VIRAL PROTEINS MEDIATE UPREGULATION OF NEGATIVE REGULATORY FACTORS CAUSING DOWN-MODULATED DENDRITIC CELL FUNCTIONS IN CHRONIC HEPATITIS C VIRUS INFECTION

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ABSTRACT

Stunted cellular immune response against a narrow range of epitopes is the hallmark of chronic hepatitis C infection, but the underneath molecular mechanisms have not been well elucidated. Suboptimal antigen presentation through defective antigen presenting cells, have been suggested. The myeloid dendritic cells as professional antigen presenting cells have been found to be phenotypically and functionally defective in chronic hepatitis C-infected patients in our recently published study. In order to find out if the maturation defects in dendritic cells (DC) are induced by the persistence of virus, we tried to differentiate CD14+ monocytes isolated from the peripheral blood of a healthy volunteer in dendritic cell culture medium containing GM-CSF and IL-4 supplemented with hepatitis C virus (HCV) proteins, core and NS5. The results indicated that a lesser number of monocytes differentiated to DC in presence of HCV proteins. Moreover, the differentiated cells depicted immature phenotype, which will not respond to the TLR-4 mediated stimulation *ex vivo* with significantly lesser upregulation of activation markers, HLA-DR, CD83, CD80 and CD86 as compared to cells differentiated in the absence of HCV proteins. Besides, these immature cells showed characteristics of defective antigen presentation, with significantly lower allostimulatory capacity towards lymphocytes from a healthy donor. Semi-quantitative reverse-transcription polymerase chain reaction (RT-PCR) showed upregulated expression of negative regulatory genes SOCS3, PDL1 and IDO in cells grown in presence of HCV proteins, suggesting the role of HCV and associated antigens in functional down-modulation of dendritic cells. This may correlate with the antigen persistence and maturation-defective status of dendritic cells in chronic HCV infection.

Keywords: Dendritic cells, hepatitis C virus, SOCS3, IDO, PDL1, HCV-NS5 protein.

INTRODUCTION

Hepatitis C Virus (HCV) poses a major global health predicament. The virus can be transmitted parenterally, sexually and through blood transfusion.[1] Infection with this morbid virus is associated with serious consequences. Chronic hepatitis in about 70–80% of cases further results in severe liver necrosis and an increased risk of cirrhosis and hepatocellular carcinoma in a smaller proportion of these individuals.[2-4] It is important to understand the mechanisms by which HCV establishes chronic infection so that better therapeutic interventions can be devised. Whilst the involvement of host genetic factors has been a major focus of research, the role of the immune system in the outcome of disease also cannot be marginalised. It has been proposed that a high rate of viral replication leads to exhaustion of the immune system through the production of overwhelming quantities of viral antigens and the production of immunomodulatory proteins by HCV.[5] Furthermore,

1	Positive for anti-HCV antibodies
2	Negative for HBV & HIV by serology
3	No prior history of any treatment for HCV
4	Negative for auto-antibodies (ANA, SMA, LKM, AMA, PCA and GBM) to exclude autoimmune hepatitis
5	Other non-viral factors responsible for liver damage like alcoholism, inherited metabolic disorders.

Table 1. Inclusion criteria for recruiting CHC patients

Parameters	CHC (n=35)
Median Age (years)	39 (19-67)
Gender M/F	31/4
Genotype 1/3/4	6/28/1
Median Viral Load (copies/ ml)	1.91×10^6
Median ALT/AST	80/95

Table 2. Demographic and clinical features of patients recruited

the inability of the innate immune response to promote timely and appropriate T-cell priming has not been well understood yet. It has been suggested that viral proteins such as NS3, NS4, E1 and core protein play an important role in impairing the generation of effective immune response against the virus.[5-7] The status of dendritic cells in chronic HCV infection(CHC), has been the focus of research for many investigators around the world. Although there are a few studies supporting the theory that dendritic cells in CHC remain functional,[8-11] there are a few who argue that during HCV infection, the primary crosstalk between virus or virus-derived proteins and the DCs may render the DCs functionally defective, and further contribute to impaired T-cell responses leading to viral persistence.[12-16] Moreover, it is not yet clear whether the establishment of a chronic carrier state is due to dysfunctional DCs, which causes inefficient priming and maintenance of HCV-specific T-cells, or whether it is a possible outcome of persistent and active HCV infection. It could very well be possible that the host-mediated immunosuppressive mechanisms might be activated in the scenario of persistent antigenic exposure. Certain negative regulatory genes have been implicated as key factors for sustaining an immunosuppressive and tolerogenic microenvironment in different viral infections and even some cancers.[17-19] We have recently demonstrated that DCs from therapy-naïve CHC patients are dysfunctional and fail to mature.[20] The present study was designed to answer the pertinent question: whether the early interactions of virus proteins with DC render them immunosuppressive instead of being immunostimulatory. We report here that the monocytes continuously exposed to viral proteins *ex vivo* during differentiation to immature DCs render these cells maturation-defective, as they fail to respond to external stimulation and we also show that this maturation and functional defect coincides with upregulation of the expression of certain immunosuppressive genes such as SOCS3, PDL1 and IDO in these cells.

MATERIALS AND METHODS

Study Subjects

This study was approved by the Institute Ethics Committee. All the patients recruited in this study were registered with the Hepatology Clinic based on the inclusion criteria **(Table 1)** after an informed consent. A cohort of 35 therapy-naïve patients with CHC infection, were recruited for the study. A total of 14 age-matched normal healthy volunteers (HC) were also recruited as controls. Major demographic and clinical features of all the patients included in this study are detailed in **Table 2**.

