Reviewer's report

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

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Reviewer: Hans Dieter Nischalke

Reviewer's report:

General:

In their revised manuscript Ruggieri et al. have considerably improved their presentation of the results. However, still some major shortcomings remain and need a more thorough revision.

Major Compulsory Revisions:

1.) It is a major shortcoming of this manuscript that control experiments (e.g. results in untransfected CHL cells and in HepG2 cells transfected with the HCV core-E1-E2-NS2-NS3 in figures 1, 2, and 5) are not reported consistently. For instance the authors showed that transfection of cell lines with HCV core alone leads to increased RANTES production in Chang liver cells but to reduction of RANTES in HepG2 cells. In CHL cells transfected with HCV core-E1-E2-NS2-NS3 this effect was completely abolished. Thus, it is important to clarify if the longer construct also abolishes the opposite effect in HepG2 cells. Therefore transfection of HepG2 cells with core-E1-E2-NS2-NS3 had been suggested as a critical control in the previous review. However, this issue is not addressed at all by the revised version of the manuscript.

2.) Figure 3: The authors state that transfection with their HCV core-containing vector (Hep39) results in downregulation of RANTES mRNA expression as compared to cells transfected with the empty vector. However, figure 3 seems to suggest that less b-actin mRNA was detected by the PCR. Thus, the presented data do not exclude the possibility that apparent changes in RANTES in mRNA reflect differences in RT efficiency or effects of PCR inhibitors. Thus, we advised the authors to corroborate their data by providing densitometry data of the gel bands together with an appropriate statistical analysis. This improved evaluation of the data is missing in the current manuscript.

3.) In figure 6, lower panel , IRF-7 data for HepG2 parental cells and CHL cells transfected with control vector (CHwt) should be presented analogous to the presentation of the IRF-1 data in the upper panel.

4.) In the results section the authors describe that in cells expressing HCV proteins C-E1-E2-NS2-NS3 (CH352) the fraction of RANTES positive cells was similar to the controls, suggesting a possible role of HCV non-structural proteins (NS2, NS3) in counteracting the effects of structural HCV proteins in RANTES induction. The authors did not consider the alternative possibility that E1-E2 counteracts the effects of core on RANTES in CH352 cell lines, since it has been recently reported by Nattermann et collaborators (J Viral Hepatitis 2004) that interaction of E2 protein with CD81 increased RANTES secretion by CD8+ lymphocytes from HCV patients. Nattermann et al. used recombinant E2 protein to stimulate T-cells extracellularly. This kind of stimulation is quite different from intracellular expression of multiple HCV proteins, because it is possible that E1 and E2 may counteract the HCV core mediated effects by direct binding to HCV core protein in the cytoplasm.

Thus, the available data do not enable to dismiss this alternative interpretation of the results, which should be taken into account in the discussion of the results.

Minor Essential Revisions

1.) Figure 2: The authors state that they omitted the misleading blue histogram in this figure which referred to CH827 cells expressing E1-E2. However, figure 2a submitted with the revised manuscript still shows this blue histogram, contrary to the statement of the authors.

What next?: Unable to decide on acceptance or rejection until the authors have responded to the major compulsory 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

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