Author's response to reviews

Title: Modulation of RANTES expression by HCV core protein in liver derived cell lines

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Author's response to reviews: see over
Dear Editor,

Thank you for reviewing the manuscript “Modulation of RANTES expression by HCV core protein in liver derived cell lines” by A. Ruggieri, M. Franco, I. Gatto, A. Kumar and M. Rapicetta, that was improved according to revisions requested by the reviewer. In particular, Fig. 1 was upgraded with the data of RANTES expression for Hep352 cell line. The minor essential revisions were also done.

Here find enclosed the point-by-point response to all the concerns. Pages and lines in point-by-point response are numbered according to the new version of the manuscript.

Wishing that this final revised version of the enclosed manuscript will fulfil the standard required by BMC Gastroenterology journal, the Authors thank you.

Best Regards

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Answer to Reviewer  Hans Dieter Nischalke report

Title: Modulation of RANTES expression by HCV core protein in liver derived cell lines

Version: 4 Date: 5 March 2007

Reviewer's report:
In their revised manuscript Ruggieri et al. have considerably improved their presentation of the results. However, still some concerns/questions remain to resolved:

Major Compulsory Revisions :
1.) Figure 1: Data illustrating RANTES expression of HepG2 cells transfected with the core-E1-E2-NS2-NS3 construct should be presented in figure 1 as an essential control experiment. However, this issue is not addressed at all by the revised version of the manuscript. Of note, the analogous control is shown for Chang Liver cells in figure 2.

   Figure 1 (A and B) was replace with the new one presenting RANTES expression in cell lines transfected with the core-E1-E2-NS2-NS3 construct. Accordingly, in results section, page 6, line 242 and 249, the following sentences were added: “However in Hep352 cell line (expressing core-E1-E2-NS3 proteins) percentage of RANTES positive cells (78.5 ± 1.5%) was close to that in control cell lines.” . . . “and that from Hep352 cells”. In legend of Figure 1, page 14 line 577, the sentence: “Hep352 cell line expressing C-E1-E2-NS2-NS3” was also added.

Minor Essential Revisions:
1.) In the original version of the manuscript the authors suggested that NS2-NS3 might counteract the effects of HCV core on RANTES induction. In our review we drew attention to the possibility that such counteracting effects could also be mediated by HCV envelope proteins. Thus in the revised version the authors have replaced NS2-NS3 by E1-E2. However, the manuscript does not report experimental data supporting E1-E2 over NS2-NS3. In order to give a balanced view of the possible interpretations the authors should state that either E1-E2 interactions or NS2-NS3 effects could counteract RANTES induction by HCV core. The words “or non-structural proteins NS2-NS3” should be incorporated on page 11 line 218, page 12, line 248 and page 13, line 253

   DONE. The Authors fully agree and the words “or non-structural proteins NS2-NS3” was inserted in pages 7, lines 250, 281 and 288.

2.) We also recommend to remove the words „since the structural E1 and E2 proteins of HCV have been reported to affect RANTES secretion through CD81 interaction [20]“ on page 11, lines 218-219, because this wording does not seem plausible in the revised context.

   Page 7, lines 250: DONE.

3.) Results section page 11, line 219-221: The sentence: „The results described above suggest that the effect of HCV structural proteins on the expression of RANTES ....“ should be changed to: „The results described above suggest that the effect of HCV core protein on the expression of RANTES ....“
4.) In the discussion section the passage:
„Co-expression of the core protein with E1-E2 and non-structural NS2-NS3 proteins of HCV resulted in counteracted effect on RANTES expression with respect to the effect of HCV core singularly expressed in both cellular systems; this suggested that E1-E2 proteins, which have been reported to affect RANTES expression by CD8+ lymphocytes in HCV patients [20] may counteract the effect of HCV core protein on RANTES, consistently to the results by Soo et al. [18], possibly by direct interaction of E1 and E2 with the core protein in the cytoplasm.“ (page 16, lines 325-330) should be reworded.

my suggested rewording is:
„The effects of HCV envelope proteins on RANTES secretion are multifaceted. Recently, Soo et al. demonstrated that the intracellular co-expression of structural proteins E1-E2 suppress the HCV core-mediated activation of RANTES promoter reporter constructs in HeLa cells [18]. In contrast, extracellular binding of HCV E2 to CD81 has been shown to increase RANTES secretion [20]. We found, that co-expression of the core protein with E1-E2 and non-structural NS2-NS3 proteins of HCV counteracts the effect of HCV core expressed alone with respect to RANTES expression in both cellular systems. Thus, in line with Soo and colleagues, our results suggest that E1-E2 proteins counteract the effect of HCV core protein on RANTES expression, possibly by direct interaction of E1-E2 with the core protein in the cytoplasm. However, the role of the non-structural proteins NS2 and NS3 remains to be clarified in this context“

What next?: Accept after minor essential revisions

Level of interest: An article whose findings are important to those with closely related research interests

Quality of written English: Acceptable

Statistical review: No, the manuscript does not need to be seen by a statistician

Declaration of competing interests:
I declare that I have no competing interests