Review Article

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Success of antiviral therapy in chronic hepatitis C infection relates to functional status of myeloid dendritic cells

Deepa Rana, Yogesh Chawla* & Sunil K. Arora

*Departments of Immunopathology & *Hepatology, Postgraduate Institute of Medical Education & Research, Chandigarh, India*

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Chronic hepatitis C infection poses a major global health predicament and appears to be potent threat to mankind. The treatment in wide use is interferon/ribavirin combination therapy which is generally effective in about 60-70 per cent of patients carrying genotype 3 and causes significant morbidity. The response to therapy is largely guided by limited number of factors such as genotype of virus, rapid virological response, ethnicity, pre-therapy viral load, *etc*. While involvement of host genetic factors has been a major focus of research in playing an important role in the outcome of disease, the role of immune system cannot be marginalized. Poor cellular trafficking and suboptimal T cell responses in liver, the hall marks of chronic hepatitis C virus infection, might be attributed to defective antigen presentation. Various immunological factors, both innate and adaptive, play role in the pathogenesis of the disease and become dysfunctional in active disease. Recent reports suggest the major impact of functional and numerical status of dendritic cells in deciding the fate of antiviral therapy. In this review we take a look at the involvement of dendritic cells in playing an important role in the response to therapy.

Key words Antiviral therapy - dendritic cells - HCV infection - hepatitis C virus - innate immune response - rapid virological response

Introduction

By the end of 20th century, hepatitis C virus (HCV) started to emerge as a global threat with around 170 million people infected worldwide[1]. The virus is a blood borne pathogen and the high risk group includes patients undergoing blood transfusions, intravenous drug users, renal patients undergoing frequent dialysis, people involved with tattooing and unsafe sexual activity. The infection with virus can follow either an acute and/or subsequently in majority of the cases a chronic clinical profile. While the acute infection is resolved spontaneously in a few weeks' time without any therapeutic intervention, the most disturbing aspect of this infection is that the spontaneous viral clearance is atypical, with nearly 54-86 per cent of the infected individuals progressing to chronic hepatitis[2-4]. Chronic HCV infection is defined as the persistence of HCV RNA in the blood for at least 6 months and in its more aggressive form in about 5-10 per cent cases progressing to cirrhosis and hepatocellular carcinoma (HCC)[3].

This virus has a single-stranded RNA of positive polarity of 9.6 kb nucleotides. The RNA contains one long open reading frame, encoding a polyprotein of approximately 3000 amino acids flanked by

un-translated regions (UTRs) on both 3' and 5' ends. Owing to high mutability due to error prone reverse-transcription, the HCV genome, has high degree of genetic variability. To date, at least six genotypes and more than 120 subtypes of the virus have been identified, and more are in the process of being characterized. Genotypes 1a (HCV-1a), 1b, 2a, 2b, and 3a are distributed globally and account for the majority of HCV infections worldwide[5]. HCV-1b, the most common genotype worldwide, is also the dominant genotype in Asia-Pacific, particularly in Japan, South Korea, China and Taiwan. HCV-2a and 2b are also commonly distributed, particularly in Japan, South Korea and Southern Taiwan[6]. While genotype-3 of HCV is more prevalent in many countries of Asia Oceania[78], the genotype-4 is predominantly found in North Africa and Middle East. Genotype-5 is limited to South Africa[9]. HCV 6-11 have been detected in South-East Asia recently. Genotypes 7-11 appear to be the variants of HCV-3 (genotype 10a) and HCV-6a (genotypes 7, 8, 9, 11), and have been re-classified as subtypes of genotypes 3 and 6[6]. In India, genotype 3 predominates in the north, east and west India while genotype 1 is more common in southern parts of India[8,10-12].

Treatment and response to antiviral therapy

Antiviral agents like interferon (IFN)-α and nucleoside analogue like ribavirin, have been widely used for the treatment of chronic HCV infection to prevent the development of cirrhosis of liver and hepatocellular carcinoma. In addition to causing direct inhibition of viral replication, these agents modulate antiviral immune responses, which greatly contribute to the successful therapeutic response. The response to the therapy is largely dependent on the genotype of the virus involved. HCV genotype 1b is less sensitive to interferon as compared to genotypes 2 and 3[13]. However, the high cost of therapy makes it far less affordable for many affected individuals, especially in the resource limited countries like India. There have been a few reports available in literature stating the superior response to IFN-based therapies in Asian patients as compared to Caucasians, Hispanics and Afro-Americans in the corresponding HCV genotype[14,15].

The outcome or the response to the treatment can be measured using three parameters: *(i)* biochemical outcome measured in terms of normalization of serum alanine transaminase (ALT) levels, *(ii)* virological outcome defined by absence of HCV RNA from serum by a sensitive PCR based assay, and *(iii)* histological outcome measured as <2 point improvement in necro-inflammatory score with no worsening in fibrosis score[16,17]. So far, the virological response is considered to be the most reliable one. Various definitions used to describe the virological response to therapy are outlined in the Table[18].