Generation of Monocyte Derived Dendritic Cells (mo-DCs)

The peripheral blood mononuclear cells (PBMCs) were isolated from venous blood drawn in heparinised vacutainer tubes, of each subject from CHC and HC groups by Ficoll-Hypaque density gradient centrifugation using Histopaque (Sigma Aldrich). Subsequently, CD14+ monocytes were separated from PBMCs using anti-human-CD14 magnetic particles [(DM-(MφP9); BD Imag TM, BD Biosciences Pharmingen, USA] according to the manufacturer's instructions. Cell suspension enriched with CD14+ monocytes (purity 95% by flow cytometry, data not shown) was cultured in Dendritic Cells Culture Medium (DCCM), consisting of RPMI 1640 supplemented with: 2mM L-glutamine, 5mM HEPES buffer, 100IU/ml penicillin and 100μg/ml streptomycin, 10% fetal bovine serum (Sigma Aldrich), along with 20ng/ml recombinant human GM-CSF and 20ng/ml recombinant human IL-4 (both from PeproTech,

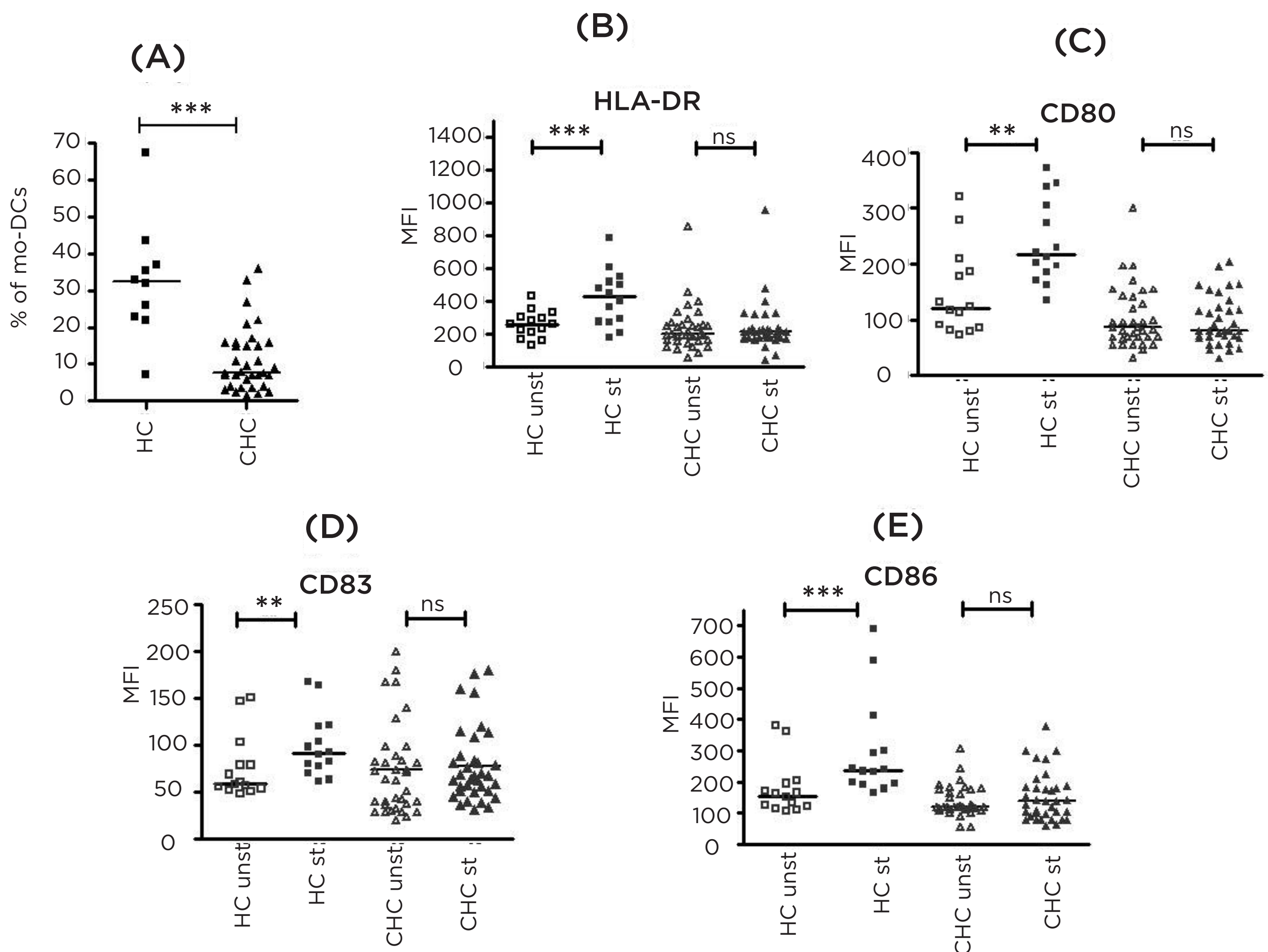

Figure 1. Frequency and expression of activation markers on mo-DCs. Cells with phenotype CD14-HLA-DR+CD11c+ were considered to be monocyte-derived dendritic cells (mo-DCs).
(A) Comparative frequency of mo-DCs in CHC and HC. Frequency of mo-DCs in CHC (n = 35) was significantly lower than HC (n = 14). Surface expression of (B) HLA-DR, (C) CD83, (D) CD80 and (E) CD86 on mo-DCs, pre and postlipopolysaccharide (LPS) stimulation. Mo-DCs from CHC, were able to upregulate expression of CD83, CD80 and CD86 upon LPS stimulation. Data presented as mean ± SD. Horizontal line represents the median value.

