Chronic hepatitis C infection blocks the ability of dendritic cells to secrete IFN-α and stimulate T-cell proliferation

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SUMMARY. Dendritic cells (DCs) are likely to play a key role in the compromised T-cell function associated with hepatitis C Virus (HCV) infection. However, studies of DC function in HCV-infected patients to date have yielded conflicting findings possibly because of patient and virus heterogeneity. Here, we report the characterization of monocyte-derived DCs obtained from a homogenous cohort of women who were infected with HCV genotype 1b following exposure to contaminated anti-D immunoglobulin from a single donor source. Patients included in the study had not received antiviral therapy and all had mild liver disease. We show that phenotypically normal monocyte-derived dendritic cells (MDDCs) (CD11c+HLA-DR+CD1a+CD14lo) can be obtained from these patients. These cells respond to both Poly(I:C) and LPS, by up-regulating expression of CD86. They secrete high levels of IL-8 and CCL5 in response to LPS, an indication that the MyD88-dependent and MyD88-independent signalling pathways downstream of TLR4 ligation are functioning normally. However, these cells are poor stimulators of T-cell proliferation in allogeneic mixed lymphocyte reactions. Furthermore, patient MDDCs fail to secrete IFN-α in response to poly(I:C) or IFN-β stimulation. Altered DC function may contribute to impaired cellular immune responses and chronicity of disease following HCV infection in this cohort. An effective therapeutic vaccine for chronic HCV infection will most likely need to target DCs to elicit an appropriate cellular response; therefore, it is important to resolve how the DCs of different patient cohorts respond to stimulation via TLRs.

Keywords: cytokines, dendritic cell, hepatitis C virus, IFN-α, T-cell proliferation, toll-like receptor.

Hepatitis C virus (HCV) identified in 1989 as the cause of non-A, non-B hepatitis [1] infects approximately 170 million people worldwide. The current therapy of pegylated IFN-α and ribavirin is not effective in 20–50% of those treated [2], and no vaccine is available. It is predicted that there will be a dramatic increase in the incidence of the complications of chronic HCV infection, such as liver cirrhosis and hepatocellular carcinoma over the next decade [3]. Consequently, there is a great clinical need to develop new anti-viral therapies and vaccines.

While the majority of patients exposed to HCV develop chronic infection, 20–40% of patients with acute hepatitis may resolve the infection and this is associated with a robust polyclonal HCV-specific CD4 T-cell response suggesting a crucial role for adaptive cellular immunity in the clearance of HCV infection [4]. Polyfunctional (i.e. IL-2, TNF-α and IFN-γ secreting) HCV-specific CD8+ T cells expressing high-avidity TCRs with broad antigen specificity are enriched in resolvers [5,6], while chronic HCV infection, on the other hand, is associated with a HCV-specific T-cell response of limited clonal diversity with an exhausted PD-1hi phenotype. Dendritic cell (DC) activity is critical for the generation of a robust polyclonal T-cell response [7]. We explored the hypothesis that the failure of an individual to mount an effective T-cell response during acute HCV infection is because of viral impairment of DC function. Despite extensive studies, there is little overall consensus on this topic. Reduced numbers of circulating DCs, reduced levels of co-stimulatory molecule expression and failure to stimulate allogeneic mixed lymphocyte reactions (MLRs) have been reported by some investigators [8–11] but not others [12–14]. Similarly, impaired responses to toll-like receptor (TLR) agonists by DCs from patients with HCV have been reported [15], while others have demonstrated their ability to induce robust responses [16]. Possible explanations for these divergent results include differing in vitro culture conditions or, more likely, variation in the patient populations studied, e.g. inclusion of patients infected by different routes with different viral genotypes with differing viral loads, variation in the
Monocyte-derived immature DCs (iDCs) were generated in using anti-CD14-labelled magnetic beads as described by the /C2112011 Blackwell Publishing Ltd (>97% CD14+) were purified from PBMCs by positive selection by density gradient centrifugation. Human monocytes

Peripheral blood mononuclear cells (PBMCs) were isolated from each patient. The study received ethical approval from the Research and Ethics Committee at St. Vincent's University Hospital, Dublin, were enrolled in this study. All were infected with HCV genotype 1b after receiving contaminated anti-D immunoglobulin from a single donor source in 1977 and 1978 [17]. All patients included in this study had mild liver disease and had not received anti-viral therapy. We evaluated the phenotype of MDDCs obtained from these patients, their ability to respond to different TLR agonists and stimulate T-cell proliferation.