Predictors of response to therapy

Clinicians and virologists across the world have been making endless efforts to look for certain reliable markers which could help in predicting the likelihood of a patient in achieving sustained virological response (SVR). As mentioned above, the viral genotype has

Table. Terminology used to describe various responses to antiviral therapy

Rapid virological response (RVR)	Clearance of HCV from serum by week 4 using a sensitive PCR-based assay
Early virological response (EVR)	≥2 log reduction in HCV RNA level compared to baseline HCV RNA level or HCV RNA negative at treatment week 12
Sustained virological response (SVR)	HCV RNA negative 24 weeks after cessation of treatment
End of treatment response (ETR)	HCV RNA negative at the end of 24 or 48 weeks of treatment
Breakthrough	Reappearance of HCV RNA in serum while still on therapy
Relapse	Reappearance of HCV RNA in serum after therapy is discontinued
Non response	Failure to clear HCV RNA from serum after 24 weeks of therapy
Partial non response	2 log decrease in HCV RNA but still HCV RNA positive at week 24
Null non response	Failure to decrease HCV RNA by < 2 logs after 24 week of therapy

Source: Ref. 18

been one of the most consistently used parameters to predict SVR. Patients infected with genotype 1 are less likely to achieve SVR as compared to those with genotypes 2 and 3. Another important parameter which decides the response to therapy is the pre-treatment HCV RNA level. It has been reported that patients with viral load less than 600,000 IU/ml have higher chances of attaining SVR[19].

Apart from these two markers there have been other less reliable baseline parameters linked with a favourable response including the doses of peginterferon (1.5 versus 0.5 µg/kg/week) and ribavirin (>10.6 mg/kg), age <40 yr, non-African-American race, female gender, lower body weight (<75 kg), the absence of insulin resistance, elevated ALT levels (three-fold higher than the upper limit of normal), and the absence of bridging fibrosis or cirrhosis on liver biopsy[20-22].

Recently, interleukin (IL) 28 polymorphism has also been highlighted as one of the reliable predictors of response to therapy. The chances of attaining an SVR with Peg-IFN and ribavirin and of spontaneous resolution of HCV infection differ depending on the nucleotide sequence near the gene for IL28B on chromosome 19[23,24]. The detection of the C or T allele at positions 12979860 is found to be highly predictive. Interestingly, the CC genotype is found more than twice as frequently in persons who have spontaneously cleared HCV infection than in those who had progressed to chronic hepatitis-C infection (CHC)[23].

Once the treatment starts, a four week rapid virological response (RVR) has been the single best predictor of an SVR for anti-HCV treatment with peg-interferon and ribavirin combination therapy, irrespective of HCV genotype[25,26]. Further, depending upon the RVR, the duration of treatment can be shortened or extended[16,25,26].

Immunopathogenesis of HCV infection

HCV is not directly cytopathic for the hepatocytes, and brings out progressive liver lesions resulting in end-stage liver disease unless eradicated effectively. It is noteworthy that the cellular immune response to virus is thought to be responsible for both viral clearance and progression to liver disease. However, the patients who successfully resolve the acute phase, mount a multi-epitopic polyclonal cytotoxic T lymphocyte (CTL) response to several viral antigens. In a study on injection drug users, those who resolved previous HCV infection were 12 times less likely to be reinfected to develop persistent infection than people infected for the first time. The median peak of HCV RNA levels in those who became re-infected, were two logs lower than individuals who got infected for the first time to develop chronic infection. These findings suggest that at least in some individuals, a protective immunity develops against the virus, which is capable of complete or partial control of HCV infection[17].

In contrast, this response is absent, short-lived or much weaker in patients who are unable to clear the virus and progress on to become chronically infected. Thus, it is rational to conclude that the outcome of HCV infection (viral clearance versus viral persistence) is determined primarily by the vigour and quality of the cellular immune response[27]. Once the virus survives the initial assault by the host immune response, it uses several strategies to nullify the selective immunological pressure during the later phases of infection. The virus alters its antigenic epitopes recognized by T cells and neutralizing antibodies to escape immune surveillance[3, 4] It may also subvert immune functions in an antigen specific manner, from innate to adaptive immunity.

Innate immune response in HCV infection

After the virus infects the liver, viral replication continues and viral particles are continuously released into the circulation. It is considered that innate immunity not only provides an immediate response to viral infection, helping to clear the virus during initial period, but also shapes the nature of the adaptive immune response to viral infection[28]. The different components of both innate and adaptive immune response are so interconnected that the defects of one or more may lead to the establishment of chronicity of HCV infection.

The first line of defense is provided by the natural killer (NK) and NKT cells, as their numbers are shown to be relatively increased in the liver compared to the periphery. These cells are activated in the liver, where expression of IFNα and IFN-inducible genes are extremely high during the early phase of hepatitis virus infection[29]. Activated NK and NKT cells eliminate virus-infected hepatocytes directly by cytolytic mechanisms as well as by secreting IFNγ, which inhibits replication of HCV through a non-cytolytic mechanism[30]. An important role for NK cells in early HCV infection has recently been suggested by Khakoo *et al*[31] who showed that the activation threshold of NK cells might be lower in HCV patients, which might support HCV clearance. Interaction of NK cells with HCV E2 protein by its