Israel), for six days in a humidified incubator (Thermo Forma, USA) at 37°C with 5% CO_2. Half medium exchange was performed on alternate days with fresh DCCM. DC viability on day six was >95% (trypan blue exclusion). To minimise the variations, we started with an equal number of monocytes in all sets of experiments. Toll-like receptor-4 (TLR-4) mediated maturation of DC was induced ex vivo by adding bacterial lipo-polysaccharide (LPS, 500ng/ml) to the culture medium on the sixth day for 48 hours. Simultaneously cells were also maintained without LPS.

Phenotyping of mo-DC

Analysis of cell surface markers on immature (iDCs) and mature DCs was performed on day eight of cell culture. The mo-DCs were stained with FITC-CD14, PE-HLA-DR/CD83/CD80/CD86 and PECy5-CD11c (all antibody-fluorochrome conjugates from BD Biosciences, USA) to characterise the phenotype of mo-DC in different conditions. Briefly, the cells were washed in staining buffer and were stained with respective fluorochrome-labelled antibodies for 20 minutes at room temperature in the dark. The cells were then washed, fixed and acquired using a three colour flow cytometer (FACS Calibur, Becton Dickinson, USA). In each case, unstained cells were also acquired to evaluate non-specific binding. Analysis was performed on gated population that included CD14 negative cells. The percentage positive cells and geometric mean fluorescence intensity (MFI) for each marker on mo-DC was

evaluated by analysis of dot plots or histograms generated by acquiring at least 100,000 cells using CellQuest software (BD Biosciences, USA). Fold increase in MFI was expressed as the ratio of MFI of stimulated cells and unstimulated cells.

Endocytosis Assay

To assess the endocytosing capacity, LPS-stimulated mo-DCs were incubated in PBS, 2% FCS, with 1mg/ml FITC-dextran (Sigma Aldrich) at 37°C to determine specific uptake, or at 4°C to determine background non-specific binding. After one hour of incubation, cells were analysed using flow cytometry. Uptake of FITC-dextran was measured in terms of fold increase in mean fluorescence intensity [MFI (i.e. ratio of MFI at 37°C to MFI at 4°C)].

Mixed Leucocyte Reaction

Allostimulatory capacity of LPS-treated mo-DCs was assessed by mixed leucocyte reaction (MLR). Briefly, LPS-stimulated DCs were treated with mitomycin (50μg/ml; Sigma Aldrich) for 30 minutes followed by co-culturing with allogenic PBMCs from a healthy, HCV negative donor at a ratio of 1:20 in triplicate wells at 37°C, with 5% CO_2 in a humidified incubator. Different healthy donor PBMCs were used in at least three different MLR experiments for mo-DC from each CHC patient. After five days of co-culture, 1μCi/well of [methyl-3H] thymidine (Bhaba Atomic Research Centre, Mumbai, India) was added for 12–16 hours. The incorporation of [methyl-3H] thymidine in proliferating PBMCs was measured using a beta-counter (Beckman-Coulter, USA) and expressed as counts per minute (cpm).

Expression of IDO, SOCS3 and PD-L1 in mo-DCs Differentiated in Presence of Viral Proteins

Peripheral blood samples were obtained from healthy uninfected donors after informed consent. CD14+ monocytes isolated from PBMCs were cultured in presence or absence of HCV proteins, core and NS5 (both 1μg/ml) of HCV genotype 3 (ProSpec-Tany TechnoGene Ltd, USA) from day zero, along with DCCM for seven days at 37°C and 5% CO_2 in a humidified incubator. On the seventh day LPS was added to the cultured mo-DCs for 48 hours for *ex vivo* stimulation through TLR4. At the end of the ninth day, flow cytometry was carried out to check for the surface expression of activation markers and co-stimulatory molecules like HLA-DR, CD83, CD80 and CD86. The expression of IDO, SOCS3 and PD-L1 genes was measured using semi-quantitative reverse-transcription polymerase chain reaction (RT-PCR). Total RNA was extracted from mo-DCs (cultured in presence or absence of HCV proteins) using Trizol reagent (Sigma, USA). RNA was reverse transcribed to make complimentary DNA copies (MBI Fermentas, European Union), which were then further amplified by normal PCR reaction. The first strand cDNA was used in PCR reactions for the amplification of SOCS3, IDO and PD-L1 genes. The estimation of constitutively expressed β-actin gene was taken as control and used to normalise the values for semi-quantitative estimation of other genes (SOCS3, IDO and PD-L1) under study.

The sequences (5'-3') of the primers (custom synthesised from Sigma Genosys, Bangalore, India) used in the study were as follows:

Primer	Sequence
β-actin F β-actin R	AGCACAGAGCCTCGCCTTTGC GCCGTGCTCGATGGGGTACTT
IDO F IDO R	GGCACACGCTATGGAAAAC ATGCATCCCAGAACTAGACG
SOCS3 F SOCS3 R	TCCGGAGGAGCCAGCTGTCC TTTCCTTCGCCAGCCCGCAG
PD-L1 F PD-L1R	TTCTGTCCGCCTGCAGGGCA ACAGCCGGGCCCTCTGTCTG

RESULTS

Phenotypic Characterisation of mo-DC

From both patients as well as controls, mo-DCs were obtained after seven days of culture. Phenotypic characterisation of differentiated immature mo-DCs was performed using flow cytometry. A significantly lesser number of monocytes from CHC patients could differentiate to DCs in presence of GM-CSF and IL-4 in CHC as compared to that of HC ($p<0.005$, **Figure 1A**). The difference observed in number of mo-DCs was not due to decreased leukocyte counts in patients, as there was no difference in total leukocyte count of CHC vs. HC (data not shown).