MATERIALS AND METHODS

Patient cohort

Forty-two patients who attend the Hepatitis C Clinic at St. Vincent's University Hospital, Dublin, were enrolled in this study. All were infected with HCV genotype 1b after receiving contaminated anti-D immunoglobulin from a single source between May 1977 and November 1978 [17]. All patients consumed less than 14 alcohol units per week and had no other risk factors for liver disease or coexisting diseases such as HIV. Informed written consent was obtained from each patient. The study received ethical approval from the Research and Ethics Committee at St. Vincent’s University Hospital and conforms to the guidelines of the 1975 Declaration of Helsinki. All 42 patients tested positive for antibodies to HCV using a third-generation enzyme immunoassay (Abbott Diagnostics, Wiesbaden, Germany), confirmed with an immunoblot assay (RIBA-3) (Chiron Corp., Emeryville, CA, USA). All patients remain chronically infected with the virus as confirmed by testing consistently positive for HCV RNA by a qualitative reverse transcriptase-polymerase chain reaction (RT-PCR) (Ambicor: Roche Diagnostic Systems, Nutley, NJ, USA). None of the patients had received anti-viral therapy at the time of entry to the study.

Leucocyte-enriched buffy coats from anonymous normal donors were obtained with permission from the Irish Blood Transfusion Board, St James's Hospital, Dublin.

Cell isolation and culture

Peripheral blood mononuclear cells (PBMCs) were isolated by density gradient centrifugation. Human monocytes (>97% CD14+) were purified from PBMCs by positive selection using anti-CD14-labelled magnetic beads as described by the manufacturer (Miltenyi Biotec, Bergisch Gladbach, Germany). Monocyte-derived immature DCs (iDCs) were generated in the presence of recombinant IL-4 (Peprotech, Rocky Hill, NJ, USA) and GM-CSF (Leukine Sargramostim, Berlex, Seattle, WA, USA) as previously described [18]. After 6 days in culture, DC phenotype was assessed by flow cytometry using the following mAbs, HLA-DR-PerCP, CD86-PE, CD11c-APC, CD1a-PE, CD14-FITC (BD Biosciences, Erembodegem, Belgium) and CD274-APC (eBioscience, Hatfield, UK). Cells were acquired using a CyAn flow cytometer (Beckman Coulter, Brea, CA, USA) and analysed using FlowJo software (Tree Star, Inc., Ashland, OR, USA).

Effect of HCV infection on dendritic cells

Peripheral blood mononuclear cells were incubated at 37 °C, 5% CO2 for 2 h, and then treated with either recombinant IL-4 or GM-CSF (20 ng/mL) for 15 min. Cells were fixed with Cytolix buffer™ (BD Biosciences) at 37 °C for 10 min, pelleted and then permeabilized with Phosflow Perm Buffer™ (BD Biosciences) for 30 min on ice. Following a washing step using PBS/1% BSA, the cells were stained with mAb specific for phospho-STAT5, phospho-ERK or phospho-STAT6 (BD Biosciences) and analysed by flow cytometry.

