Reviewer's report

Title: Soluble egg antigen of Schistosoma hematothium induces HCV replication in PBMC's from patients with chronic HCV infection.

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Reviewer: Sanaa Kamal

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In this manuscript, El-Awady et al performed analysis of the effect of SEA on intracellular HCV replication in PBMC on cell proliferation in 26 patients with chronic HCV infection. The main result of this report is that SEA induction resulted in stimulation of viral replication. They conclude SEA stimulation is associated with proliferative activity on PBMCs which might provide explanation to the higher morbidity observed in patients co-infected with schistosomiasis and HCV.

This study addresses the role of host-viral interactions in determining the disease pattern in chronic hepatitis C. The study of proliferative responses in HCV and schistosomiasis coinfection has been addressed previously in several studies (Kamal et al, J Infect Dis. 2004 Apr 1;189(7):1140-50.; Elrefaei M et al J Hepatol. 2004 Feb;40(2):313-8., Kamal et al Infect Dis. 2001 Oct 15;184(8):972-82...Kamal et al Gastroenterology. 2001 Sep;121(3):646-56 and several others).. Theses studies demonstrated a decrease in HCV-specific immune responses in HCV/schistosomiasis coinfection

The study has several problems in the design, sample size and the methods. A serious concern is that these conclusions are not necessarily the only ones that fit with the data. The following areas need to be addressed:

Major Compulsory Revisions

A. Study Design

1. Patient selection:
   • It is not clear how the patients were selected. Were they consecutively enrolled?
   • Baseline demographic and clinical characteristics of subjects were not shown. The authors did not mention important information such as the mean disease duration and stage of liver disease at enrollment and if some already have cirrhosis. These criteria are particularly important since there are many factors that influence the immune responses. It is known that the magnitude and breadth of T cell responses differ markedly with the stage of liver disease. Well matched homogenous cohort is needed to settle this issue.
   • It is stated that the patients were coinfected with HCV and schistosomiasis. It is not clear whether it is S. hematobium or S. mansoni. It is not mentioned if it is only urinary or intestinal schistosomiasis or it is hepatic schistosomiasis and whether they were treated or untreated. There is no mention on whether schistosomiasis was active or not and if the schistosomiasis preceded HCV infection. These data are very important because the immune responses are affected more with active and hepatic S. mansoni infection. Absence of these data represent a major weakness of the study.
   • Sample size: The number of patients recruited is extremely small (only 26 patients). This is not
adequate given that this study is cross-sectional and limited number of immunological and virological experiments at one time point and the T cell responses are hard to detect in chronic hepatitis C. Also enrolling a larger cohort of chronic hepatitis C patients is not hard in a community with high prevalence of HCV. A larger sample would definitely increase the impact and credibility of the findings.

B. Methods:

1. It is not shown how the blood was collected and whether the blood was on heparin or EDETA.
2. The amount of blood drawn (10ml) may not be sufficient for production of enough PBMCs for the flowcytometry and proliferative assays particularly that the authors mention that 2 million cells were plated at 2 million cells per well. What was the average numbers of cells produced. This information is crucial. Typically, at least 20 ml are required to perform the assays mentioned in the study. These details are obviously important since the quality of functional assays may differ depending on the number of cells and the conditions in which the cells are processed.
3. Where the assays for all patients performed at the same time with the same laboratory and experimental settings?
4. What is the rationale for using only Schitosoma heamatobium SEA?
5. Cells were incubated for 7 days. This duration is long since proliferation occurs within 48 hours.
6. The authors tested the effect of SEA on cell proliferation only in 5 healthy control subjects. This is very confusing because it is not clear how the authors measured PBMCs proliferation in chronic HCV patients? How could the authors demonstrate the influence of SEA on the cell proliferation if they did not perform proliferation assay, ELISpot or FACs to the chronic HCV patients?
7. T cell responses are hard to detect in chronic hepatitis C. The technologies to detect them have led to important improvements especially by introducing ELISPOT assays using overlapping peptides or known CD4 and CD8 epitopes on pre selected subpopulations of PBMC's. The assays used in this study are not state of the art and represent a weakness in the stud. ELISPOT assays for CD4 and CD8 responses are much more sensitive and produce more reproducible results than the tests used in this study.
8. FACs analysis: the specificities of FACs analysis are not mentioned.
9. In general the immunological component of the study is much weaker than the virological component.
10. Primers: The genotypes represented in this report are G4 in 24 patients and G1b in only 2 patients. Although the primers used were from a conserved region, yet some differences exist between G4 and G1 given that commercial kits are based on G1. In order not to miss positive results it would it have been better to design and use specific primers for genotype 4. Do the authors have experience with such primers? If so, did the carry experiments comparing the sensitivity and specificity of G4 primers with the commercially available primers?