The surface expression of HLA-DR, CD80, CD86 and CD83 molecules was used as a hallmark of activation and maturation of DCs. While none of the molecules got upregulated significantly on the surface of mo-DCs from CHC in response to LPS stimulation, a uniformly significant upregulation in the surface expression of HLA-DR ($p<0.005$), CD80 ($p<0.005$), CD86 ($p<0.005$)and CD83 ($p<0.05$) was seen on the mo-DCs from HC **(Figure 1 B,C,D,E)**. These data clearly suggest that the mo-DCs from CHC patients were functionally defective and were incapable of

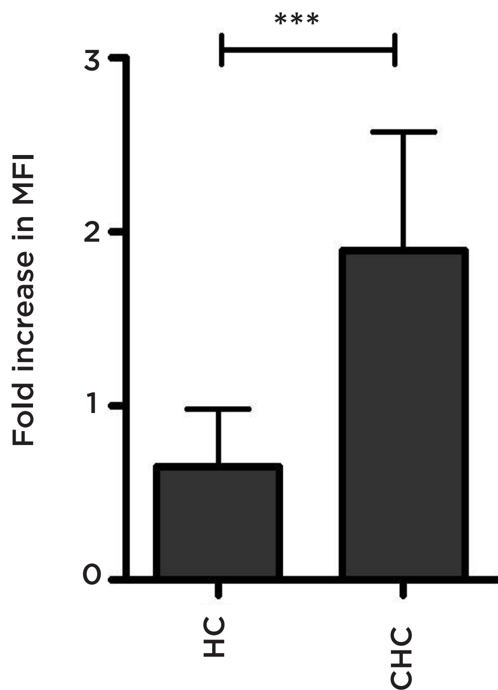

Figure 2. Endocytosing capacity of lipopolysaccharide-stimulated mo-DCs. Uptake of FITC-dextran by mo-DCs from CHC was significantly higher than HC.

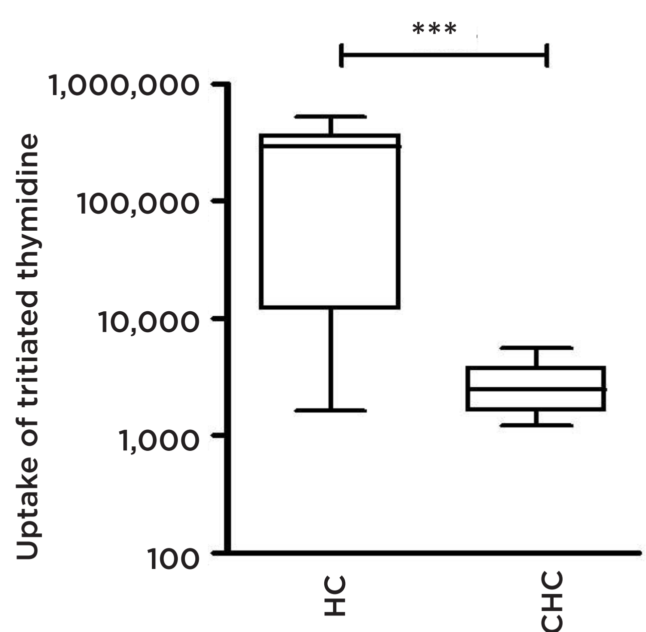

Figure 3. Comparative allostimulatory capacity of mo-DCs. Allostimulatory capacity of mo-DCs from CHC is significantly impaired as compared to HC.

maturing in the presence of an external stimulus.

Antigen Uptake Efficiency of Differentiated mo-DC

Immature dendritic cells are characterised by their marked capability of phagocytosing the invading foreign antigen. As they mature and become efficient, antigen-presenting cells they tend to lose this property. To evaluate antigen uptake efficiency of LPS-stimulated DCs, endocytosis assay was performed using FITC-conjugated dextran particles. Flowcytometric analysis revealed that the amount of FITC-dextran endocytosed by mo-DCs from CHC was significantly higher ($p<0.005$) than those of HC **(Figure 2)**, suggesting that mo-DCs from CHC did not respond to maturation stimulus and remained functionally immature.

Impaired Allostimulatory Capacity of mo-DCs from CHC

The allostimulatory capacity of mo-DCs from different groups was measured by performing Mixed Lymphocyte Reaction (MLR) using mitomycin treated mo-DCs from patients/controls and PBMCs from a single healthy donor. The degree of stimulation of allogeneic lymphocytes induced by mo-DCs from CHC was significantly lower than that of HC ($p<0.005$) **(Figure 3)**. This data is in concordance with the low surface expression of activation and maturation markers on CHC indicating that DCs remained phenotypically as well as functionally immature even upon *ex vivo* stimulation. These data also suggest that viral persistence could be having a negative effect on the allostimulatory potential of DCs.

Assessing the Role of HCV Core and NS5 Proteins on Differentiation of Monocytes to Dendritic Cells

To ascertain whether the defect in dendritic cells is virus mediated, an *in vitro* experiment was performed by exposing monocytes from healthy individuals to HCV Core or NS5 antigen in DCCM during their differentiation to dendritic cells. Simultaneously, a control experiment was run in parallel in which only DCCM was added to mo-DC culture without the addition of viral antigens. The differentiated mo-DCs were then stimulated with LPS for 48 hours, after which surface expression of HLA-DR, CD83, CD80 and CD86 was evaluated. It was observed that mo-DCs exposed to NS5 and core antigens during differentiation, showed characteristics of maturation defect as they expressed lesser amounts of HLA-DR, CD83, CD80 and CD86 on their surface as compared to cells grown in medium without viral proteins, and there was no upregulation of these molecules even after

