Dendritic cell-based immunity and vaccination against hepatitis C virus infection

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Summary

Hepatitis C virus (HCV) has chronically infected an estimated 170 million people worldwide. There are many impediments to the development of an effective vaccine for HCV infection. Dendritic cells (DC) remain the most important antigen-presenting cells for host immune responses, and are capable of either inducing productive immunity or maintaining the state of tolerance to self and non-self antigens. Researchers have recently explored the mechanisms by which DC function is regulated during HCV infection, leading to impaired antiviral T-cell responses and so to persistent viral infection. Recently, DC-based vaccines against HCV have been developed. This review summarizes the current understanding of DC function during HCV infection and explores the prospects of DC-based HCV vaccine. In particular, it describes the biology of DC, the phenotype of DC in HCV-infected patients, the effect of HCV on DC development and function, the studies on new DC-based vaccines against HCV infection, and strategies to improve the efficacy of DC-based vaccines.

Keywords: dendritic cells; hepatitis C virus; vaccine development

Introduction

Hepatitis C virus (HCV) is a blood-borne pathogen and has led to chronic infection in an estimated 170 million people worldwide. It is a major cause of chronic liver diseases with a substantial morbidity and mortality.1 People infected with HCV serve as a reservoir for transmission to others and are at risk for developing chronic liver diseases, such as liver cirrhosis and primary hepatocellular carcinoma. It has been estimated that HCV accounts for 27% of cirrhosis and 25% of hepatocellular carcinoma worldwide.2 Therapy for chronically HCV-infected patients has involved a combination of a pegylated interferon-α and ribavirin (pegIFN/RBV).3 The choice of this regimen was based upon the results of three pivotal, randomized, clinical trials that demonstrated the superiority of this combination treatment over standard IFN-α and RBV.4–6 However, this therapy is expensive, non-specific, toxic, and only effective in about 50% of genotype-1 HCV patients.7 Specific targeted antiviral therapies for HCV using directly acting antiviral agents or inhibitors are at different phases of development and clinical trials.8 These inhibitors target HCV receptors, HCV-IRES, NS3/4A, NS5A and NS5B.9 Two protease inhibitors (boceprevir and telaprevir) have recently been approved and are increasingly used in combination with pegIFN/RBV for type-1 HCV mono-infection.

An effective HCV vaccine would reduce the number of new infections and thereby reduce the burden on healthcare systems. However, there are many impediments to the development of an effective HCV vaccine including the existence of multiple HCV genotypes, limited availability of animal models and the complex nature of the immunological response to HCV.10 Clearance of HCV infection appears to require strong and broadly cross-reactive CD4+ and CD8+ T-cell responses11–13 and neutralizing antibody responses.14 With the variability of HCV, a combination approach including vaccination and antiviral therapy or immune modulation might be necessary for management of HCV infection.15 Several HCV vaccines have been developed. Although most of them are still at the preclinical stages, some have advanced into phase I or phase II clinical trials to determine the safety and efficacy of the candidate vaccines. The approaches or classifications of HCV vaccine development include: (i) recombinant proteins such as HCV core protein and non-structural proteins emulsified with MF59,16 HCV gpE1/E2 emulsified with MF59,17 GI-5005: HCV NS3 and core proteins,18 HCV core protein/ISCOMATRIX;19 (ii) synthetic peptides such as IC4120 and a peptide (core)
emulsified with ISA51\textsuperscript{,21} (iii) DNA-based vaccine such as CIGB-230\textsuperscript{22} and others\textsuperscript{,23–26} (iv) virus-based vaccine such as modified vaccinia Ankara virus-based HCV vaccine: TG4040,\textsuperscript{27,28} recombinant adenoviral HCV vaccines,\textsuperscript{29–31} lentiviral vector-based HCV vaccine.\textsuperscript{32} These approaches have limited effectiveness for a number of reasons including: the delivery of a limited number of protective viral epitopes, the inclusion of incorrectly folded recombinant proteins, the limited humoral and cell-mediated responses that are associated with DNA vaccines, and the use of adjuvants with relatively poor potency.

Recently, dendritic cell (DC) -based vaccines against HCV has been developed.\textsuperscript{31–37} Dendritic cells (DC) are the most effective antigen-presentation cells and are professionalized to capture and process antigens, converting proteins to peptides that are presented on MHC molecules and recognized by T cells.\textsuperscript{38,39} The medical implications of DC that control a spectrum of innate and adaptive responses have been reviewed.\textsuperscript{40} The present review summarizes the current understanding of DC functions in HCV infection and explores the prospects of DC-based HCV vaccine development. In particular, it describes the biology of DC, the phenotype of DC in HCV-infected patients, the effect of HCV on DC, the studies on new DC-based vaccines against HCV, and strategies to improve the efficacy of DC-based vaccines.

