ANSWERS TO THE COMMENTS OF REVIEWERS

Reviewer's code: 00004603

1. Quality of figures and figure legends are poor. In certain cases, it is almost impossible to read what is presented. Figure legends also do not shed the light on how experiment has been done. Instead of guessing, the reader should clearly see and understand the results.

Response: As suggested by the reviewers, the figures and figure legends has been improved and the way in which the experiment was carried out has been mentioned.

2. Using Dendritic Cell Isolation kit, the enrichment of DC was 65% vs 10% in PBMC. It is definitely, DC-enriched population, but we should not forget that 35% of PBMC were not DC, which needs to be taken into account in the results interpretation.

Response: The population which we have used for further experiments are the enriched DC population only as the eluted fraction of cells contain only the cells positive for CD1c marker. In the first step, CD1c (BDCA-1)–expressing B cells are magnetically labeled with CD19 MicroBeads and, subsequently, depleted by separation over a MACS Column which is placed in the magnetic field of a MACS Separator. In the second step, CD1c (BDCA-1)+ myeloid dendritic cells in the B cell–depleted flow-through fraction are indirectly magnetically labeled with CD1c (BDCA-1)-Biotin and Anti-Biotin MicroBeads.

3. There is intensive discussion in the paper regarding HCV in dendritic cells. Even if some researches showed very low expression of HCV in PBMC or DC, it is not obvious, since hepatocytes are the cells that replicate the virus. If you want to claim the infection in DC, at HCV RNA should be measured in extensively washed DC; otherwise, since usual stimulation of macrophages through TLR2 by HCV core protein takes place, it cannot be named "infection". Furthermore, if these DC are not infected, there is no reason to measure anti-viral interferon-stimulated genes (ISGs) like OAS1, OAS2, etc) in the cells and definitely, there is no sense in the interpretation that authors provided.

Response: We appreciate the concern shown by the learned 'reviewer' regarding the main cells being hepatocytes and not DC that get infected and promote the replication of HCV in human body. In the current paper we by no means claim that DC are infected, but the DC being one of the professional antigen presenting cells, do phagocytose the free HCV or the HCV proteins that are released due to persistent replication of HCV in chronically infected host. It has also been well accepted now that besides hepatocytes there are some other reservoir cells including DCs, also harbor the virus, although at a very low level as compared to hepatocytes. We and many other workers have earlier shown that the DCs are functionally defective in chronic hepatitis-C infection as they do not mature in response to TLR stimulation (ref). We have also recently shown that the responsiveness to anti-viral therapy is also associated with functional status of DC in these patients (ref). In order to understand the mechanism of this defective phenotype, in

the current study we hypothesized that HCV or its proteins modulates some genes in DCs that may be responsible for the regulation of the functional status of dendritic cells and thus affecting the overall maturation capability of these cells. By using a custom designed gene array we have tried to find out the pathways involved. The genes selected in the custom designed array involve the immune response genes and genes associated with maturation and antigen presentation.

4. Gene array always is a great option when you can then confirm the dysfunction of certain signal transduction pathways. As an example, in the IFN-induced JAK-STAT1 pathway, this dysfunction is not based on the general expression of STAT1, STAT2, PIAS1 or ISG15, but on posttranslational modifications of phosphorylated STAT1, which cannot be addressed by using this gene array. If there is no chance to study it in a proper way, there is no reason to start it at all.

Response: We do agree with the suggestion of reviewer regarding JAK-STAT1 signaling pathway. Since the findings of our study reveal some signature genes that are upregulated or downregulated during the chronic Hep-C infection, the phosphorylation or other post-translational changes are deemed to be checked subsequently. Although we do envisage that, there can be other regulatory genes, which may influence the transcription of Interferon inducible genes such as IRF7.

5. It is difficult to judge which changes in gene array are related to response to treatment, since they were not examined before treatment has been started. It is known that overstimulation of ISGs at a baseline may be of a poor prognostic value; however, we do not know whether the changes observed in the paper existed before or are the result of treatment. If PBMC of patients that undergo treatment were examined at a baseline, with further retrospective distribution of them to responders and non-responders, this study would make more sense and really would be able to answer the questions that were asked by the authors.

Response: Yes this is not a longitudinal study and was not planned that way. This being a cross-sectional study, the comparisons have been made between the gene expression arrays of cells from patients who responded to treatment vis-à-vis those who did not respond to treatment. This is again a good study design and gives a very important information regarding differential expression of some important genes that may influence the functional status of DCs which ultimately may explain our previous findings published previously (refs). However the point suggested by the worthy reviewer is well taken and will be implemented in our future study design.

6. In the Discussion, the authors tried to parallel the changes in gene activation induced by HCV proteins in healthy donors and in what they found studying responders vs non-responders. First, the arrays are not completely the same; second, the interpretation is wrong (they mentioned the things they only want to see), so this comparison proves nothing. Collectively,

the attempt to do basic-translational research is certainly appreciated; however, this type of studies should be planned well and allow generating interpretable data.

Response: It is obvious that the genes differentially expressed are not the same in-vivo and ex-vivo model, but if we carefully observe the nature and functional characteristics of the gens in the two systems, we find that the ultimate effect of up-regulated or down-regulated genes in the two models remain the same viz: the cells from chronic Hep-C infected individuals (in vivo model) or the cells differentiated in presence of Hep-C [proteins (Ex-vivo model) have the genes differentially expressed causing the maturation and functional defects in these cells. Moreover there may be some other factors responsible for this as it is difficult to match exactly the same conditions in *ex-vivo* model.

Reviewer's code: 00162042

GENERAL COMMENTS: Rationale for the study is clearly presented with appropriate selection of study subjects, cellular isolation/differentiation methodology, and set up for microarray analysis of relevant gene expression. Data presented generally support the authors' conclusions and speculation, but some additional discussion and interpretation should be included. Given that the numbers are relatively low, objective criteria for considering a two fold or some other change in gene expression level significant should be stated and justified. Many of the gene expression changes in the non-responders seem to represent continued exposure to IFN-?? as would be expected with unresolved infection. As stated by the authors, there was not much overlap in the dysregulated DC genes observed in vivo versus ex vivo. This should be discussed in greater length as to an explanation or as to the general validity of the ex vivo model. It's not clear what information is being conveyed in figure 4, which is barely referred to in the text and figure 6 requires considerably more explanation as to the information represented by the individual elements and its overall interpretation.

Response: Generally a fold change greater than one represents a significant difference in expression level of genes. We have taken at-least a 2-fold change as significant, so on this basis we have considered these as up-regulated or down-regulated genes in comparison to normal or other group.

As explained above (Response to Point No. 6 of Reviewer #1)