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December 10th, 2014

Dear Editor in Chief,

Please find enclosed the edited manuscript 15278 in Word format (file name: 15278-edited.docx).

Title: HCV-specific cytotoxic T cell response restoration after treatment-induced HCV control,

Authors: Juan Ramón Larrubia, Elia Moreno-Cubero, Joaquín Miquel, and Eduardo Sanz-de-Villalobos,

Name of Journal: *World Journal of Gastroenterology*,

ESPS Manuscript NO: 15278,

to be considered for publication in *World Journal of Gastroenterology*, after receiving the kind invitation from the WJG Editor in Chief to carry-out a review for this journal.

The manuscript has been improved according to the suggestions of reviewers and Editors:

1.- According to the Editor's comments: the title has been improved to make it more specific and informative, the citation number format has been modified according to WJG publishing rules and finally, the figure format has been changed into ptx, in order to allow the figure editing.

2.- Revision has been made according to the suggestions of the reviewers and the changes have been highlighted in yellow in the main text:

(1) According to **00504141** reviewer's comments the following comments/changes has been made:

- The review is well written although there are a few places which need sentences to be rewritten.

Answer: English style has been polished, in spite of the fact that, according to the three reviewers the language evaluation has been A (priority publishing), B (minor language polishing) and B (minor language polishing).

- The sectional structure is unusual, i.e., the authors need to introduce paragraphs into the review.

Answer: to our knowledge, we have followed WJG format and according to it, paragraphs are not allowed into the same sub-heading. In case the editors accept the inclusion of paragraphs in a text belonging to a sub-heading, we would be more than happy to do that.

- The authors on page 9 suggest that HCV specific CTL responses at 12/52 of treatment has a 100% PPV. The authors should comment on the practicalities of performing CTL responses and perhaps how this could be maneuvered into the clinical management of HCV.

Answer: We think that this information is already given in the main text at the following sentence, which has been modified to make it clearer:

“This data could be useful to encourage these patients to finish treatment, but it could also be a decision rule to maintain double therapy in cases with positive cells or to add a DAA in patients without detectable HCV-specific CTLs at w12, which could have interest from an economical point of view in countries restricting the free use of DAA”.

- The authors mention and reference in Figure 2 that occasionally they can detect HCV 5'UTR in sera from some SVR patients several months after the EOT. The COBAS AMPLICOR HCV assay has been superseded by a TAQMAN based assay with a notable improvement in the LOD to ~10-15 IU per mL with a 95% CI. I wonder if these sera were tested with these more sensitive diagnostic assays would the previously mentioned non-detectable "SRV" patients, still remain SVR!

Answer: These samples were checked few years ago using a PCR test with a 40 IU/ml LOD. Nevertheless, those patients have been followed up for several years, and tested again using TAQMAN based assay and they remained SVR. Unfortunately, we do not have frozen sample left to repeat those experiments at that time point with this more sensitive PCR technique. In any case, if HCV viral load was lower than 40 IU/ml or negative in those SVR patients at that moment is not a big issue in my opinion, because since a clinical point of view they remained as infection resolvers during follow-up. The point is that after a successful treatment, other and we have been able to detect viral traces that correlates with HCV-specific CTL response, which could suggest the need of this response for a complete and sustainable viral clearance (Gastroenterology: 2011; 676-685).

- The authors should support their UTR findings by amplification of another region of the genome and publishing the resultant genome sequences. Ultralow copy PCR is more prone to difficulties of interpretation than high titre samples. By supporting the UTR findings with additional genomic information, the authors would provide enhanced evidence that their findings are robust.

Answer: we thank the reviewer for this suggestion and we will perform this analysis in future studies. Unfortunately, we do not keep frozen samples of these cases to carry-out this analysis now, in order to add this information to the review.

- Figure 4 needs a more expansive legend.

Answer: following reviewer advice, we have increased the information encompassed in figure 4 legend.

(2) According to 00181536 reviewer's comments the following comments/changes has been made:

- In 'IS IT NECESSARY TO RESTORE THE HCV-SPECIFIC CTL RESPONSE TO OBTAIN A SUSTAINED VIRAL RESPONSE?', the authors mentioned many evidences of HCV detection even in sustained viral responders to interferon based treatment. However, in clinical, most of the

patients show sustained free of serum viremia with usual PCR assay and sustained clinical remission that are different from HBV. This part should have overestimated the HCV positive results in experimental assays. The authors are recommended to mention that usual sustained viral responders show clinical remission and the meaning is different from HBV reactivation.

