

Ze-Mao Gong

Scientific Editor, Editorial Office

Baishideng Publishing Group Inc

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4 March, 2016

Dear Professor Ze-Mao Gong,

We were grateful for the excellent comments of the reviewers on our manuscript PONE-D-13-48674 titled “**Restored NKG2D expression, altered cytokine production and increased cytotoxicity by NK cells in patients with chronic HCV infection with persistently normal ALT**”.

We made a minor revision of our article considering all the suggestions of the reviewers.

The following correction was made:

Scientific Editor’s comments:

Comment 1. *“Title should not appear abbreviation, please correct it, also should not be more than 12 words and including no less than 2 key words.”*

According to the suggestion we altered the title to “Immunological changes in different patient populations with chronic Hepatitis C virus infection”

Comment 2. *“Please add postcode”*

We inserted the postcode as suggested.

Comment 3. As suggested we changed “Ethical considerations” to “Institutional review board statement”

Comment 4. We inserted the Telephone and Fax number as suggested.

Comment 5. *“AIM: No more than 20 words, and start with "To..."”*

We corrected and shortened the AIM part in the abstract as suggested. “To better characterize the immune defects underlying chronic viral persistence we focus our analysis on NK, NKT-like and CD8+T cells.”

Comment 6. *“Abbreviations and acronyms are often defined the first time they are used within the main text and then used throughout the remainder of the manuscript. Please consider adhering to this convention.”*

According to the suggestion we altered the text.

Comment 7. As suggested we inserted the COMMENTS section with Background, Research frontiers, Innovations and breakthrough, Applications, Terminology and Peer-review

Comment 8. “References”

We used Mendeley and applied the style for World Journal of Gastroenterology.

Reviewer #1 (Reviewer’s code: 00012216)

Comment 1. *“In the description of quantitative variables would be more representative of the sample to give the mean plus the standard deviation instead of the SEM, and it would be even better to use non parametric data such as median plus interquartile range.”*

According to the suggestion of the reviewer we altered the tables by adding standard deviation instead of standard error.

Comment 2. *“When authors analyse CD8+ and CD4+ cells they should state if they label PBMC with CD3 as well, since NK also express CD8 and macrophages do also express CD4.”*

During our measurements we used anti-CD3 antibody as well together with CD4 and CD8 labeling and altered the text according to this comment.

Comment 3. *“Therefore, to analyse in HCV specific CTLs these phenotypic differences observed in total CD8+/CD3+ cells could add impact to the results.”*

Comment 4. *“Authors should show representative examples of the FACS dot-plots obtained, to illustrate the phenotypical differences observed between chronic and PNAL groups.”*

As suggested, we included a figure (Figure 1.) with dot-plots to illustrate the differences.

Reviewer #2 (Reviewer’s code: 00036624)

Reviewer #3 (Reviewer’s code: 00504882)

Comment 1. *“All the abbreviations should be expanded for the first time stated in the text.”*

According to the suggestion we altered the text.

Comment 2. *“ALT is alanine aminotransferase (not aminotransferase as mentioned in the introduction)”*

We corrected the sentence in Introduction as suggested: “aminotransferase” to “alanine aminotransferase”

Comment 3. *“anti-HCV should be indicated as “anti-HCV antibody”.”*

According to the suggestion of the reviewer we corrected “anti-HCV” to “anti-HCV antibody”.

Comment 4. *“Page 9:, 2nd para “Than we determined NKG2D, CD160 and KIR2DL3 expression by NK cells by flow cytometry”. This sentence should be restructured.”*

According to the suggestion of the reviewer we corrected the sentence to “NKG2D, CD160 and KIR2DL3 expression by NK cells was determined by flow cytometry.”

Comment 5. *“Page 11, line 3: “Expression of NKG2C was significantly increased on NK cells of patients with chronic HCV infection compared to healthy controls (Fig. 1g)”. This statement is ambiguous and in contrast to the conclusion. It should be stated as; “Expression of NKG2C was higher in NK cells from patients with chronic HCV infection as compared to healthy controls but significantly lower than HCV carriers (Fig. 1g).”*

As suggested we corrected the sentence to “Expression of NKG2C was higher in NK cells from patients with chronic HCV infection as compared to healthy controls but significantly lower than HCV carriers (Fig. 2g).”