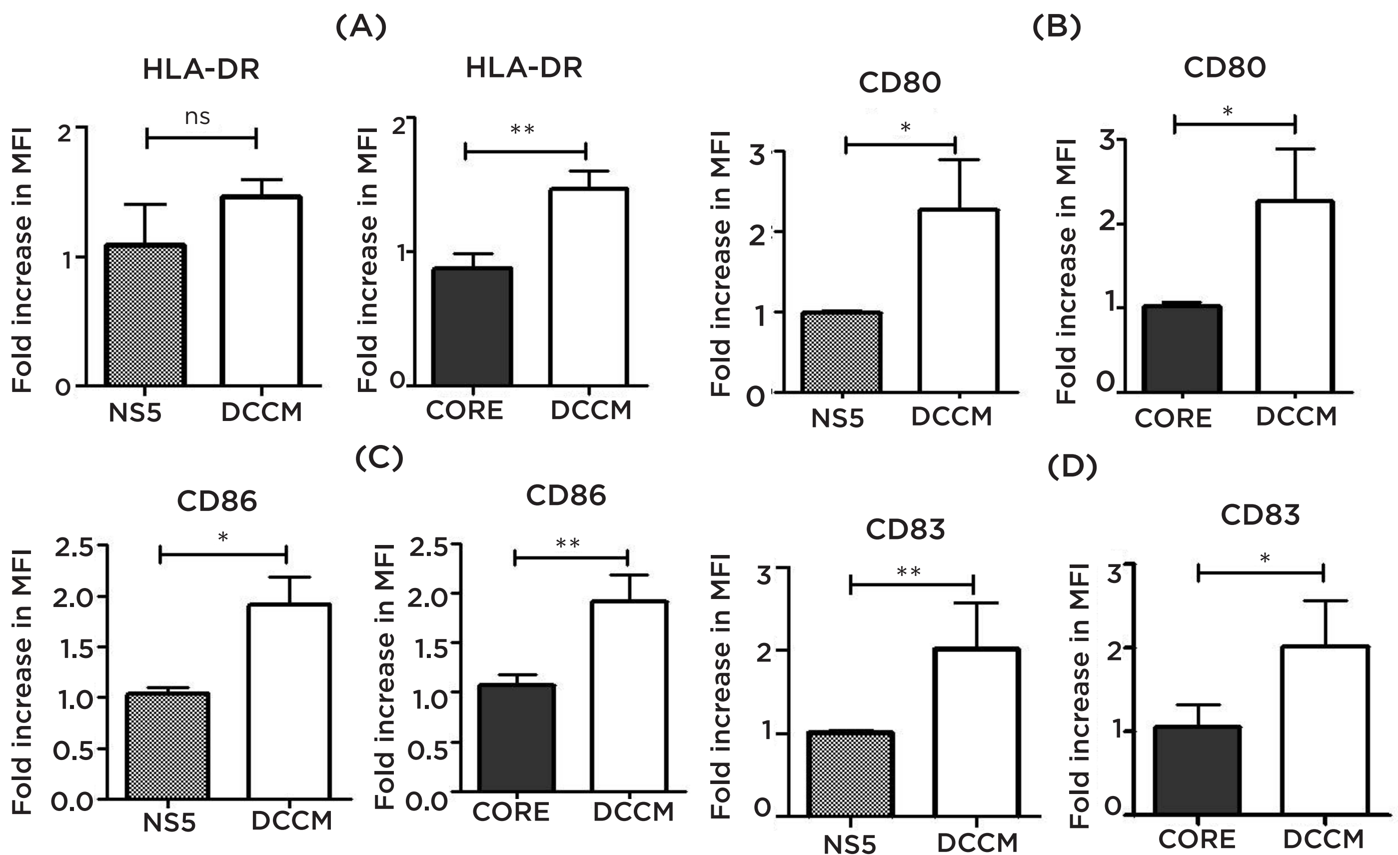

Figure 4. Effect of LPS stimulation on expression of (A) HLA-DR, (B) CD83, (C) CD80 and (D) CD86 on mo-DCs exposed to HCV-NS5 and Core. Each figure represents the fold increase in MFI as compared to mo-DCs raised in absence of LPS stimulation denoted as DCCM only. Both NS5 and Core antigens have deleterious effect on maturation of mo-DCs.

stimulation with LPS. After LPS stimulation, the upregulation of CD83, CD80 and CD86 on mo-DCs exposed to NS5 antigen was significantly lower ($p<0.05$ in all three) as compared to that of medium alone. Similarly, after LPS stimulation, the upregulation of HLA-DR, CD83, CD80 and CD86 on mo-DCs exposed to core antigen was significantly lower ($p<0.05$ in all four, **Figure 4**) as compared to that of DCCM only.

Expression of SOCS3, IDO and PD-L1 in mo-DCs Differentiated in Presence of HCV Core and NS5

To check whether the exposure to HCV antigens caused an increase in expression of down-modulatory genes such as SOCS3, PD-L1 and IDO in mo-DCs, semi-quantitative RT-PCR revealed that in presence of NS5 and Core proteins, the expression of PD-L1 and IDO genes got significantly upregulated ($p<0.05$ for all) in the mo-DC, while only NS5, and not Core, caused a significant increase in the expression of SOCS3 ($p<0.05$) **(Figure 5)**.