Mixed lymphocyte reaction

CD3+ T cells were isolated from PBMCs of healthy donors using CD3-labelled magnetic beads (Miltenyi Biotec), and the cells were routinely >95% pure. The T cells were labelled with CFSE (Molecular Probes, Eugene, OR, USA) as previously described [19]. iDCs were treated with 5 μg/mL of poly(I:C) or medium alone for 24 h. iDCs- or poly(I:C)-treated DCs (5000 cells per well) were cultured in triplicate with 50,000 allogeneic T cells in round-bottomed 96-well plates for 5 days. All cells were cultured in RPMI 1640 supplemented with 10% foetal bovine serum, 1% penicillin/streptomycin and 50 μM 2-mercaptoethanol (Invitrogen, Paisley, UK). At day 5, cells were stained with a CD3-PerCP-labelled antibody and the number of cell divisions determined by assessing CFSE dye dilution on the CD3+ cell gate by flow cytometry. Supernatants were removed and frozen at −20 °C for cytokine analyses. Levels of IL-10 (R&D Systems, Minneapolis, MN, USA) and IFN-γ (Peprotech) were determined by enzyme-linked immunoabsorbent assay (ELISA) using commercially available matched antibody pairs.

Analysis of dendritic cell response to toll-like receptor agonists

Immature DCs were adjusted to a concentration of 1 × 10^6 cells/mL and stimulated with optimized concentrations of the following TLR agonists: poly(I:C) (5 μg/mL), poly (I:C) complexed with the transfection reagent LyoVec (1 μg/mL), CL097 (2 μg/mL) or ultrapure E. coli LPS (1 μg/mL) (Invivogen, Toulouse, France) for 18 h. Levels of CCL5, IFN-10, CXCL10 (R&D Systems, Minneapolis, MN, USA), IL-8,
TGF-β (Peprotech) and IFN-α (Bender Medsystems, Vienna, Austria) in cell culture supernatants were determined by ELISA using commercially available matched pairs of mAbs. Expression of HLA-DR, CD86 (BD Biosciences) and CD274 (eBioscience) was assayed by flow cytometry.

In some experiments, cells were stimulated for 4 h and gene expression examined by quantitative RT-PCR. Total RNA was extracted from DCs using the RNeasy kit (Qiagen Ltd, West Sussex, UK) according to the manufacturer’s instructions. RNA yield and quality were assessed using an Agilent 2100 Bioanalyzer (Agilent Technologies, West Lothian, UK). Two micrograms of total RNA from each sample was reverse transcribed to cDNA with oligo-dT primers using SuperScript III first strand synthesis kit (Invitrogen). Reactions were carried out in a total volume of 25 µL with 2 µL of cDNA, 12.5 µL 2x PCR master mix (Stratagene Corp., La Jolla, CA, USA) and 10.5 µL primer/H2O. qRT-PCR was performed using an MX3000P quantitative PCR system (Stratagene Corp.) with the following cycling parameters: 95 °C for 10 min followed by 40 cycles of 95 °C for 30 s, 60 °C for 1 min and 72 °C for 30 s.

Primer sequences used were as follows: IFN-β forward, 5′-gtctctggacaacagggaggttctcaaca-3′, IFN-β reverse, 5′-aagccagaggttctcaaca-3′, IL-29 forward, 5′-ctaggctcctccaggatg-3′, IL-29 reverse, 5′-agtagggctcagcgcataaa-3′, IFN-α2 forward, 5′-gctacacatccacacaggt-3′ and IFN-α2 reverse, 5′-agatgggttcagccttgga-3′. All gene amplifications were performed in duplicate and normalized to peptidylprolyl isomerase A (PPIA) that was selected as most stable reference gene across duplicate and normalized to peptidylprolyl isomerase A of potential genes analysed using GeNORM [20]. PPIA primers using SuperScript III first strand synthesis kit (Invitrogen).

Statistical analysis

Statistical analysis was performed using GraphPad Prism version 5.01 for Windows (GraphPad Software, La Jolla, CA, USA). The median or mean values of a given variable were compared between groups by the unpaired Student’s t test, nonparametric Mann–Whitney U-test or ANOVA, as appropriate. A P value of <0.05 was considered significant.