Comment 6. *“Fig. 1 has several panels but not organized properly, and their description is missing in the legend. Figure legends should walk through the figure. Each of the three rows has same ‘healthy control, chronic HCV with elevated ALT and HCV carriers with PNALT’ labeling. This could be a single label for all the three rows at the bottom panel only. Similarly other figures should also be organized.”*

According to the suggestion of the reviewer we organized the figures and inserted the missing description in the legends.

Comment 7. *“Figure 5 panel a, b and c are confusing. Each panel should have its subtitle in the figure legend. For example (a) Cytotoxicity of NK cells isolated from healthy, HCV carrier and Chronic HepC patients. (b) Cytotoxicity of TGFbeta-treated NK cells isolated from healthy individuals. (c) Expression of NKG2D, KIR2DL3 and CD160 receptors by TGF beta-treated NK cells In Fig 5, the number of patients is indicated. Does it mean that NK cells from these individuals were mixed and then used in the experiment? If this is so, then it is unacceptable.”*

As suggested we inserted the subtitles in the figure legend. Also indicated the number of the patients on Fig.6c. NK cells from each individual were separately analyzed during our experiments not mixed.

We hope that the revised manuscript has now been sufficiently improved to merit publication.

Sincerely,

Laszlo Szereday

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Roberto J Firpi

Editor-in-Chief (Associate Editor)

Baishideng Publishing Group Inc

5 April, 2016

Dear Professor Roberto J Firpi,

We were grateful for the excellent comments on our manuscript PONE-D-13-48674 titled **“Immunological changes in different patient populations with chronic hepatitis C virus infection”**.

We made a minor revision of our article considering all the suggestions.

The following correction was made:

Comment 1. *“Your AIM is not clear. To better characterize the immune defects...we focus. Delete “we focus”. It should read “To better characters natural killer cells, NKT-likeetc”.*”

According to the suggestion we altered the Aim section to “The aim of our study was to investigate the possible role of natural killer, NKT-like and CD8+ T cells during the immunological changes in HCV infection.”

Comment 2. *“You have too many abbreviations in the abstract. Abbreviations should be avoided if possible in the abstract.”*

We deleted the unnecessary abbreviations.

Comment 3. *“In Introduction, eliminate the parenthesis after “A minority...” so you should say “Approximately 20% of HCV...”*”

We corrected the sentence as suggested.

Comment 4. *“The definition of HCV carriers is really a controversial one. You can have chronic HCV with normal ALT with advance disease or even cirrhosis. You don't have liver biopsies on these patients, only a few fibroscan that may help, but not the best.”*

Due to ethical considerations we cannot get liver biopsy samples from HCV carriers, that is why we used the following classification and excluded patients with Fibroscan result suggesting > F1 liver fibrosis (Ls> 7.0 kPa) “Persistently normal ALT was defined as ALT < 30 IU/L in men, ALT < 19 IU/L in women measured every 3 mo over an 18-mo period.”

Comment 5. *“Be consistent in the manuscript. If are using the abbreviation HCV for chronic HCV, then you have to use it all the time”*

We used the abbreviation CHC for “chronic HCV” infection and HCV for “hepatitis c virus”.

Comment 6. *“In Material and Methods: The first sentence is really a description of your population that will go in your results not here”*

According to the suggestion we moved the description of our patient population part to the Result section.

Comment 7. *“In Results, you need a description of your population first.”*

We inserted the description of our population first.

Comment 8. *“In Discussion, your 1st paragraph should be a description of your findings or results, the 2nd a comparison with other papers and what your results mean. The 3rd should be your limitations and your last the conclusions”*

We altered the text as suggested and inserted a new paragraph dealing with the limitations.

We hope that the revised manuscript has now been sufficiently improved to merit publication.

Sincerely,

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