receptor, CD81, inhibits NK cell activity[32], whereas it causes stimulation of T and B lymphocytes. NK cells from HCV-infected patients are impaired in their capacity to activate dendritic cells due to the production of IL-10 and tumour growth factor (TGF)-β[33]. Not surprisingly, HCV has evolved multiple strategies to counter the host's NK cell response. Peripheral blood NK cells are reduced in chronic HCV patients compared to healthy individuals[33]. The reduction in NK cell frequency may be a consequence of HCV infection, or a predisposing factor to chronic HCV disease, and both hypotheses have some scientific support. In individuals with chronic HCV infection, a reduction in peripheral blood NK cell frequency in individuals with chronic HCV as compared to spontaneous resolvers has been noted, while NK cell frequency increases following successful antiviral therapy[34,35]. IL-15, a pivotal cytokine for NK cell development, proliferation and function, may be relevant to this observation. Meier *et al*[36] showed a significant reduction in IL-15 levels in HCV patients as compared to healthy controls and demonstrated that exogenous IL-15 rescued their NK cells from apoptosis, increasing *ex vivo* proliferation and function. Several studies have reported an increase in circulating $CD56^{bright}$ NK cells, but not $CD56^{dim}$ component in this population in chronic HCV infection as compared to acute cases[37,38].

Adaptive immune response in HCV infection

While most studies on the human adaptive immune responses against HCV are concentrated on the T cell responses, the role of neutralizing antibodies in the protective immunity against HCV has only recently gained attention[39,40]. Even though there are less number of cases with self-limited HCV infection that have been studied so far, a pattern of vigorous and strong HCV specific T cell response has been observed[40-42]. Such responses were observed only during the early phase of acute disease[41,43] and sustained for long after the clearance of HCV[40]. These responses were generally targeted at multiple major histocompatibility complex (MHC) restricted epitopes rather than a dominant epitope[42]. In contrast, patients with chronic HCV infection usually have weak or defective T cell responses against HCV, as indicated by low frequencies of the specific T cells[44], short-lived responses[44,45], narrowly targeted epitopes[44], as well as defects in the effector functions of the specific T cells[45]. HCV specific $CD8^+$ T cells in chronic hepatitis C patients possess lesser capacity to proliferate and produce lesser amounts of IFNγ in response to HCV antigens[46] (Fig. 1).

Taken together, these studies strongly suggest that the host T cell responses are a key factor in determining the outcome of HCV infection. Interestingly, during the acute phase of self-limiting HCV infection, there is a brief span of dysfunction of HCV specific $CD8^+$ T cells[43,44], suggesting that a transient down-modulation of the effector functions of specific $CD8^+$ T cells may be a host strategy to keep a check at the tissue damage, caused by the cytotoxic $CD8^+$ T cells at the early stage of infection when viral replication is at its peak. On the contrary, there is a report which failed to find a relationship between CTL response and HCV eradication[47].

Although, little is known regarding the exact cause for the failure of the host's adaptive immunity against HCV, many of these defects can be attributed to deficiencies in innate immune responses leading to progression to chronic HCV infection. To understand the mechanism behind apparently weak or abnormal T cell immunity to HCV in chronic hepatitis, it is important to explore the initial events leading to the antiviral adaptive immunity, which mainly pertains to the processing and presentation of viral antigens and onward signaling. In this scenario it seems pertinent to investigate the role of dendritic cells during this situation.

Dendritic cells in HCV related chronic hepatitis

The immune system works as a well-coordinated squad of different types of cells whose final mission is to eliminate invading pathogens. Dendritic cells (DCs) orchestrate the entire course of events as these are essential for activation of both T helper cells and cytotoxic T-lymphocytes. Dendritic cells not only form an important component of the innate immune surveillance but also play a significant role in shaping the nature of adaptive immune response against various pathogens and tumour cells. Among different subsets of DCs, myeloid-derived DCs (mDCs) are the most important antigen presenting cells with the capability to capture and process antigens, express lymphocyte co-stimulatory molecules, migrate to lymphoid organs and secrete cytokines to initiate B and T cell responses[48]. While the plasmacytoid dendritic cells (pDCs) play a unique role in secreting large amounts of type I interferons, their efficiency as antigen-presenting cells (APCs) has not been completely understood. However, it has been reported that during viral infection pDCs not only secrete immunomodulatory cytokines, but also recognize infected cells and function as antigen

