ESPS manuscript NO: 19573

Rebuttal letter

Dear Editor

We thanks for considering our manuscript entitled "Anti-rods/rings autoantibody generation in hepatitis C patients during interferon-α/ribavirin therapy" for publication in the World Journal of Gastroenterology.

Reviewers' comments have been carefully considered and the manuscript revised accordingly.

A point-by-point answer to the editor's/reviewer's comments is provided below.

We appreciate the Journal's efforts in handling the manuscript.

Sincerely
All Authors

EDITOR COMMENTS/QUERIES:

1- Please provide language a certificate letter from a professional English language editing company.
Reply: One of the authors, S. John Calise, is a native English speaker from Florida, US. He carefully reviews the entire manuscript to improve the language editing. We hope that this can meet level A for language quality.

2- Please read the core tip then provide the audio core tip:
Reply: The audio core tip was recorded and provided.

3- Please add PubMed citation numbers and DOI citation to the reference list and list all authors.
Reply: We revise the reference list according to the Journal criteria, and provide the PMID and/or DOI when available for each reference. We did not found the DOI for some references, however the PMID is provided for all of them.

4- For the figures, the fonts and lines can be edited or moved. It can be made by ppt.
Reply: The Figures will be supply as ppt independent files.

REVIEWERS COMMENTS/QUERIES:

Reviewer 1 code: 00225318

1- The review "Anti-rods / rings autoantibody generation in hepatitis C patients during interferon-α / ribavirin therapy" of Keppeke et al. It is very interesting and it is well documented with respect to the proposed title and objective. However, at present the treatment of infection by the hepatitis C virus (HCV) with interferon free therapies (direct antivirals) it is increasingly widespread and strongly recommended by international societies. By this reason a review about the autoimmune pattern of "rods and rings" cannot exclude this type of treatment and discuss their possible effect on this curious autoimmune pattern. Therefore, the main limitation of this review is the complete absence of comments regarding the new and expected major treatments of HVC infection without interferon but including Ribavirin. Authors should explore these cases and possible studies of the presence of "rods and rings" in patients treated in interferon free therapies. The fact that this type of autoimmune response observed in patients treated with interferon and ribavirin cannot exclude the role of interferon in the phenomenon. Therefore, it is essential discuss this type of treatments "without interferon".
Reply: We appreciate the reviewer's comment; however, data with new anti-viral treatment for HCV, without interferon, but still including ribavirin, is not yet available. We have planned such investigation but the results are not yet available.
However, we do have some preliminary information on the potential role of interferon on induction of anti-RR antibodies. After an extensive search, we have recovered samples from 10 patients treated only with ribavirin due to IFN side effects in our clinic, and none presented anti-RR autoantibodies, indeed supporting the potential importance of IFN as coadjutant in the induction of anti-RR autoantibodies.

2- When the hypothesis that ribavirin may act as inhibitor of the viral polymerase is discussed, the mechanism commented, although feasible, should be noted that the Ribavirin is a possible "indirect inhibitor" of the viral polymerase raised on its competitive inhibition of inosine-5'-monophosphate dehydrogenase 2 (IMPDH2). It should be also commented as possible antiviral action mechanisms the "hipermutagenic" activity of Ribavirin due to its ability to hybridize with both purines and pyrimidines.
Reply: We have included a new sentence discussing the viral polymerase inhibition and hypermutagenic activity of ribavirin. Please, refer to the second paragraph of 'Introduction.'

3- With regard to the possible mechanism of autoimmune response associated with infection by the hepatitis C virus (HCV) (page 5): Do the authors refer to the possibility that the interaction of HCV E2 protein with CD81 on the surface of B lymphocytes action emulated BAFF? This point should be clarified.
Reply: We have rewritten the sentence to make it clear.

"CD81 on the surface of B-lymphocytes is a natural ligand for HCV envelope 2 (E2) protein. B lymphocyte-specific protein CD21, a receptor for the complement C3d fragment, is closely related to CD81. The B cell threshold for polyclonal activation is lowered considerably when HCV E2 coated by C3d engages CD81 and CD21, favoring misleading B cell activation against autoantigens. In addition, the B lymphocyte activating factor (BAFF) is up regulated during HCV infection. BAFF binds CD19, a transducer of activation signal into the cell, adding towards the production of autoantibodies and cryoglobulins".