RESULTS

Phenotype of monocyte-derived dendritic cells obtained from patients with chronic HCV

Previous reports have demonstrated reduced numbers of circulating DCs in patients with chronic HCV infection [10], suggesting that HCV may disrupt myelopoiesis. However, we have previously shown that chronic HCV infection does not have an effect on the numbers of circulating monocytes [21]. We observed no significant differences in the frequencies of myeloid (Lin1+CD14+) and plasmacytoid (Lin1+BDCA2+) DCs in the circulation of patients with chronic viral infection, patients who had resolved infection and healthy controls (data not shown). The low frequency of DCs in the circulation and the limited volume of blood available from patients precluded us from assessing the functional properties of these cells directly. However, MDDCs can be obtained in reasonable numbers from patients. They have potent antigen presentation capacity and are the cell type most likely to be used in a DC vaccine [22].

Monocytes cultured in the presence of IL-4 and GM-CSF for a period of 6 days differentiate into CD11c+HLA-DR+CD14+ immature MDDCs. We investigated the ability of monocytes obtained from patients with chronic HCV infection to respond to GM-CSF or IL-4. We found that monocytes obtained from healthy controls or patients with HCV+ up-regulated expression of pSTAT5 (Fig. 1a) and pERK (Fig. 1b) in response to GM-CSF and pSTAT6 in response to IL-4 (Fig. 1c). Furthermore, after 6 days of culture in the presence of GM-CSF and IL-4, CD14+ monocytes of both healthy controls and patients with chronic HCV infection had acquired a typical iDC phenotype (CD11chigh, CD1ahigh, HLA-DR+ and CD14low, Fig. 2a). No statistical differences were found in the frequencies of cells expressing CD1a, CD11c, HLA-DR or CD14 between healthy controls (n = 10) and patients with HCV+ (n = 17) (Fig. 2b). These data clearly show that phenotypically normal MDDCs can be obtained from patients with chronic HCV infection. All the patients included here were treatment naïve, had mild disease (Ishak Score ≤2) and had no other risk factors for the development of liver disease (Table 1).

Poly(I:C)-stimulated dendritic cells of patients with HCV+ are poor stimulators of T-cell proliferation

Having demonstrated that MDDCs could be obtained from this homogenous cohort of women with chronic HCV infection, we assessed the functionality of these cells, by investigating their capacity to stimulate T-cell proliferation in allogeneic MLRs. Poly(I:C) treatment of DCs from healthy controls enhanced their ability to stimulate T-cell proliferation (Fig. 3a). In contrast, DCs of patients with HCV+ poorly stimulated T-cell proliferation, and this was not enhanced by the addition of poly(I:C). Interestingly, we found significantly elevated IL-10 levels in HCV patient DC:T-cell co-cultures, while IFN-γ levels were significantly lower (Fig. 3b), suggesting an important role for IL-10 in limiting T-cell proliferation.

CD86 expression in response to toll-like receptor agonists

The effect of chronic HCV infection on the ability of patients’ DCs to respond to TLR agonists is not well understood. Failure of DCs from HCV-infected patients to up-regulate expression of the co-stimulatory molecule CD86 in response to poly(I:C) may explain their poor performance in activating...
Therefore, we investigated the ability of iDCs obtained from patients with HCV + to respond to TLR agonists, including: poly(I:C) (5 or 25 μg/mL), poly(I:C) complexed with the transfection reagent LyoVec (1 μg/mL), CL097 (1 μg/mL) or LPS (1 μg/mL). DCs were incubated in the absence or presence of TLR agonists for 18 h, and the expression of the DC maturation markers CD86 and HLA-DR was examined by flow cytometry. We discovered that both CD86 (Fig. 4a,b) and HLA-DR (Fig. 4c) expressions were significantly elevated by all agonists in cells from healthy controls or patients with HCV. There were no significant differences observed in the expression levels of CD86 between the iDCs of patients with HCV + and healthy controls; however, HLA-DR expression of HCV patients’ DCs was consistently lower than controls (Fig. 4c). Reduced HLA-DR expression of the DCs obtained from patients with HCV may contribute to the poor ability to stimulate T-cell proliferation.