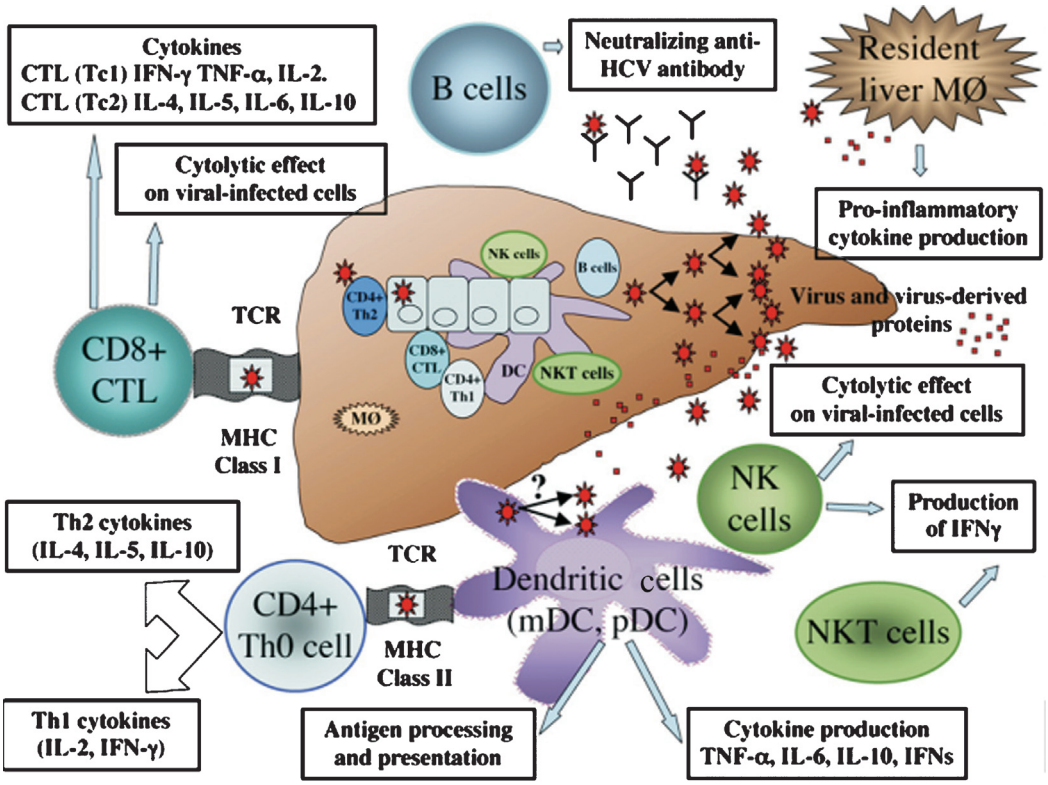

Fig. 1. The immune response in viral hepatitis C involves both the innate and adaptive immune system. Innate immunity involves activation of resident liver macrophages (MØ), dendritic cells (DCs), natural killer (NK) cells, and NKT cells, whereas $CD4^+$ T cells, $CD8^+$ T cells, and B lymphocytes are effectors of adaptive immunity. CTL, cytotoxic T lymphocyte; HCV, hepatitis C virus; IFN, interferon; IL, interleukin; MHC, major histocompatibility complex; TNF, tumour necrosis factor.
(Reproduced with permission from John Wiley and Sons Ltd., New Jersey, USA [*Clin Liver Dis* 2006; *10* : 753-71][17].

cross-presenting cells to trigger the antiviral immune response with specific reference to activation of virus specific CD8+ cells[49,50].

Besides activating lymphocytes, DCs also have the important function as mediators of peripheral immune tolerance and maintenance of immune homeostasis[48]. The two conflicting roles of DCs have been related to their different patterns of maturation and cytokine production[51,52]. The functions of DCs are precisely controlled to either activate or tolarize the T cells depending on the nature of antigens which may be foreign or self. Any perturbation to this control may lead to serious consequences including impaired immunity to infecting pathogens, causing persistent infections. Many viruses have devised strategies to counteract antiviral immunity by down-modulating the functions of DCs.

The response of dendritic cells to any infection in general and initial HCV infection in particular, at early stage is critical in determining the course and outcome of the disease. However, studying the immune responses in patients at early stage of HCV infection is difficult because the acute infection is usually asymptomatic, and by the time the disease is identified, it has already passed the initial acute phase and progressed into chronic infection. It has been shown that HCV specific T cells appear early after infection inducing strong T cell response during acute HCV infection[40]. To activate HCV specific T cells and induce strong antiviral immune responses, DCs have to respond to HCV viral proteins. Moreover, HCV seems to successfully disrupt the coordinated activity of the innate immune cells to result in deficient adaptive immune response and prevent pathogen elimination.

There are controversial reports on the function of myeloid DCs in chronic HCV infection. While a couple of studies have observed no functional defects in mDCs in HCV-infected individuals[53-55], the findings from other studies indicate that blood-derived mDCs from HCV infected patients are functionally and

numerically impaired[55-60]. An increase in the frequency of mDCs during acute HCV infection has been shown to be associated with viral clearance in a small study, whereas the deficiency of mDC might be an important factor in the development of chronic state of the infection[61]. The mDCs of patients with chronic HCV infection have impaired abilities to stimulate allogeneic $CD4^+$ T cells and to produce IL-12p70 compared with those from healthy volunteers[62]. Although DCs can bind to HCV and may even get infected with this virus and possibly act as HCV reservoirs[63], but there has not been much evidence of viral replication within these cells.

The phenotype and cytokine production profile of bone marrow–originated mDCs can be generated from peripheral blood monocytes [monocyte-derived DCs (mo-DCs)] in the presence of IL-4, granulocytes-macrophage colony-stimulating factor (GM-CSF) and serve as an *ex vivo* model for mDCs. Data on the function/phenotype of mo-DCs during acute HCV infection are scarce, and the opinions on the function of mo-DCs in chronic HCV are divided. Although Longman *et al*[53] reported normal phenotypic characteristics and allogeneic functions in mo-DCs in humans and Larsson *et al*[64] identified similar findings in a non human primate model of chronic HCV infection, the majority of researchers find functional abnormalities in mo-DCs of humans with chronic HCV infection[65-67]. We and others have demonstrated that decreased expression of the co-stimulatory molecules CD83 and CD86, increased production of IL-10 and decreased levels of IL-12, may lead to impaired capacity to stimulate allogeneic T cells *in vitro*, and may cause defective maturation of mDCs in patients with chronic HCV infection regardless of viral genotype or patient age/sex across a wide geographic localization of patients[65-68].