Answer: Following reviewer's advice, we have clarified this issue by adding this sentence.- "In fact, during clinical practice most of the HCV patients who show sustained undetectable viraemia with usual PCR assay develop a permanent clinical control, while in HBV infection is more common the virus reactivation under immune-suppressive conditions. Nevertheless, this does not mandatory mean that after SVR, HCV is completely cleared by interferon/ribavirin treatment..."

- The function of HCV proteins such as NS3/4A protease to interfere the innate immune system such as RIG-I pathway should be added to explain the anti-viral immune system defect in chronic hepatitis C (such as Meylan E, et al. Nature 437).

Answer: we disagree in this point with the reviewer's opinion. There are many components of the innate and adaptive immune response impaired during persistent HCV infection (MU. Lokhande, J Miquel, S Benito, JR Larrubia (2011). HBV & HCV Immunopathogenesis, Viral Hepatitis - Selected Issues of Pathogenesis and Diagnostics, Dr. Sergey Mukomolov (Ed.), ISBN: 978-953-307-760-4, InTech, DOI: 10.5772/25832). Nevertheless, our review focuses specifically on a particular element of the adaptive immune response, which is the role of anti-HCV treatment in HCV-specific CTL response. Therefore, we think adding information about other impaired elements of the immune response, such as innate response, could distract the reader attention. We have only included information about innate response regarding NK cells, since a restoration of their function during IFN-based treatment has been reported to impact on HCV viral load and consequently, this could help in HCV-specific CTL response restoration.

(3) According to 00225318 reviewer's comments the following comments/changes has been made:

- The authors should add that this type of analysis would be useful for tracking those patients with situations of immunosuppression that having achieved sustained virologic response who will be in risk of recurrence until his immune system is unable to activate appropriately, as has been observed in liver transplant patients or in cancer treatment.

Answer: Following reviewer's advice, we have included this information by adding this sentence.- "Thus, checking the quality of the HCV-specific CTL-response would be useful for tracking those patients who develop immunosuppression soon after developing SVR and consequently, could be at risk of recurrence until their immune system is restored^[38, 39]."

- The paragraph on page 4: "In our group, we have also been able to amplify HCV 5'-untranslated region (UTR) RNA by an in-house nested RT-PCR in sera from some SVR patients several months after end of treatment, even though they had been classified as SVR based on undetectable plasma HCV RNA by Cobas Amplicor HCV test (Not published data, Fig.-2) "must be removed because it is a not contrasted or reviewed result and is not necessary for the right argument of the bearer data from the literature

Answer: we agree with the reviewer that the shown experiments have not been peer reviewed and this decrease the value of them. Nevertheless, we have used the same methodology than Veerapu NS, et al in their work reported at Gastroenterology 2011; 140: 676-685. We think that, as a way to illustrate the idea of having HCV traces after treatment that should be eliminated by the immune system, is a good point to add these experimental data but attesting they have not been published previously. We think this is a better option than not reporting them. We also agree with the reviewer that to sustain our hypothesis is not necessary to show these experiments, since the data from published studies are enough to enforce our discussion, but this figure can increase the reader interest on this review. In any case, if the WJG Editors

consider that the figure 2 and the text referred to it should be removed, we will be more than happy to do that. In that case, the figures left should be re-numbered.

Thank you again for considering our manuscript for publishing in the *World Journal of Gastroenterology*.

Sincerely yours,

A handwritten signature in black ink, appearing to read 'Juan R Larrubia', with a long horizontal flourish extending to the right.

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January 28th, 2015

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for publication in *World Journal of Gastroenterology*, after receiving the kind invitation from the WJG Editor in Chief to carry-out a review for this journal.

The manuscript has been improved according to the suggestion of the Editor-in-Chief:

The review is well written and will be published once Fig. 2 is removed as recommended by one of the reviewers.

Answer: we agree with the reviewer and the Editor-in-Chief that the experiments shown in Figure 2 have not been peer reviewed and this decrease the value of them. For this reason, the figure 2 and the text referred to it have been removed and the figures left has been re-numbered.

Thank you again for publishing our manuscript in the *World Journal of Gastroenterology*.

Sincerely yours,

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