DISCUSSION

Viruses are known to devise different strategies to counteract antiviral immunity by manipulating the functions of various components of the immune system. It has been demonstrated that infection of DCs by measles virus resulted in diminished IL-12 production and inhibition of DC maturation,[21,22] while HIV-infected subjects have been reported to have defects in the number and functions of circulating DC subsets.[23,24] CMV infection has also been shown to cause inhibition of DC maturation and T-cell activation, as well as increased apoptosis in T-cells and downregulation of MHC class I molecules.[25, 26]

In recent years there have been some reports describing the role of DCs in HBV or HCV-induced hepatitis.[27,28] However, these reports have been diverse and conflicting. While some studies have reported that HBV or HCV-related chronic hepatitis displayed a phenotype defect and functional deficiency of DCs,[29,30] others reported no observable defect in DCs from CHC subjects as well as chimpanzee models.[8,9,31]

This has brought in a state of ambiguity to the scenario of CHC infection as far as functional status of DCs is concerned and justified for a systematically designed study to further evaluate this aspect. Our results from the present study regarding status of mo-DC from CHC patients clearly demonstrate that there is a defect in terms of number and function in this population during the chronic HCV infection. We found that lesser number of monocytes from CHC patients' blood could be differentiated to DCs in presence of IL-4 and GM-CSF as compared to HC, which further fail to mature upon *ex vivo* stimulation. This observation is coherent with our observation (data not shown) and other studies where reduced number of DCs has been reported in CHC patients.[20,32-35] Moreover the DCs also displayed immature phenotype which was demonstrated by their low allostimulatory potential and high phagocytosing capacity after LPS stimulation *ex vivo*. This could be attributed to the viral persistence in these patients as we have earlier reported that the DCs from patients who cleared virus after therapeutic intervention showed a marked improvement in their numerical as well as functional status.[20]

By exposing the monocytes from healthy individuals to HCV-3 specific core and NS5 antigens during differentiation to immature DCs *in vitro*, and by further inducing maturation in presence of LPS, we found that both core and NS5 antigens induced maturation and activation defects in healthy mo-DCs as they failed to upregulate surface expression of HLA-DR, CD83, CD80 and CD86 upon *ex vivo* LPS stimulation. These results clearly elucidate the direct evidence of virus-mediated downmodulation of DC functions in chronic viral hepatitis. Cell culture grown HCV (HCVcc) when incubated with immature mo-DCs has been shown to have deleterious effects on their ability to present the antigen.[15] Recently Landi et al. found that HCV core, when transfected in immature mo-DCs, does not have any inhibitory effect on human DC maturation,[36] while monocytes exposed to high concentrations of HCV proteins during differentiation to dendritic cells had diminished capacity to present the antigen.[37] These observations corroborate our findings, indicating virus-driven mechanisms causing maturation and functional defects in dendritic cells. Moreover, this approach seems to be convincingly closer to the actual *in vivo* situation in which the precursor cells in chronically infected patients would be exposed to various HCV proteins present in the milieu over a long period of time while differentiating, leading to development of defective dendritic cells. Our study, as well as the findings of Krishnadas et al. supports this hypothesis.[37]

Trying to elucidate the possible mechanism, we further investigated the effect of these viral proteins on expression of certain down-modulatory genes such as SOCS3, IDO and PD-L1, which might be playing a significant role in rendering the DCs tolerogenic or maturation defective. We found that in the presence of NS5 and core antigens, the expression of PD-L1 and IDO got upregulated while only NS5, and not core, caused the increase in expression of SOCS3 in the mo-DC that were differentiated in the presence of these viral proteins right from day one.

The HCV protein has earlier been shown to cause upregulation of SOCS3[38] and it has recently been proposed that non-responsiveness to antiviral therapy

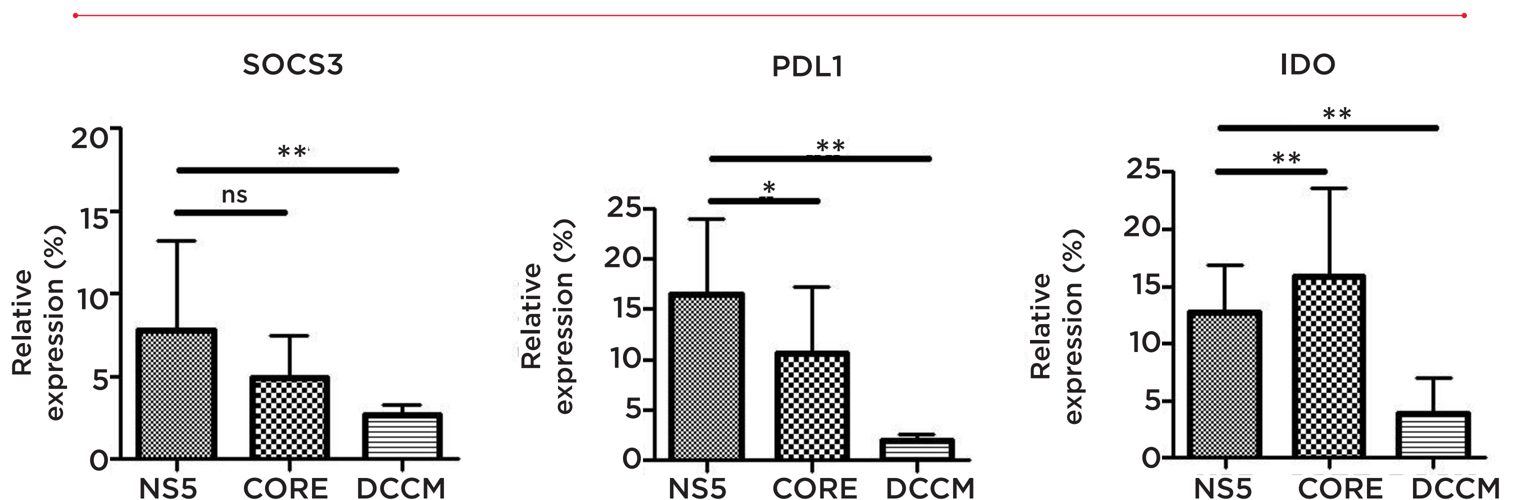

Figure 5. Relative gene expression of SOCS3, IDO and PD-L1 in mo-DCs exposed to HCV-NS5 and Core antigen.

