Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 38968-Revised manuscript.docx).

Title: Ambiguous roles of innate lymphoid cells in chronic development of liver diseases

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Name of Journal: *World Journal of Gastroenterology*

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1. Reviewer 1 00004603:

This is an excellent review. It is clearly written and has the need message to researchers. My only suggestion is to add the paragraph about the methods to detect these ILCs and measure their activity: is it something that can be done exclusively in vivo/ex vivo or some in vitro approaches (as cell lines or purified primary cells) are available.

Reply:

Dear Reviewer 1:

Thank you for your kind remarks and constructive comments. The paragraph about the methods to detect ILCs and measure their activities has been added.

Firstly, isolation of intrahepatic as well as peripheral blood mononuclear cells to further detect ILCs according to the cluster of differentiation on the surface of different ILCs subsets and intracellular contents by flow cytometry *in vitro* is the most common methods applied in ILC research. Secondly, considering the limited accessibility of primary intrahepatic ILCs, the expansion of cell lines of primary intrahepatic ILCs is also an alternative to assess and monitor the activities and functions of ILCs. Thirdly, *in vivo* depletion of ILCs using specific antibodies or targeted transcription factor gene-deficient mice is also an important method to determine the functions of

ILCs in the liver which can be indicated by the severity of liver injury before and after the treatment of ConA or carbon tetrachloride. Last but not least, *in vivo* blockade of upstream cytokines or cell surface receptors via targeted gene-knock mice to distribute the signaling pathway of ILCs as well as adoptive transfer experiments of ILCs sorted by MACS/FACS have all been applied to investigate the role of ILCs in chronic liver diseases.

Please refer to the section '**CONCLUSIONS AND FUTURE PERSPECTIVES**' as highlighted on page 24-25 in the revised manuscript. The section has been expanded to include the following contents.

'Different methods have been applied to detect ILCs and measure their activities. Intrahepatic as well as peripheral blood mononuclear cells are isolated for further *in vitro* staining with fluorescence-labeled antibodies according to the cluster of differentiation on the surface of different ILC subsets and intracellular contents. Flow cytometry is further applied to detect the frequency and cellularity of ILCs and analyse the expression of their transcription factors and effector cytokines induced by PMA/ionomycin once they have been sorted *in vitro*. By observation of the differences of these factors between patients with chronic liver diseases and healthy control groups, their changes before and after the inducing factors and their consistency with liver injury, the researchers could validate the activities and functions of ILCs in the liver[23,38]. Considering limited accessibility of primary intrahepatic ILCs, the expansion of cell lines of primary intrahepatic ILCs is also an alternative to assess the function of this small cell population and to seek their secretion profile through the stimulation of PMA/ionomycin[96]. Besides, there have been studies exploring the roles of ILCs in initiation of liver injury including both hepatitis and liver fibrosis by the mechanism of *in vivo* depletion of ILCs using specific antibodies[22,73] or targeted transcription factor gene-deficient mice[72]. The protective or pathological roles of ILCs are determined by comparison of the severity of liver injury before and after the depletion of ILCs as indicated by histological analysis of liver tissue and

expression of liver injury serum biomarkers as well as inflammatory cytokines in *RAG1*$^{-/-}$ mice which are reconstituted with CD4^{+} T cells. *In vivo* experiments to distribute the signaling pathway of ILCs through the blockade of upstream cytokines and surface receptors of ILCs via targeted gene-knock mice are also important methods, in which the expansion of ILCs and their expression of transcription factors and downstream effector cytokines are further detected by flow cytometry and quantitative real-time PCR analysis[24,52]. Additionally, the activities of ILCs could also be monitored by transfer experiments, in which purified ILCs sorted by MACS/FACS are adoptively transferred into recipient mice before the challenge of stimulus including ConA and carbon tetrachloride to further investigate the function of ILCs in the liver[22,24,58].'

2. Reviewer 2 00053433:

This is an interesting review on innate lymphoid cells (ILCs), which are mononuclear hematopoietic cells involved in immunity and tissue remodeling. The topic is relevant since it is well known that dysregulation of those cells can lead to severe inflammation and injury in many organs. Authors review the existing knowledge on ILCs' characteristics and effects. In addition, potential roles of ILCs in physiopathological mechanisms underlying chronic liver diseases are explored, providing hypothetical therapeutic perspectives. The manuscript is well written and structured and contains a faire number of pertinent references on the subject.

Reply:

Dear Reviewer 2:

Thank you for your kind remarks.

Thank you again for your kind remarks and guidance. We look forward to contributing our manuscript to WJG.

Sincerely yours,

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