In vitro experiments have effectively shown that upon exposure of human mo-DCs to cell culture-grown HCV (HCVcc) genotype 1a or 2a, there was a significant inhibition of DC maturation and was associated with low HLA-DR expression and high production of IL-10. Furthermore, DCs exposed to HCVcc were impaired in their functional ability to stimulate antigen-specific $CD4^+$ and $CD8^+$ T cell responses[69]. DCs, upon exposure to HCV core protein, showed downregulation of major histocompatibility molecules (HLA-DR) and co-stimulatory molecules (CD80, CD86) and induction of IL-10 producing T cells[70]. The HCV may induce modulation of the cytokine response, especially of increase in IL-10 and decrease in IL-12 production that may result in altered HCV specific T cell responses in chronic HCV infection. In another report, lipopolysaccharide (LPS)-stimulated mo-DC from HCV patients showed a mature phenotype but with a significantly decreased expression of IL-12, could be responsible for the Th1 defect since the intensity of the Th1 defect was directly related to the intensity of IL-12 decrease[71]. We have also recently shown that the monocytes from healthy donors when differentiated in the presence of HCV core and NS5 proteins developed maturation defects as these could not upregulate the HLA-DR alongwith CD83, CD80 and CD86 on toll like receptor (TLR)-4 mediated stimulation[68]. Peripheral blood DC of chronically infected patients have been shown to express less IL-12 than control cells from healthy individuals[60] and a small population of circulating myeloid DC displayed an impaired response to specific TLR stimulation[72]. Viral components can either bind to TLR and activate their signaling pathway or block TLR function by interfering with intracellular intermediates.

Dendritic cells and antiviral therapy

There have been a few studies in which role of DCs have been studied in context of antiviral therapy[57,66,73]. In general, it has been demonstrated that the early response to antiviral treatment leads to the improvement of DC functions which was not observed in patients who remained viraemic on therapy. Evaluating the defects in maturation and chemotaxis in peripheral mDCs and pDCs from therapeutic responders and non-responders, revealed that successful antiviral therapy normalized many phenotypic and functional abnormalities of pDCs from patients with HCV infection[74].

Maturation defects were detected in the mo-DCs from genotype-3 infected chronic hepatitis C (CHC) patients when checked before the initiation of therapy, however, it was very interesting to find that all those CHC patients whose mo-DCs could upregulate the surface expression of activation (CD83), and co-stimulatory (CD80 and CD86) molecules on their surface after TLR-4 mediated stimulation *ex-vivo* even before the initiation of therapy, were able to subsequently achieve SVR after completion of antiviral treatment, while the patients whose cells showed an inability to upregulate these molecules initially, did not achieve SVR later on as well[68]. This suggested that the response to antiviral therapy in CHC is somehow related to the functional status of DCs. This finding is in agreement with a previous report wherein it was demonstrated that the pDCs were functionally activated in terms of increased

chemotaxis even before therapy in patients who subsequently achieved SVR, suggesting that increased baseline inflammation is associated with failure to respond to the antiviral therapy[74] (Fig. 2).

Furthermore, DCs from patients who achieved SVR in our study[68] demonstrated an improvement in the maturation capacity after completion of treatment. On the other hand, non responders to therapy continued to show maturation defects in their mo-DC. The functional improvement of mo-DCs was directly related to the successful clearance of virus in responders upon completion of therapy[68] (Fig. 3). It clearly suggests that defect seen in chronically infected patients is limited to the period of active viral replication but can be resolved after viral clearance. This observation is corroborated by similar reports demonstrating clearance of HCV RNA from circulating DCs in CHC patients who achieved rapid virological response (RVR)[57] indicating that therapy with IFNα may induce improved DC function by reducing the load of HCV *in vivo*. However, the role of antiviral agents, especially that of type-1 IFNs on maturation and activation of DCs, is controversial. It has been proven that *in vitro* addition of IFNα to DC cultures of CHC patients improves their function in terms of increased expression of activation and co-stimulatory molecules[75,76]. On the other hand, McRae *et al*[77] have shown that IFNα and β inhibit the *in vitro* differentiation of mo-DCs from normal subjects. It has been suggested that ribavirin is not strictly antiviral in its action, but rather alters the T-cell balance in the immune system and attenuates the DC function *in vitro* and *in vivo*[78].