Cytokine secretion in response to LPS and poly(I:C)

Human MDDCs express high levels of pathogen recognition receptors such as TLR3 and TLR4 [23]. As previous studies in hepatocytes have demonstrated that HCV can degrade key intermediates of the signalling pathways downstream of TLR ligation, such as the adaptor molecule TRIF [24], we investigated the ability of DCs obtained from patients with chronic HCV infection to respond to TLR agonists. LPS initiates a complex signalling cascade in DCs downstream of TLR4, with two main signalling pathways: a MyD88-dependent and a MyD88-independent pathway. The MyD88-dependent pathway leads to the activation of the transcription factor NF-κB, which induces expression of genes with a κB-binding motif in their promoter region e.g. IL-8. The MyD88-independent pathway involves the recruitment of the adaptors TRAM and TRIF to TLR4, resulting in activation of the transcription factor IRF3, which induces the expression of genes with the interferon-sensitive response element (IRSE) e.g. CCL5 [25]. MDDCs obtained from both healthy controls and patients with HCV + stimulated with LPS secreted high levels of IL-8 and CCL5, indicating that the signalling pathways downstream of TLR4 remain functional in the cells obtained from patients with chronic HCV infection (Fig. 5a,b).

Next, we investigated cytokine production by DCs of patients with HCV in response to Poly(I:C) that was directly added to cells (primarily activates TLR3 initiated pathway) or added complexed with a transfection reagent, LyoVec (primarily activates RIG-I initiated pathway). CXCL10 (Fig. 5c) and CCL5 (Fig. 5d) were secreted by DCs of patients with chronic HCV infection in response to Poly(I:C)/LyoVec and Poly(I:C), respectively. However, levels of both chemokines secreted by patient DCs were significantly reduced compared to levels secreted by DCs obtained from healthy controls. Furthermore, higher levels of IL-10 secretion were observed following stimulation of DCs obtained from patients with HCV with poly(I:C) compared to those of healthy controls (Fig. 5e). Impaired secretion of the key T-cell-attracting chemokines, CXCL10 and CCL5, by DCs from patients with HCV, coupled with their high levels of IL-10 secretion may contribute to their poor ability to activate robust T-cell responses in patients.

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IFN-α secretion and IFN-α2, IFN-β and IFN-λ1 (IL-29) gene expression

In the early phase of the response to poly(I:C), DCs up-regulate the expression of the mRNA for IFN-α/β, and the type I IFN signal, in turn, amplifies and sustains the secretion of cytokines, including IFN-α itself. As both CXCL10 and CCL5 are secreted by DCs in response to stimulation by type I IFN [26], we investigated whether the observed reduction in chemokine secretion by DCs from patients with HCV in response to poly(I:C) was because of a defect in IFN-α/β transcription or a defect in their response to type I IFN.

Fig. 2 Immature DCs (iDC) phenotype of patients with chronic patients with HCV. CD14+ monocytes were purified from PBMCs of healthy controls (n = 10) and patients with chronic HCV infection (n = 17) and cultured in vitro for 6 days with IL-4 and GM-CSF. At day 6, the expression of CD14, CD1a, CD11c and HLA-DR was determined by flow cytometry. Representative dot plots of the iDC phenotype of a healthy control and a HCV patient are shown (a). Similar percentages of the iDCs of healthy controls (n = 10) and patients with HCV (n = 17) expressed these markers (b).
Levels of IFN-α2, IFN-β and IL-29 gene transcription in response to poly(I:C) stimulation were assessed in MDDCs obtained from healthy controls and patients with HCV. Transcription of IFN-β (Fig. 6c) and IL-29 (Fig. 6d) was significantly induced by poly(I:C) in DCs from patients and controls. However, we found that IFN-α expression in response to poly(I:C) was significantly reduced in patients compared to controls (Fig. 6b). In addition, we found that DCs obtained from patients with HCV secreted almost no IFN-α (0–23.5 pg/mL) following 18-h culture in the presence of poly(I:C), while DCs obtained from healthy controls secreted significant amounts of this cytokine (56–258 pg/mL) (Fig. 6a).

