Author's response to reviews

Title: Hepatic inflammation mediated by hepatitis C virus core protein is ameliorated by blocking complement activation

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Version: 2 Date: 2 February 2009

Author's response to reviews: see over
Dear reviewers and editor,

Thank you for your kindly reviewing of “Hepatic inflammation mediated by hepatitis C virus core protein is ameliorated by blocking complement activation”. My point-by-point response to the concerns is as follows:

**Reviewer’s report**

**Title**: Hepatic inflammation mediated by hepatitis C virus core protein is ameliorated by blocking complement activation

**Version**: 1  **Date**: 26 September 2008

**Reviewer**: Jefferson Chan

**Reviewer’s report:**

BMS-Medical Genomics Manuscript-Chang, Bissell et al.

In this manuscript, Chang et. al. examined the pathogenesis of inflammation and fibrosis associated with chronic hepatitis C infection. A Tet-inducible model was used in which HCV core protein is expressed in adult mouse hepatocytes to mimic more closely the situation in human HCV infection. The main findings in this paper are: 1) expression of HCV core at intermediate levels leads to liver damage, oxidative stress and steatohepatitis; 2) expression at high or low levels leads to steatosis only; 3) degree of steatosis correlated with level of core expression; 4) complement pathway genes (and other genes) were found to be altered by Microarray analysis; 5) treatment with CD55 (decay accelerating factor for complement) protects against liver damage in transgenics.

**Major points**

1) Inflammation was minimal to none (at least histologically) in transgenics expressing high levels of core protein. Yet gene expression analysis showed changes in complement pathway genes as in transgenics expressing intermediate levels of core. Is the complement pathway activated only in intermediate core-expressor but not in high expressors? Could pathology in this model mediated by another (or more than one) pathway?

- According our previous work (BMC Genomics. 2008 Feb 29;9:109.), complement pathway is also down-regulated in the high expressors, whose C3 is down-regulated rather than up-regulated as in the intermediate expressors. Surely there maybe another pathway responsible for the pathology in this model, but, after comparing the array data between the
non-, high-, and modest-expressors, as well as blocking the inflammation by complement blockers (CD55), we trusted complement pathway is one of the potential pathways responsible for the pathology.

2) Genes identified in the Microarray studies should be shown instead of just listing the categories.
   ➔ In a cross-comparison of data from experiments II and III, 30 genes were significantly changed and the 30 identified genes was listed in the table 3.

3. Show qPCR results in a table or graph for each of reading.
   ➔ The data of qPCR of the 30 genes was listed in table 4.

Minor points
4) Organ names should be used in labeling of figure 1G, H, I, instead of Number.
   ➔ The organ names had been listed.

5) Label for Y-axis is missing in Figure 1J.
   ➔ Y-axis had been labeled.

6) Upper labels are in Table 1 are not separated.
   ➔ I am sorry that I cannot follow you since the upper labels are separated already.

7) Errors, missing words, etc
   ➔ The missing words had been added.

- page 8, line 12..."total 30 genes.." should be “a total of 30 genes…”
- page 8, line 13 ..."they were potential" should be “they are…. ” Run on sentence
- page 10, line 11.....
- page 10, line 12.....
   ➔ I am sorry that I cannot follow you since I didn’t figure out what need to be added for line 11 and 12 in page 10.

Level of interest: An article of importance in its field
Quality of written English: Acceptable
Statistical review: No, the manuscript does not need to be seen by a statistician.

Reviewer’s report
Title: Hepatic inflammation mediated by hepatitis C virus core protein is ameliorated by blocking complement activation
Version: 1 Date: 28 November 2008
Reviewer: Maria Rapicetta

Reviewer's report:
The subject of the paper is dealing with pathogenetic aspects of HCV infection with particular reference to liver inflammation fibrosis and steatosis and relationship with HCV Core protein expression. The experiments were based on the use of transgenic mouse model.
The experimental model was able to express low, intermediated and high levels of HCV and to show differences in inflammation steatosis and fibrosis patterns. CD55 administration seems to have effect in inflammation reduction indicating possible involvement of complement pathways.
The proposed model seems interesting however a precise standardization is lacking and the numbers of the animals applied to show the significance of experiments is not clear. As a major revision, the paper should be completed to clearly indicate the numbers of applied animals and show the statistical significance of experimental results.

➔ The numbers of the experiment animals had been added in the 1st, 5th and 8th paragraphs of the methods and materials.

Level of interest: An article whose findings are important to those with closely related research interests
Quality of written English: Acceptable
Statistical review: Yes, but I do not feel adequately qualified to assess the statistics.
Declaration of competing interests:
I declare that I have no competing interests

Reviewer's report
Title: Hepatic inflammation mediated by hepatitis C virus core protein is ameliorated by blocking complement activation
Version: 1 Date: 21 November 2008
Reviewer: Tarik Asselah

Reviewer's report:
In the article "Hepatic inflammation mediated by hepatitis C virus core protein is ameliorated by blocking complement activation", a mouse with conditional expression of HCV core was developed and the effect of core protein production in the adult liver examined. They concluded that transgenic mice that conditionally express intermediate HCV core protein develop inflammation, steatosis, and fibrosis. These effects mediated by HCV core are reduced by
administrating CD55, a regulator of the complement pathway.

Minor Essential Revisions

(1) How the authors explain that mice with the more advanced changes all had intermediate levels of HCV core protein in the liver; while mice with high levels of the protein had minimal findings? Since mice with high expression of HCV core display markers of oxidant stress, we should suspected advanced disease (steatohepatitis).

- In our previous studies (World J Gastroenterol. 2007 Jul 7;13(25):3472-7.; Scand J Gastroenterol. 2008;43(6):747-55.), robust HCV core expression increased oxidative stress and led to subsequent hepatocellular apoptosis, which may account for the lack of advanced disease (steatohepatitis) in the mice with high core expression.

(2) Regarding the fact that core protein is marked by the complement proteins, initiating the complement pathways. Is it a non specific mechanism with just HCV core initially elicits hepatic inflammation and subsequently activates the coagulation pathway; or a more specific mechanism that links HCV and complement pathway?

- Asides from our previous studies regarding the relationship between HCV core protein and complement pathway (BMC Genomics. 2008 Feb 29;9:109.), Yao, et al. had demonstrated that Hepatitis C virus core protein inhibits human T lymphocyte responses by a complement-dependent regulatory pathway (J Immunol. 2001 Nov 1;167(9):5264-72.); the cumulative data convinced us that there is specific mechanism links HCV core and complement pathway.

(3) The conclusion (of the abstract) is too speculative and global “The model may be valuable in investigating the pathogenesis of liver injury in chronic hepatitis C and in designing therapeutic interventions”.

- The conclusion of the abstract had been modified.

**Level of interest:** An article of outstanding merit and interest in its field

**Quality of written English:** Acceptable

**Statistical review:** Yes, but I do not feel adequately qualified to assess the statistics.

**Declaration of competing interests:**
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I declare that I have no competing interests' below