might be related to up-regulation of SOCS3.[39] These reports have used human hepatoma cell line HepG2 or Epstein-Barr virus–transformed lymphoblastoid cell lines derived from peripheral lymphocytes from patients. None of these studies have been executed on human blood derived dendritic cells so far. In addition, a recent study has suggested that increased PD-L1 expression and PD-L1/CD86 ratio may be responsible for the dysfunction of DCs in chronic HCV infection, as it might be correlated with exhaustion of T-cell function.[40] Our results confirm the same hypothesis. Moreover, another recent study highlights the dual nature of dendritic cells in ovarian cancers, where there is strong expression of PD-L1 by the immunosuppressive DCs.[19] The tumour microenvironment resembles the chronic disease as the continuous presence of antigen leads to immune exhaustion in chronic disease as well as cancer. Although IDO has been implicated in causing tolerogencity in DCs elsewhere it has not been linked so far with HCV.[41] Nevertheless, it cannot be denied that HCV might upregulate this gene for its own survival. We have demonstrated for the first time that this gene gets upregulated in mo-DCs raised in presence of HCV proteins suggesting that IDO might also be involved in causing dysfunction in DCs. These results also suggest that various HCV proteins may coordinate their negative modulatory action on DCs to impair T-cell stimulation and facilitate the establishment of chronic HCV infections. It would be really interesting to conduct similar studies on DCs from patients recovering from acute HCV infection and to find whether they have an immunostimulatory profile which might be instrumental in resolving the infection. Nevertheless, the findings elucidate the mechanisms of immune dysfunction in chronic HCV infection, which may be helpful in designing newer strategies of intervention in future.

REFERENCES

1. Donahue JG, Munoz A, Ness PM, Brown DE, Jr., Yawn DH, McAllister HA, Jr., et al. The declining risk of post-transfusion hepatitis C virus infection. N Engl J Med. 1992;327:369-73.

2. Liang TJ, Rehermann B, Seeff LB, Hoofnagle JH. Pathogenesis, natural history, treatment, and prevention of hepatitis C. Ann Intern Med. 2000;132:296-305.

3. Lauer GM, Walker BD. Hepatitis C virus infection. N Engl J Med. 2001;345:41-52.

4. Chen SL, Morgan TR. The natural history of hepatitis C virus (HCV) infection. Int J Med Sci. 2006;3:47-52.

5. Szabo G, Dolganiuc A. HCV immunopathogenesis: virus-induced strategies against host immunity. Clin Liver Dis. 2006;10:753-71.

6. Crotta S, Stilla A, Wack A, D'Andrea A, Nuti S, D'Oro U, et al. Inhibition of natural killer cells through engagement of CD81 by the major hepatitis C virus envelope protein. J Exp Med. 2002;195:35-41.

7. Tseng CT, Klimpel GR. Binding of the hepatitis C virus envelope protein E2 to CD81 inhibits natural killer cell functions. J Exp Med. 2002;195:43-9.

8. Longman RS, Talal AH, Jacobson IM, Albert ML, Rice CM. Presence of functional dendritic cells in patients chronically infected with hepatitis C virus. Blood. 2004;103:1026-9.

9. Longman RS, Talal AH, Jacobson IM, Rice CM, Albert ML. Normal functional capacity in circulating myeloid and plasmacytoid dendritic cells in patients with chronic hepatitis C. J Infect Dis. 2005;192:497-503.

10. Piccioli D, Tavarini S, Nuti S, Colombatto P, Brunetto M, Bonino F, et al. Comparable functions of plasmacytoid and monocyte-derived dendritic cells in chronic hepatitis C patients and healthy donors. J Hepatol. 2005;42:61-7.

11. Barnes E, Salio M, Cerundolo V, Francesco L, Pardoll D, Klenerman P, et al. Monocyte derived dendritic cells retain their functional capacity in patients following infection with hepatitis C virus. J Viral Hepat. 2008;15:219-28.

12. Kanto T, Hyashi N, Takehara T, et al. Impaired allostimulatory capacity of peripheral blood dendritic cells recovered from hepatitis C virus-infected individuals. J Immunol 1999;162:5584–91.

13. Auffermann-Gretzinger S, Keeffe E, Levy S. Impaired dendritic cell maturation in patients with chronic, but not resolved, hepatitis C virus infection. Blood 2001;97:3171–6.

14. Bain C, Fatmi A, Zoulim F, et al. Impaired allostimulatory function of dendritic cells in chronic hepatitis C infection. Gastroenterology 2001;120:512–24.

15. Kaimori A, Kanto T, Kwang Limn C, et al. Pseudotype hepatitis C virus enters immature myeloid dendritic cells through the interaction with lectin. Virology 2004;324:74–83.

16. Miyazaki M, Kanto T, Inoue M, et al. Impaired cytokine response in myeloid dendritic cells in chronic hepatitis C virus infection regardless of enhanced expression of Toll like receptors and retinoic acid inducible gene-I. J Med Virol. 2008;80:980–8.

17. Krebs DL, Hilton DJ. SOCS proteins: negative regulators of cytokine signaling. Stem Cells. 2001;19:378-87.

18. Martin-Orozco N, Wang, YH, Yagita H, Dong C. Cutting Edge:Programmed death (PD) ligand-1/PD-1 interaction is required for CD8+T cell tolerance to tissue antigens. J Immunol 2006;177:8291-5.

19. Scarlett UK, Rutkowski MR, Rauwerdink AM, et al. Ovarian cancer progression is controlled by phenotypic changes in dendritic cells. J Exp Med. 2012;209:495-506.