<table>
<thead>
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<td>42</td>
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</tr>
<tr>
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<td>23 (20–36)</td>
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<td>AST (U/L)</td>
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<td>27 (17–36)</td>
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Table 1 Characteristics of patients used for monocyte-derived dendritic cell preparation

![Image](image_url)

Fig. 3 Poly(I:C) pretreatment enhances the T-cell stimulatory capacity of healthy control dendritic cells (DCs) but not HCV-infected patient DCs. Immature DCs of healthy controls (n = 4) and chronically infected patients with HCV (HCV RNA+, n = 5) and women who had resolved infection (HCV RNA–, n = 3) were cultured in media alone or with 5 μg/mL Poly(I:C) for 18 h. cells were washed and 5000 DCs were cultured in round-bottomed 96-well plates in triplicate with 50 000 allogeneic CD3⁺ T cells that had been labelled with CFSE. After 5 days, T-cell proliferation was assessed by measuring CFSE dye dilution by flow cytometry, a representative plot is shown (a). After 5 days, supernatants were harvested from the cultures and the levels of IL-10 and IFN-γ were determined by ELISA. No detectable cytokines were secreted in control cultures (i.e. T cells only or DCs only) (b). *P < 0.05; determined by the Mann–Whitney U-test.

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Next, we tested the ability of DCs isolated from patients with chronic HCV to respond to type I IFN. DCs obtained from patients with HCV that were stimulated for 4 h with recombinant IFN-β failed to up-regulate IFN-α expression in contrast to cells obtained from healthy controls (Fig. 6e). To further confirm that IFN-α mRNA expression is amplified by
the IFN-β induced by poly(I:C), we stimulated MDDCs obtained from healthy donors with poly(I:C) in the absence or presence of an IFN-α/β neutralizing mAb and assessed IFN-α2, IFN-β and IL-29 expression by real-time PCR. The blocking mAb had no effect on IFN-β or IL-29 expression in response to poly(I:C) (Fig. 6f), indicating that expression of these genes in response to poly(I:C) was independent of the induction of type I IFN. However, up-regulation of IFN-α expression in response to poly(I:C) was significantly reduced in the presence of the blocking mAb (Fig. 6f), confirming a role for type I IFN in the induction of IFN-α expression under these conditions.

These data show that MDDCs obtained from patients with chronic HCV infection secrete virtually no IFN-α in response to poly(I:C), most likely because of the failure of these cells to respond to the initial wave of IFN-β produced.

**DISCUSSION**

In this study, we show that phenotypically normal MDDCs (CD11c⁺HLA-DR⁺CD1a⁺CD14⁻) can be obtained from patients with chronic HCV infection. These cells respond to both Poly(I:C) and LPS, by up-regulating their expression of CD86. They secrete high levels of IL-8 and CCL5 in response to LPS, an indication that the MyD88-dependent and MyD88-independent signalling pathways downstream of TLR4 ligation are functioning. However, these cells are poor stimulators of T-cell proliferation in allogeneic MLRs. Fur-
thermore, they fail to secrete IFN-α in response to poly(I:C) and secrete significant levels of IL-10.

Low IFN-α production and impaired ability of myeloid and plasmacytoid DCs from patients with chronic HCV to drive a polarized Th1 response have been demonstrated in other patient cohorts [27,28]. Impairment of the ability of DCs to drive Th1 responses was restored in vitro by the addition of the anti-viral agent, ribavirin [29], suggesting that the virus directly affects DC function. Further evidence for a direct effect of the virus on DC function comes from studies where MDDCs are infected with cell culture–adapted strains of HCV, such as JFH-1 (genotype 2a) or H77 (genotype 1a). One study demonstrated that JFH-1- or H77-infected MDDCs secreted increased levels of IL-10 and had limited ability to activate T-cell responses [30]. A further study, in which MDDCs were infected with JFH-1, showed that MDDCs infected with HCV for 24 h significantly up-regulate IFN-β transcription, while IFN-α gene transcription remained at basal levels [31]. Individual HCV proteins have significant immunomodulatory properties that could be responsible for DC defects in chronic HCV infection, e.g. high levels of the inhibitory cytokines TGF-β and IL-10 are secreted by PBMCs and monocytes in response to the HCV NS4 protein [19]. HCV core protein reduced TLR9-triggered IFN-α while increasing IL-10 secretion by PBMCs [32]. However, the effects of chronic HCV infection on DC function are likely to vary depending on either the viral strain, length of infection, genotype of the virus or genetic background of the host, as some studies report no defects [14]. Interestingly, the patients with HCV studied by Piccioli et al. [14] were pre-
dominantly male, perhaps contributing to the differences observed between our reports, as our cohort is entirely female. We have previously demonstrated that oestrogen exerts significant effects on DC function, altering chemokine and cytokine secretion patterns [18].

