that IL-18 and IL-36 inhibitory proteins are inhibitors of IL-18 and IL-36 rather than that IL-18 can inhibit NK cell activation. This expression may be misleading in some sense, however, it is the expression coming from the original paper.

6. Not all studies demonstrate that mDC and pDC frequency and function are impaired by HCV. All these studies should be discussed as well to give a balanced view.

Reply: Thanks for the reviewer' s good suggestion. We have extended this part just as the reviewer suggested.

7. The statement "Therefore, persistent viral infection and inflammation could be attributed to the decrease in IFN-α–producing" is too simplistic and not supported by data. Similarly, "Altered TLR signaling is the most probable cause for abnormal

cytokine production in HCV infection." Also this statement is not based on facts

Reply: The statement "Therefore, persistent viral infection and inflammation could be attributed to the decrease in IFN-α–producing pDCs and increase in proinflammatory cytokine-producing mDCs, respectively" is replaced by the statement "In summary, lower amount of IFN-α and lower levels of IL-10 can contribute to persistent viral infection and inflammation in HCV infection, respectively." , and we cited a reference additionally.

The statement "Altered TLR signaling is the most probable cause for abnormal cytokine production in HCV infection." is not a speculation and is supported by the "Effect of HCV on TLR signaling" part and "Impact of HCV on cytokine production from monocytes" part. We all know that TLR is one of the most important PRRs in immune cells. After activation, TLR signalings give rise to both inflammatory and antiinflammatory cytokines. It was widely reported that TLR signalings of monocyte are influenced by HCV. Combined with the abnormal production of both IL-10 and IL-12, it is logical to conclude that "Altered TLR signaling is the most probable cause for abnormal cytokine production in HCV infection."

8. "monocytes are the major contributors to the regulation of the immune system following HCV infection" Why is this not true for macrophages, and why are macrophages "tightly associated with HCV-induced inflammation and cirrhosis" , but not monocytes??

Reply: Monocyte is the precusor of macrophage. Monocyte circulates in blood whereas macrophage is tissue-resident cell. When the host is challenged, such as during infection and injury, macrophage detect the PAMP and/or DAMP to initiate the inflammatory response. When tissue-resident macrophages are not enough to deal with the challenges, monocytes are recruited from the blood to differentiate into macrophage and strengthen the firepower. As a matter of fact, the interface between monocyte and macrophage is elusive, for they share the same marker such as CD14. We can not exclude the contribution of monocyte to inflammation, however, monocyte contribute to inflammation by differentiating into macrophage. So in our review, we concluded that macrophages are tightly associated with HCV-induced inflammation and cirrhosis. On the other hand, monocyte circulates in blood and is convenient to contact with other immune cells. Just as described in "Regulatory function of monocytes following HCV infection" part, monocyte can regulate functions of NK cell and CD4 T cell. However, macrophage is the progeny of monocyte and definitely inherit some properties of monocyte, of course including immune regulatory function. Just like the macrophage in inflammation, monocyte is the major contributor to the regulation of the immune system following HCV infection.