Besides the expression of co-stimulatory and MHC class II molecules on their surface, DCs also secret many cytokines, as a direct evidence of their maturation and activation. A few reports have demonstrated that purified DCs or mo-DCs from CHC patients showed lower IL-12 and higher IL-10 production in response to poly-IC or TNFα stimulation *ex vivo*[79,80]. However, no difference in cytokine profile from CHC and HC was found in another report[55]. IL-10 has been shown to be an important player in the pathogenesis of HCV infection, being induced by various HCV antigens[81]. We also reported recently that the mo-DCs from non-responders to antiviral therapy were not able to secrete proinflammatory cytokines such as IL-1β and IL-12 in response to LPS stimulation but showed an increased IL-10 and TNFα production[68] (Fig. 4). This aberrant cytokine profile also conforms to immature phenotype of mo-DCs in these patients. Consequently, the patients who did not achieve SVR also had high IL-10 secreting mo-DCs, even prior to the start of antiviral treatment and continued to have that trend even after completion of therapy. This finding implies the possibility of the tolerogenic signals by immature DCs under the influence of IL-10 in these individuals which might be responsible for their inability to ultimately clear the virus. The persistence of high vireamia in CHC patients who failed to achieve SVR might be a possible reason behind the abnormal cytokine secretion. On the other hand, the viral clearance in SVR+ patients alleviated the 'stress' causing the DCs to secrete pro-inflammatory cytokines. However, the immunomodulatory role of IFNα/ribavirin therapy cannot be ignored. A previous *in vitro* study had revealed that IFNα and ribavirin act synergistically to normalize the functions of defective dendritic cells, while the IFNα enhances the production of IL-12 and TNFα, the ribavirin at the same time suppresses the production of IL-10, thereby tilting the balance towards Th1 response[55].

Divergent conclusions have been drawn on *in vitro* established mo-DCs with regard to allostimulatory capacity of these cells in mixed lymphocyte reaction (MLR) assay[53,55]. In our study, LPS stimulated mo-DCs from CHC patients exhibited low allostimulatory capacity indicating impaired antigen presentation on *ex vivo* stimulation. This was expected as DCs from CHC patients exhibited low abundance of co-stimulatory molecules (CD80 and CD86) and MHC-II on their surface making them weak antigen presenters[68]. We also observed a significant improvement in allostimulatory capacity of mo-DC in patients who achieved SVR in sharp contrast to those who did not. It has also been shown in other studies that allostimulatory capacity of DC from therapy naïve CHC patients was compromised[80,82]. Yet another report had revealed that DCs from patients who became HCV RNA negative after four weeks of combination therapy improved allogenic T cell stimulating capacity[57]. Some groups have reported that the priming capacity of DCs from CHC towards allogeneic T lymphocytes was comparable to that of HC[53,55]. However, McDonald *et al*[83] using MLR assay have suggested that the defect in DCs is subtle and can be overcome by increasing DC numbers. This would only be possible if patients are given autologous mo-DC therapy in which large numbers of DCs are administered to the patients.

Dendritic cells play a key role in shaping up the adaptive immune response as they can drive the Th1 or Th2 cell responses. The mo-DCs from CHC patients

