ANSWERING REVIEWERS

May 9, 2014

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 6843-review_.docx).

Title: Hepatitis D virus infection, replication and cross-talk with the hepatitis B virus
Author: Chi-Ruei Huang and Szecheng John Lo
Name of Journal: *World Journal of Gastroenterology*
ESPS Manuscript NO: 6843

Thank you and your colleagues for re-reviewing a submitted review paper entitled "hepatitis D virus infection, replication and cross-talk with the hepatitis B virus" to *World Journal of Gastroenterology* (ESPS Manuscript NO: 6843) and giving excellent comments and suggestions.

We have gone through the entire revision of manuscript according two reviewers' comments and suggestions. The current form of manuscript has been critically read and edited by a visiting professor Michael J. Leibowitz who is a professor of Medical Microbiology and Immunology at University of California, Davis campus.

The point-to point responses to two reviewers are shown in the following pages. I hope the revised manuscript is now acceptable by *World Journal of Gastroenterology*.

Sincerely yours,

Szecheng J. Lo, Ph.D.
Professor and Chairman
Department of Biomedical Sciences
Chang Gung University

Point-to-point responses:

#Reviewer 1

This review addresses interesting questions of the interaction between HBV and its satellite virus HDV. However, there is a need for full grammar and spelling corrections. Besides this, a number of imprecisions and incorrections are present throughout the manuscript. Some examples include:

Lane 26: "Hepatitis D virus (HDV) is mandatory to either co-infect or post-infect with hepatitis B virus (HBV) because HDV contains a small size of RNA (about 1.7 kb) with a single open reading frame (ORF)…" is not correct. There is no direct relationship between the size of the HDV genome and the interaction between HBV and HDV.

Responses: We apologize for English grammar and spelling. The new version of manuscript has been edited by a professional English editing company. The original description on lane 26 was changed into two sentences. The "because" was removed.

Lane 48: "Approximately, 350 million people are HBV carriers, among them, 15 to 20 million people are infected by HDV, which remains a major world-wide public health problem". This is not correct. HDV infection is not a worldwide problem. HDV is endemic in some regions of the globe but is almost eradicated in countries where vaccines against HBV are routinely administered to the population.

Responses: Thanks for the reviewer's comments. We added more information in HDV infection to indicate the divergent infection rate ranging from 0% to 84.9%. We also added a decline of prevalence of HDV infection in those countries conducted HBV vaccination.

Lanes 60-62: Since this is a review on the cross-talk between HBV and HDV I believe that a more detailed discussion on the relationship between different virus genotypes and clinical outcome would improve the manuscript and be of interest to the readers.

Responses: Thanks for the reviewer's suggestion. We added two more paragraphs regarding the relationship between different HDV genotypes and clinical outcome. We also discussed whether the combination of HBV-HDV genotypes plays a role in HDV-induced liver diseases.

Lane 65: "As all viruses that enter the host cell are receptor-mediated…". This is redundancy. All viruses enter the host cell; otherwise they would be no viruses but something else.

Responses: We thank the reviewer's comment and deleted the sentence.

Lane 97: "The trans-activating role of SDAg in HDV replication has been demonstrated that SDAg has an ability to recruit the host's DNA-dependent RNA polymerases to drive the replication of HDV…" Although there is evidence for a role of SHDAg in the accumulation of virus RNA species, it is unclear and controversial which exact role(s) it plays. It is not proven that SHDAg recruits host DNA-dependent RNA polymerases. Moreover, it has not been proved that different polymerases participate in HDV RNA synthesis. These issues should be discussed by the authors.

Responses: Thanks for the reviewer's comments. We rephrased into "Three species of host RNAPI, II, and III have been demonstrated to interact with the HDV genome,[66, 72] but the role of RNAPI and III in HDV replication remains undetermined, except for that of RNAPII.[63, 69-71] The role of RNAPII in HDV replication involves the de novo synthesis of HDV genomic RNA and HDAg-coding mRNA.[63, 69] Whether RNAPI plays a role in HDV replication is debatable. The studies conducted by Lai and his colleagues demonstrated that newly synthesized HDV antigenomic RNA is present in the treatment of a specific inhibitor of RNAPII, alpha-amanitin, in contrast to the de novo synthesis of HDV antigenomic RNA, which decreases after the treatment of actinomycin D, an inhibitor of RNAPI.[63, 70] However, Taylor and coworkers provided evidence suggesting that RNAPII is the only host RNAP that is required for the amplification of both HDV genomic and antigenomic RNA.[69, 71] No evidence has verified the role of RNAPIII in HDV replication except for its interaction with the HDV genome.[72]" and discussed the role of three polymerase in HDV replication.