20. Rana D, Chawla YK, Duseja A, Dhiman RK, Arora SK. Functional reconstitution of defective myeloid dendritic cells in chronic hepatitis C infection on successful antiviral treatment. Liver International. 2012;32:1128-37.

21. Schneider-Schaulies S, Klagge IM, ter Meulen V. Dendritic cells and measles virus infection. Curr Top Microbiol Immunol. 2003;276:77-101.

22. Servet-Delprat C, Vidalain PO, Valentin H, Rabourdin-Combe C. Measles virus and dendritic cell functions: how specific response cohabits with immunosuppression. Curr Top Microbiol Immunol. 2003;276:103-23.

23. Barron MA, Blyveis N, Palmer BE,

MaWhinney S, Wilson CC. Influence of plasma viremia on defects in number and immunophenotype of blood dendritic cell subsets in human immunodeficiency virus 1-infected individuals. J Infect Dis. 2003;187:26-37

24. Donaghy H, Gazzard B, Gotch F, Patterson S. Dysfunction and infection of freshly isolated blood myeloid and plasmacytoid dendritic cells in patients infected with HIV-1. Blood. 2003;101:4505-11.

25. Moutaftsi M, Mehl AM, Borysiewicz LK, Tabi Z. Human cytomegalovirus inhibits maturation and impairs function of monocyte-derived dendritic cells. Blood.2002;99:2913-21.

26. Raftery MJ, Schwab M, Eibert SM, Samstag Y, Walczak H, Schonrich G. Targeting the function of mature dendritic cells by human cytomegalovirus: a multi-layered viral defense strategy. Immunity. 2001;15:997-1009.

27. Beckebaum S, Cicinnati VR, Dworacki G, Muller-Berghaus J, Stolz D, Harnaha J, et al. Reduction in the circulating pDC1/pDC2 ratio and impaired function of ex vivo-generated DC1 in chronic hepatitis B infection. Clin Immunol. 2002;104:138-50.

28. Beckebaum S, Cicinnati VR, Zhang X, Ferencik S, Frilling A, Grosse-Wilde H, et al. Hepatitis B virus-induced defect of monocyte-derived dendritic cells leads to impaired T helper type 1 response in vitro: mechanisms for viral immune escape. Immunology. 2003;109:487-95.

29. Ninomiya T, Yoon S, Sugano M, Kumon Y, Seo Y, Shimizu K, et al. Improvement of molar ratio of branched-chain amino acids to tyrosine (BTR) associated with liver fibrosis in chronic hepatitis C patients treated with interferon-alpha. Dig Dis Sci. 1999;44:1027-33.

30. Kanto T, Hayashi N. Involvement of dendritic cell dysfunction in the persistence of hepatitis C virus infection. Nippon Rinsho. 2004;62 Suppl 7(Pt 1):170-4.

31. Larsson M, Babcock E, Grakoui A, Shoukry N, Lauer G, Rice C, et al. Lack of phenotypic and functional impairment in dendritic cells from chimpanzees chronically infected with hepatitis C virus. J Virol. 2004;78:6151-61.

32. Wertheimer AM, Bakke A, Rosen HR. Direct enumeration and functional assessment of circulating dendritic cells in patients with liver disease. Hepatology. 2004;40:335-45.

33. Della Bella S, Riva A, Tanzi E, Nicola S, Amendola A, Vecchi L, et al. Hepatitis C virus-specific reactivity of CD4+-lymphocytes in children born from HCV-infected women. J Hepatol. 2005;43:394-402.

34. Della Bella S, Crosignani A, Riva A, Presicce P, Benetti A, Longhi R, et al. Decrease and dysfunction of dendritic cells correlate with impaired hepatitis C virus-specific CD4+ T-cell proliferation in patients with hepatitis C virus infection. Immunology. 2007;121:283-92.

35. Saito K, Ait-Goughoulte M, Truscott SM, Meyer K, Blazevic A, Abate G, et al. Hepatitis C virus inhibits cell surface expression of HLA-DR, prevents dendritic cell maturation, and induces interleukin-10 production. J Virol. 2008;82:3320-8.

36. Landi A, Yu H, Babiuk LA, van Drunen Littel-van den Hurk S. Human dendritic cells expressing hepatitis C virus core protein display transcriptional and functional changes consistent with maturation. J Viral Hepat. 2010; doi:10.1111/j.1365-2893.2010.01357.

37. Krishnadas DK, Ahn JS, Han J, Kumar R, Agrawal B. Immunomodulation by hepatitis C virus-derived proteins: targeting human dendritic cells by multiple mechanisms. Int Immunol. 2010;22:491-502.

38. Persico M, Capasso M, Persico E, Svelto M, Russo R, Spano D, et al. Suppressor of cytokine signaling 3 (SOCS3) expression and hepatitis C virus-related chronic hepatitis: Insulin resistance and response to antiviral therapy. Hepatology. 2007;46:1009-15.

39. Kim KA, Lin W, Tai AW, Shao RX, Weinberg E, De Sa Borges CB, et al. Hepatic SOCS3 expression is strongly associated with non-response to therapy and race in HCV and HCV/HIV infection. J Hepatol. 2009;50:705-11.

40. Shen T, Chen X, Chen U, Xu Q, Lu F, Liu S. Increased PD-L1 expression and PDL1/CD86 ratio on dendritic cells were associated with impaired dendritic cells function in HCV infection. J Med Virol. 2010;82:1152–9.

41. Munn DH. Indoleamine 2,3-dioxygenase, tumor-induced tolerance and counterregulation. Curr Opin Immunol. 2006;18:220-25.

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