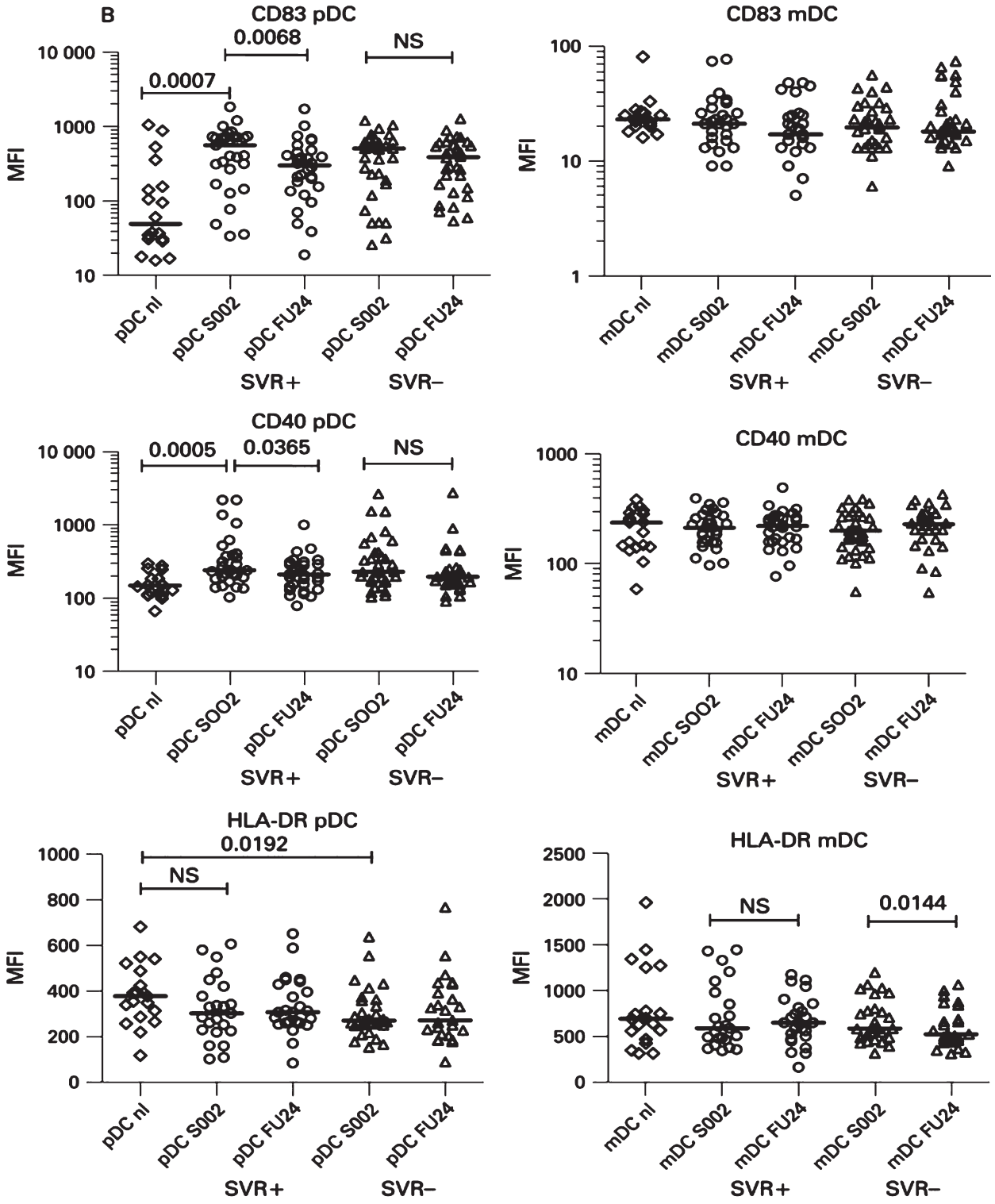

Fig. 2. Differential elevations of chemokine receptors and maturation markers in dendritic cell (DC) subtypes before and after therapy. SOO2 is screening visit 2, 2 wk before initiation of therapy. FU24 is 24 wk after cessation of treatment. Median fluorescence intensity (MFI) values for each patient are shown. The horizontal line is the median. A two-tailed non parametric Wilcoxon signed rank test was used for paired data or the two-tailed Mann-Whitney test was used to calculate *P* values. (B) CD83 MFI on plasmacytoid and myeloid DCs (pDCs, mDCs); CD40 MFI for pDCs, mDCs; HLA-DR for pDCs and mDCs. SVR, sustained virological response.
(Reproduced with permission from British Medical Association, London, UK [*Gut* 2009; *58* : 964-73][74].

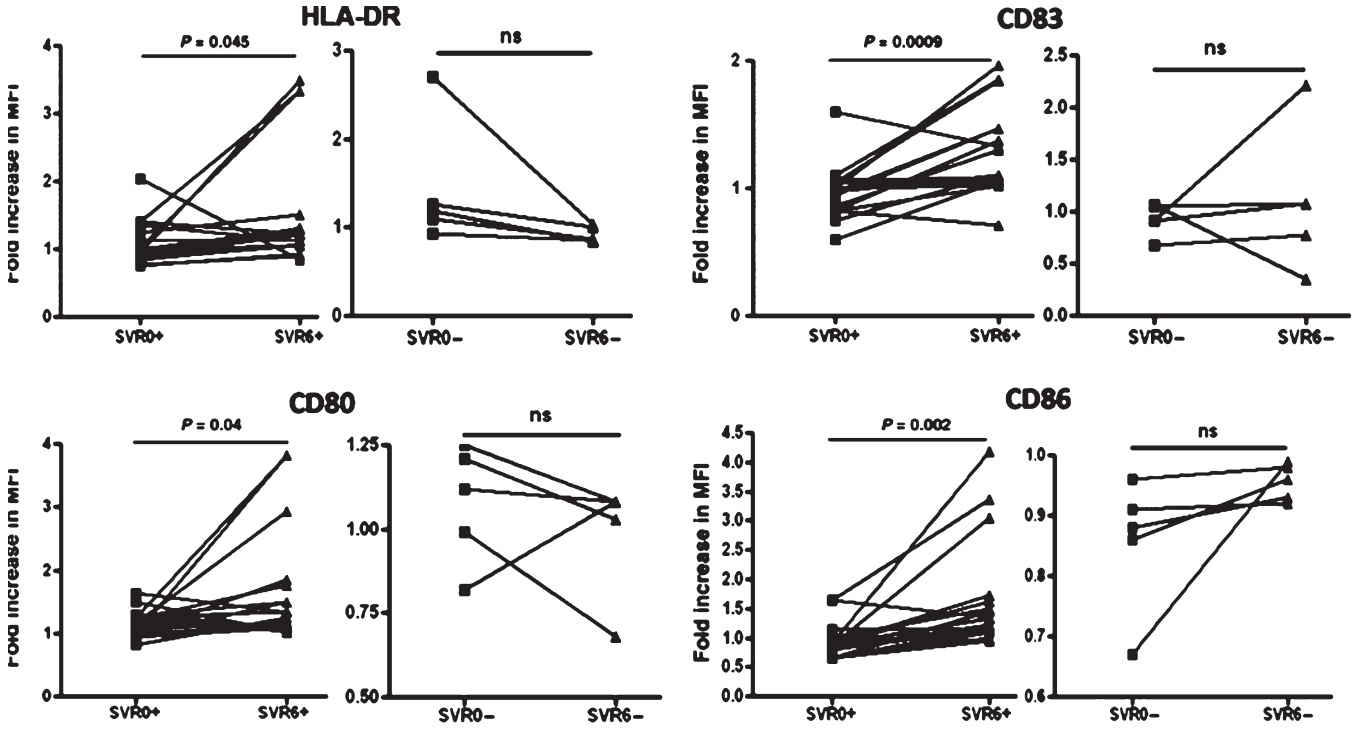

Fig. 3. Effect of treatment on expression of (A) HLA-DR, (B) CD83, (C) CD80 and (D) CD86 on monocyte-derived dendritic cells (mo-DCs) from chronic hepatitis-C patient (CHC)-II pre and post-treatment (in terms of fold increase in mean fluorescence intensity, MFI). Expression of all molecules increases significantly in SVR^+ although no change is seen in SVR^- patients. SVR, sustained virology response.
(Reproduced with permission from John Wiley and Sons Ltd., Philadelphia, USA [*Liver Int* 2012; *32* : 1128-37][68].