RESULTS

1. Several important data such as the clinical background of patients is not mentioned. This is not made explicit, and some more information is needed.
2. The authors repeat here that the predominant genotype among the studied cases is G4 and this has been already mentioned in the methods section.
3. The authors detected negative strand in 21 patients while only plus strand was detected in 5 patients. How do the patients explain that? Could be this attributed to potential technical aspects of the experiment? Were the testing for negative strands repeated for these 5 patients?
4. The authors mention that stimulation of the 5 control cultures with PHA and SEA resulted in a significant increase in DNA index to 16.7 ± 2.5 % and 16.84 ± 1.7 % respectively. How did they
prove that the difference is significant? What is the P value? In general the statistics need to be reviewed by a biostatistician - it seems to me that a lot of post-hoc analysis were applied to the data sets.

5. Again the authors mention that stimulation of patients' PBMC in culture for 72 hours resulted in increased PBMC count in all cases ranging from 1.3 – 4 fold increase with a mean value of 4.26± 1.5. What does this mean value indicate? This is an ambiguous statement. Is it mean fold increase? If so how come the range is 1.3-4 folds and the mean is 4.26???

6. Proliferation assays, ELispots or FACs were not shown to be performed in chronic patients How did the authors get these results? There is plenty of missing information that need to be clarified. 7. The paradoxical finding that after 24 hours of PBMC cultures, 5 cultures (23.8%) lost both plus and minus RNA strands which were restored after addition of SEA is interesting, however, how could that be explained?

8. The authors mention that the goal of the study was to test the effect of soluble egg antigen (SEA) on intracellular HCV RNA load in peripheral mononuclear cells (PBMC`s) as well as on cell proliferation in patients with chronic HCV infection. While the answers for these goals were not properly presented in the results section.

Discussion:
1. The discussion includes previous published data and repetition of the results, speculations. There is only limited analysis of the data or findings in the manuscript. The authors do not state how this improves upon previously published cross sectional reports, which have already suggested these findings. While the issue of explaining the role of schistosomiasis on HCV replication is of substantial interest, the authors have not provided credible information that would enhance our understanding of how this happens.

2. The authors state that the dual stimulatory function on both PBMCs and viral copies per cell, the magnitude of total viral pool response in SEA stimulated culture is obviously larger than the presented values. Given that the tests used for measuring PBMCs stimulation are not efficient and given the small samples size, it is not possible to make such statement and thus the interpretation of the results is hardly acceptable.

3. Although the finding that SEA was capable of restoring either one or both strands in cultured cells from those patients, is interesting the authors do not provide convincing explanation or even speculation.

4. The conclusion is not supported by the data

Data representations:
1. Tables: Important data are missing particularly clinical and demographic background.

2. The figures are not of good quality and need to be improved. The legend need to be sufficiently informative to the reader to get the information without getting back to the text.

References:

Recent references relevant to the study are not included.
What next?: Unable to decide on acceptance or rejection until the authors have responded to the major compulsory revisions

Level of interest: An article of limited interest

Quality of written English: Acceptable

Statistical review: Yes

Declaration of competing interests:

'I declare that I have no competing interests'