Lane 107: "The LDAg together with SDAg and genomic RNA, but not antigenomic RNA, assemble into RNP…". This has not been proved. There are evidences that RNPs are formed, may be even transiently, between the antigenome and Delta antigens. Shall I be wrong, than the authors should provide at least one reference supporting their statement.

Responses: Thanks for the reviewer's comments. The original description was simplified into "The LDAg interacts with SDAg and genomic RNA to form RNP that can be exported from the nucleus and assembled with HBsAgs."

Lanes 113-115: Please, provide a reference

Responses: Yes, we added three references [77-79] in the revised version.

Lanes 119-121: The two questions raised by the authors are not sufficiently sustained by facts besides the experiments described on their own paper (reference 43) which, in my opinion, includes conclusions not supported by the performed experiments.

Responses: We rephrased a part of paragraph including the second question as follows. "However, the predominant cellular distributions of LDAgs are in the nucleus, whereas the major location of HBsAgs is in the cytoplasm;[79, 81-83] two questions are thus raised: 1) how the LDAg which contains a nuclear exporting signal (NES) at the C-terminus receives signals to export from the nucleus to the cytoplasm and 2) whether the HBsAgs play a role in LDAg nuclear exportation. Furthermore, the exact location, ER or Golgi, where LDAgs interact with HBsAgs to form particles remains undetermined. The study conducted by Carmo-Fonseca et al. demonstrated that the HDV RNP can be shuttled between the nucleus and cytoplasm,[84] suggesting that the interaction between HBsAg and HDV RNP is the consequence of HDV RNP being shuttled from the nucleus to the cytoplasm. The isoprenylation of LDAg is the key event for HDV assembly when HBsAgs interact with HDV RNP.[79, 83, 85] However, the location of isoprenylated LDAg occurs inside the nucleus even in the absence of HBsAgs[86], suggesting that the HBsAgs do not play a role in the event of LDAg isoprenylation."

Lanes 149-150: There is strong evidence that detection of LHDAg in the nucleoli cultured cells is an artifact since it does not occur in the presence of virus RNA. Delta antigens are basic proteins that were already shown to bind unspecifically to several nucleic acids, including DNA. If the authors do not agree, at least should mention other works that show exactly opposite evidences.

Responses: Thanks for the reviewer's comment. Indeed, whether the LHDAg enters into nucleoli is unsolved question. Localization of LHDAg in different subcellular regions may depend on its conformation. Various post-translational modifications of LDAg, i.e., phosphorylation, various amino acid sequences of LDAg encoded by different genotypes, or LDAg binding to different partners, i.e, virus RNA can influence LDAg conformation which results in different locations. No LHDAg observed in the nucleolus in the presence of virus RNA could be explained that the complex of LHDAg and virus RNA does not favor LHDAg in the nucleolus. However, the in vitro systems that using GFP fused with LHDAg (GFP-LD) shows the nucleolus localization of GFP-LD.

Lane 164-165: what do the authors mean by "HBsAgs is detected in the ER where the HBsAg is required for glycosylation." Please, clarify this sentence.

Responses: Thanks for the reviewer's comment. We deleted the sentence in the new version.

On page 8 the authors discuss a putative influence of different factors, including the different forms of HBsAgs, on the efficiency of LHDAg export. The available data are, at least, controversial and therefore should be discussed with care and include other hypothesis and experimental evidences. For instance, it has been shown that HDV RNPs shuttle between the nucleus and the cytoplasm. The observation of distinct amounts of LHDAg in the cytoplasm may represent just a consequence of its retention after interaction with HBsAgs. These and other issues should be discussed in more depth by the authors.

Responses: Thanks for the reviewer's suggestion. Though the shuttling of HDV RNPs between the nucleus and cytoplasm has been observed, it s unknown whether the HBsAgs-interaction of LDAg is the consequent result after HDV RNPs shuttling from the nucleus to cytoplasm. Furthermore, the detail mechanism regarding the shuttling of HDV RNPs in presence of HBsAgs is not proved. From another viewpoint, the isoprenylation is required for the HDV package through interacting with HBsAgs. Previous studies showed that the deficiency of LDAg isoprenylation could not be exported into the cytoplasm from the nucleus. Therefore, we would like to focus on the role of post-translation modification of LDAg during HDV package in this review.

Finally, on lanes 194-197, it is not clear what the authors mean. This sentence needs to be clarified

Responses: Thanks for the comments. We rephrased the statement as "However, the efficiency of LDAg nuclear exportation induced by TNF- treatment is indiscernible when cells are treated with or without cycloheximide, a translation inhibitor, [78] suggesting that the LDAg nuclear exportation is not dependent on *de novo* gene transcription for HDV assembly when the signals are induced by TNF- . Although both TNF- and HBsAgs-induced ER stress are linked to the activation of NF- B, the link between enzymes that modify LDAgs (PP2A and ERK1/2) and the downstream molecules either activated or suppressed by NF- B remains unclear."