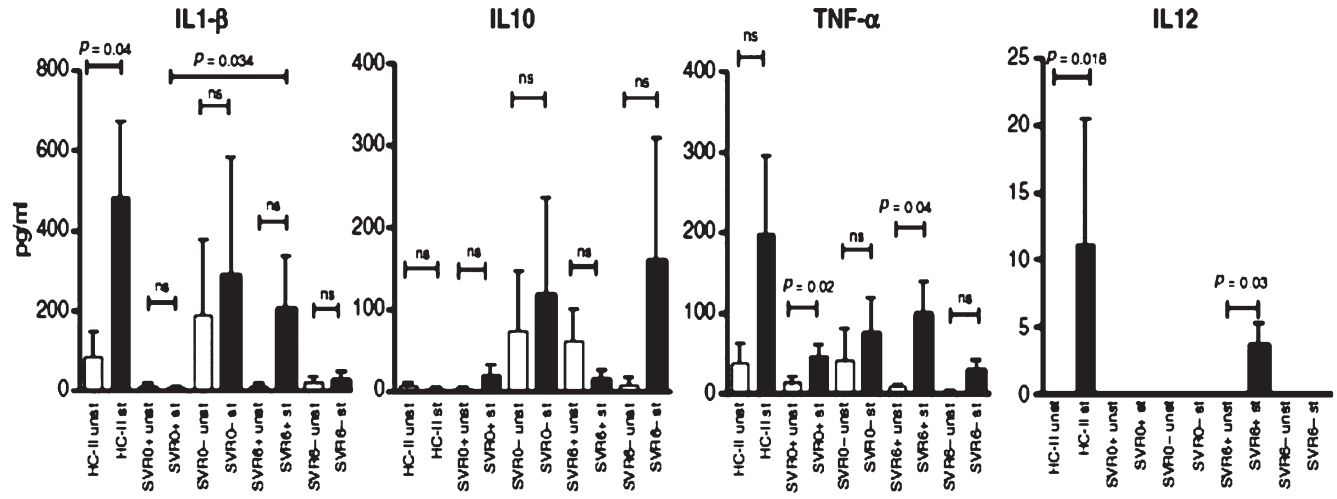

Fig. 4. Cytokine profile of monocyte-derived dendritic (mo-DCs) cells pre- and post-lipopolysaccharide (LPS) stimulation. Secretion of IL1β, IL12 and TNF-α increased significantly in LPS stimulated mo-DCs after therapy in SVR^+ patients, although those from SVR^- patients secreted high IL10 before and after therapy. HC, healthy controls; SVRO+, treatment naïve sustained virological responders; SVR6+, responders post 6 months treatment; SVR6-, non-responders post 6 months treatment.
(Reproduced with permission from John Wiley and Sons Ltd., Philadelphia, USA [*Liver Int* 2012; *32* : 1128-37][68].

secreted lower quantities of Th1 cytokines like IFNγ, IL-2, IL-12 but higher quantities of Th2 cytokines like IL4 and IL10, in MLR supernatants which got reversed on successful treatment in therapy responders, suggesting that the combination therapy of IFNα and ribavirin alters the cytokine profile of maturing DCs to induce Th1 reactivity[55,68]. This highlights the dual role of IL-10 in chronic HCV patients. In fact, the IL-10 released from DC may hamper the Th1 responses, by inducing tolerogenic T-cell responses. The activated T cells, on one hand are fundamental to the eradication of HCV infection, are also responsible for injury to the liver tissue due to activated cytotoxic T cell response against HCV-infected hepatocytes[84]. Therefore, the production of IL-10 in CHC patients may be detrimental to the control of viral infection on one hand but may also be favourable to the prevention of overwhelming liver inflammation and injury on the other.

Based on these observations it can be proposed that the inflammatory state in chronic viral hepatitis impairs the DC functioning by making them incapable to mature. The viral clearance in CHC patients after cessation of treatment improves the status of DCs which further helps in rejuvenating the adaptive immune response in these individuals. The observation that DCs from patients achieving SVR in response to antiviral treatment projected an active and mature phenotype upon LPS stimulation even before start of treatment emphasizes the use of immunological markers in predicting response to therapy. Further studies are warranted to identify the factors that may be responsible for the sustained functional defects in DCs in non-responders, to find an appropriate agent that may help in reversing these defects and achieve viral clearance.

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Reprint requests: Dr Sunil K. Arora, Professor, Department of Immunopathology, Postgraduate Institute of Medical Education & Research, Chandigarh 160 012, India
e-mail: skarora_in@yahoo.com