Our study differs from previous investigations of the effect of chronic HCV infection on DC phenotype and function; in that, our cohort were homogeneous for age, sex and ethnicity and were exposed to the same type/subtype of HCV from a single source for the same duration (~30 years) [17]. We have previously shown that chronic HCV infection perturbs the immune response in this cohort, with depleted levels of circulating cytotoxic CD56<sup>dim</sup> NK cells and an expansion of IFN-γ-producing CD56<sup>bright</sup> NK cells [33]. In vitro studies have shown a critical role for type I IFN in the induction of NK cell cytotoxicity [34]. Therefore, the lack of IFN-α secretion by DCs in response to poly(I:C) suggests that the failure of DCs to produce IFN-α in response to viral stimuli results in reduced NK cell cytotoxicity thus contributing to viral persistence. However, a study of NK cell phenotype and function in a separate cohort of patients found evidence of increased NK cytotoxicity and reduced IFN-γ production [35]. The authors suggested that this polarized NK cell phenotype (TRAIL<sup>+</sup> CD107<sup>+</sup>) was because of chronic exposure to HCV-induced IFN-α in vivo and that this excessive NK cell cytotoxicity may contribute to liver injury. In general, the women of our cohort have mild liver disease [17,36]; low levels of virus driven IFN-α secretion by their DCs accompanied by reduced NK cell cytotoxicity may explain their mild disease.

In vitro studies of hepatocytes demonstrated disruption of the retinoic acid-inducible gene I (RIG-I) [37] and Toll-IL-1 receptor domain-containing adaptor inducing IFN-β (TRIF or TICAM-1)-dependent [24] signalling by the viral protease NS3/4A, thus rendering the HCV-transfected cells hyporesponsive to Poly(I:C). TLR agonists such as Poly(I:C), alone or in combination have demonstrated potential as vaccine adjuvants or agents to induce DC maturation in cancer-diagnosis of the inhibitory co-stimulatory molecule CD274 by DCs failed to activate T-cell proliferation in an allogeneic MLR (Fig. 3). Previous reports have demonstrated expansion of antigen-specific Tregs in HCV-infected patients [44,45], which may be because of high levels of IL-10 secreted by DCs in response to TLR agonists (Fig. 5). The interaction of PD1 with PDL1 (CD274) expressed by DCs is thought to play a crucial role in mediating T-cell exhaustion in chronic viral infection [46,47]. We assessed the expression of the inhibitory co-stimulatory molecule CD274 by DCs and found no differences in the basal level of expression between healthy controls and patients with HCV. In addition, levels of CD274 expression, which were up-regulated by control cells following poly(I:C) stimulation, were unaltered on HCV patients’ cells (Fig. S1). This may be due in part to the role that IFN-α plays in the regulation of expression of this molecule [48].

These data reveal the important role of IFN-α in generating mature DCs capable of activating a robust T-cell response. In the absence of IFN-α, DC activity is driven towards IL-10 production that suppresses T-cell activity. Therefore, immunotherapeutic strategies that target DCs, such as TLR agonists, might amplify standard treatment regimens of pegylated IFN-α in combination with ribavirin, currently used to treat chronic HCV infection.

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STATEMENT OF INTERESTS

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## SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

**Fig. S1** DCs obtained from patients with chronic HCV infection fail to up-regulate CD274 expression in response to poly(I:C).

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