#Reviewer 2

The paper reviews the current state of knowledge on the interactions between Hepatitis B and Hepatitis D in together causing liver disease. However the paper needs a lot of revision in terms of language. The authors also need to add why the

interaction between the two viruses are important, if at all, in an era when HBV can be eradicated or at least suppressed with potent antiviral therapy, which will adversely influence the survival of latter virus also.

Responses: Thanks for the reviewer's comments. We apologize for English grammar and spelling in the first manuscript. The current manuscript has been edited by a professional English editing company. At mean time, we added two new paragraphs to illustrate why studying the interaction between the HBV and HDV are important as the reviewer suggested.

#Reviewer 3

Q1. Please get a native English speaker to check the English used in the paper. Various sentences should be reviewed due to grammatical error.

Yes, the current form of manuscript has been edited by a visiting professor of Chang Gung University, Dr. Michael J. Leibowitz, who is from the Department of Medical Microbiology and Immunology, University of California—Davis.

Q2. The space between the words should be reviewed throughout the manuscript.

Thanks for the comment. The manuscript was reviewed by both two authors and Professor Leibowitz.

Line 50 – Please, substitute the bracket style [hepatitis A (HAV).

Professor Leibowitz suggests that it is not necessary to substitute the bracket style.

Please, the mechanisms expounded between lines 294 and 306 are under-elaborated. There is insufficient information about the cross-talk of HDV and HBV molecules and inflammatory mediators. A clear description of the hypothesis is needed.

Yes, it has been re-written as the reviewer suggested.

Q3. References

The references should be revised due to different aspects. First they are not in the style adopted by the journal. Second they should be reviewed due uncorrected journal abbreviation.

The entire references have been double checked to fit the style adopted by *WJG.*

Q4. Figure **1 and 2** – Please, the figure (legend) should be able to "stand alone" from the manuscript and still make sense to readers. Legends should contain sufficient information to provide an adequate understanding of the figure/graphic by the reader without reference to the text. The figures are under-elaborated and should be reviewed.

We added an image to show various locations of GFP-LDAg, such as SC35 nuclear speckles which is not familiar by general readers. Professor Leibowitz also carefully read the figure legend.

#Reviewer 4

Q1. This paper is well built in concepts, but needs a lot of language corrections and Literature must be up-dated with particular reference with recent improvements in anti-viral therapy. These improvements should be cited also in discussion.

Thanks for the reviewer's comments. As we stated in the letter, we have asked a visiting professor for editing the manuscript. We do not put references with recent improvements in anti-HDV therapy in the revised manuscript because anti-viral therapy is not the scope of paper. Secondly, some studies indicated the treatment of the chronic HDV infection is difficult due to the drugs have a limit efficiency in clearance of viral RNA.[1, 2] Besides, the mechanism of antiviral drug in HDV infection or HDV viral RNA clearance is poorly to clarify. Therefore, we did not describe the part of anti-viral therapy in this review article.

1 Hsieh TH, Liu CJ, Chen DS, Chen PJ. Natural course and treatment of hepatitis D virus infection. *J Formos Med Assoc* 2006; **105**(11): 869-881 [PMID: 17098688]

2 Abbas Z, Memon MS, Mithani H, Jafri W, Hamid S. Treatment of chronic hepatitis D patients with pegylated interferon: a real world experience. *Antivir Ther* 2014 [PMID: 24423484 DOI: 10.3851/IMP2727]

#Reviewer 5

Q1. The review was quite comprehensive, however there was no clear message about this linkage pathogenesis of these two viruses to the impact of clinical outcome and management.

Thanks for the reviewer's comment. The clinical outcome is described from lane 80 to 98. The clinical management is not described because it is not the scope of paper. The linkage pathogenesis of HDV and HBV was briefly reviewed from the lane 99 to lane 115.

3 References and typesetting were corrected

Thank you and your colleagues for reviewing a submitted review paper to *World Journal of Gastroenterology* (ESPS Manuscript NO: 6843) and giving excellent comments.

We have gone through the entire revision of manuscript according two reviewers' comments and suggestions. Additionally, we modified the title and added two figures to increase readership. This current form of manuscript has been edited by a professional English language editing company (please see the attached certificate from the company of Wallace Academic Editing).

You will find point-to point response to two reviewers in the following pages. I hope the revised manuscript is now acceptable by *World Journal of Gastroenterology.*

Sincerely yours,

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Department of Biomedical Sciences
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