4- In page 5 paragraph: "despite occurring in high titers, anti-RR autoantibodies have yet to be linked with demographic, clinical, or Virological features [18, 20-22]" should be rewritten as: "despite occurring in high titers, anti-RR autoantibodies have not yet been clearly linked with demographic, clinical, or virological features [18, 20-22].
Reply: We have adjusted the sentence as suggested by the reviewer.

Reviewer 2 code: 00053556

1- Conflict of interest is missing.
Reply: We have included the statement that there is no conflict of interest.

2- ABSTRACT. Gives a clear delineation of the research background, including important data and conclusions; however, the following are better to be considered:

a) The aim of the work is better to be clearly identified at the beginning of the abstract.
Reply: We have included a statement on the aim of the article in the abstract.

b) IMPDH2 has to be fully written, when mentioned for the first time.
Reply: The full name is now given.

c) Conclusion has to be more concise and clearly identified.
Reply: We have reduced and revised the conclusion in the abstract.

3- INTRODUCTION. Provides sufficient background regarding the studied topic, however, the following points have to be addressed:

a) First paragraph, last sentence: The last sentence has to be revised and Ref. 2 is better to be updated. The virus replication in liver cells is not cytolytic, but it causes hepatitis secondary to antiviral immunopathology and inflammation of hepatocytes.
Reply: We have revised the sentence and updated the reference.

b) The aim of the work is not clearly identified.
Reply: We have included a new paragraph at the end of 'Introduction' describing the aim of the review.

4- TEXT. The section is almost well organized; an overall theoretical analysis

concerning the provided data is well covered to great extent, however and in order to satisfy the reader, the followings points have to be considered:

a) HCV patient treatment induces autoantibodies against RR structures: "This difference between the studies may be related to the origin of the cohorts studied, since Covini et al. and Novembrino et al. studied Italian patients whereas we studied Brazilian patients." The provided explanation is not convincing enough.
Reply: We appreciate the comment from the reviewer; unfortunately, we do not have a more convincing explanation at this moment. We included a more accurate information in the sentence about the sustained virological response (SVR) rates, since this could influence statistical analysis. In the Italian cohorts, SVR is about 60%, in the Brazilian cohort is about 30%. Once more reports from different cohorts are published, this point may be better clarified.

b) RR structures: The subheading is better to be RR structures and functions to cope with what have been actually discussed
Reply: Revised as recommended.

c) Subheadings: RR structures & Aggregation of IMPDH2 versus CTPS: The scientific content under these two subheadings are purely related to biochemistry and does not correspond to the journal's aims and scope. In order not to be boring to the reader, it has to be summarized in more plausible way.
Reply: This part of the section has been re-written to make it more concise and reduced by 30-40% in each subheading section.

5- Figure 1: The source of the provided assay is missing and has to be mentioned.
Reply: We have included the information in the Figure Legend.

6- Table 1: The number of the cited reference is better to be added.
Reply: Revised.

7- References: Finally relevant adequate references, especially the most current literatures were cited (30/59 references were cited from publications ≥ 2010). The journal style for writing names of authors has to be followed for Ref.No:12. PMID is not maintained for all references.
Reply: We have revised the references to include all the information as suggested.

Reviewer 3 code: 00503590

1- IMPDH2 should be defined in the abstract
Reply: The full name is now given.

2- Permission from Eurimmun for re-publication of figure 1 should be stated in the caption.

Reply: All data presented in Figure 1 was obtained in our own laboratory, with experiments performed by Keppeke GD. We have included the information in the Figure Legend.

3- Does the RR phenomenon occur in hepatocytes of the HCV patients, or is it solely an in vitro event? Authors present no data to this end, but I suggest this could be an area for future study.
Reply: We appreciate the reviewer comment. Indeed unpublished observations from our laboratory show RR structures in hepatocytes of ribavirin-treated mice. We include this information in the second paragraph of the section "The clinical relevance of anti-RR antibody".

4- Introduction, first sentence in second section: The term "decades" is a bit misleading as the introduction studies for IFN/Ribavirin therapy were published in the period 2001-2004.
Reply: We have adjusted the sentence as suggested by the reviewer.

5- Introduction, third sentence in the second section: The primary IFN A response is a non-specific antiviral response inducing apoptosis in infected cells, inhibition of viral replication and induction of NK killing of infected cells. These are general innate mechanisms and not related to HCV specifically.
Reply: We have corrected the term as suggested by the reviewer.

6- Authors suggest the RR phenomenon as a model for autoimmunity. A few suggestions as to which aspects should be studied in future studies would be nice.
Reply: We have included a sentence at the end of the manuscript with suggestions for future studies.