**DC function and generation in culture**

Dendritic cells are the most efficient inducers of all immune responses, and are capable of either inducing productive immunity or maintaining a state of tolerance to self and non-self antigens. Two major DC subsets have been characterized to date in humans, based on their development from myeloid or lymphoid precursors of bone marrow pluripotent cells.\textsuperscript{41} Myeloid dendritic cells (MDC) are CD1\textsubscript{a}, CD11c, CD13, CD14, CD33\textsuperscript{,37}, whereas lymphoid descendants, also called plasmacytoid dendritic cells (PDC) express CD123 and BDCA-2 on their surface. Both MDC and PDC are derived from bone marrow and can be found in peripheral blood in an immature stage. Immature dendritic cells (iDC) express low levels of MHC class I and II and co-stimulatory molecules on their surface and are proficient in endocytosis and antigen processing. Maturation of DC occurs after detecting microbial or host-derived danger signals, or upon contact with pro-inflammatory cytokines, such as tumour necrosis factor-$\alpha$ (TNF-$\alpha$), interleukin-1 (IL-1), or after engagement of the CD40/CD40 ligand (CD40L) system. The DC play a key role in regulating immunity, serving as the sentinels that capture antigens in the periphery, process these antigens into peptides, and present these peptides to lymphocytes within lymph nodes. The maturation process includes a series of transformations that lead to a reduction of antigen-capturing capacity, an increase in MHC and co-stimulatory molecule expression and, most importantly, the development of an exceptional efficiency in presenting antigens to T cells, activating natural killer cells, and producing interferons, so linking the innate and adaptive immune responses.\textsuperscript{42} Although both MDC and PDC are potent in antigen uptake, processing and presentation, they have fairly distinct cytokine profiles: MDC produce large amounts of IL-12 and IL-10 and make small amounts of IFNs, while PDC are specialized type-I IFN-producing machines and express much lower levels of other cytokines (Table 1).

As the frequencies of DC in the peripheral circulation are low, alternative approaches to DC generation for research purposes were sought.\textsuperscript{43} The classic strategy for the *ex vivo* generation of monocyte-derived DC (MDDC) consists of a two-step culture protocol, in which monocytes are differentiated towards iDC, followed by the induction of mature DC. Monocytes may be isolated from blood by adherence or positive selection using immunomagnetic beads.\textsuperscript{44} Differentiation of DC is induced by using granulocyte-macrophage colony-stimulating factor and IL-4,\textsuperscript{45} but the doses of each reagent, the culture conditions (flask or closed plastic bag\textsuperscript{46,47}), the composition of the culture medium, the cocktail of reagents such as CD40L\textsuperscript{48} and poly(I:C)\textsuperscript{49} used to induce maturation, and the methods used to antigen-load DCs all vary substantially.\textsuperscript{40} The total *in vitro* culture duration lasts 1 week but there is increasing evidence that maturation of MDDC can be generated even after short-term cell culture for 2–3 days\textsuperscript{51–54} with several advantages: it simplifies the laborious and time-consuming process of DC manufacture and it reduces the actual risk of microbial contamination related to *in vitro* culture.

**DC in HCV-infected patients**

Many researchers have explored the hypothesis that the failure of HCV-infected individuals to mount an effective T-cell response, and so lead to the development of chronic HCV infection, is the result of a virus-mediated impairment of DC function. This impairment may include a

| Table 1. Characteristics of myeloid and plasmacytoid dendritic cells |
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| **Myeloid dendritic cell** | **Plasmacytoid dendritic cell** |
| Surface molecular | CD1\textsubscript{a}, CD11c, CD14, CD33 | CD123, BDCA-2 |
| Cytokine secreted | IL-12, IL-10, IFN | Type-I IFN |
| Ex vivo inducer | GM-CSF, IL-4, CD40L/poly(I:C) | GM-CSF, IL-4, Flt-3 |
| Mature marker | CD80, CD86, CD83 | CD80, CD86, CD83 |
| | GM-CSF, granulocyte–macrophage colony-stimulating factor; IFN, interferon; IL, interleukin. | |
reduced frequency of MDC and PDC, reduced IL-12 and IFN-α, and increased IL-10 production, accompanied by an impaired capacity to prime naive T cells. In human studies, findings related to DC functions are controversial. Complex defects such as reduced number of DC, deficiency in co-stimulatory molecules, decreased T-cell stimulatory capacity, overproduction of the immunoregulatory cytokine IL-10/transforming growth factor-β, and proliferation of regulatory T lymphocytes were detected in patients with chronic HCV infection, while others failed to identify any DC abnormalities. One analysis suggested that DC from HCV-infected subjects have a normal capacity to stimulate CD4+ T cells, and so the functional effectiveness of DCs derived from HCV-infected individuals provides a rationale for the DC-based immunotherapy of chronic HCV infection. Another study demonstrated that DC retained the same allostimulatory capacity before and following the establishment of persistent HCV infection. The surface phenotype and the amount of IL-10 and IL-12p70 produced during DC maturation did not differ between HCV-infected individuals and healthy controls. Maturation of DC from HCV-infected individuals performed comparably in an allogenic MLR compared with healthy individuals. Mature MDDC from HCV-infected individuals stimulated the expansion of peptide-specific naive CD8+ T cells. The MDDC from HCV-infected and healthy individuals were phenotypically indistinguishable and performed comparably in functional assays. Such discrepancies most possibly derive from different patient cohorts who had taken ribavirin either at the time of study or in the past, or from cohorts with different amounts of liver inflammation/fibrosis, assessment of non-human primate models of HCV infection, different experimental approaches, and distinct read-outs.

Various approaches have been used to clarify the discrepancies and possible underlying mechanisms, including generation of MDDC or the analysis of peripheral blood DCs in patients with chronic HCV, by studying the effectiveness of recombinant HCV proteins or cell-culture-adapted strains of HCV on DC in vitro. Some researchers also reported that HCV-infected cells trigger a robust IFN response in PDC by a mechanism that requires active viral replication, direct cell–cell contact, and Toll-like receptor 7 (TLR7) signalling, and showed that the activated PDC supernatant inhibits HCV infection in an IFN receptor-dependent manner. As there is clearly controversy regarding MDC’s ability to activate T cells, it is unclear whether on a per cell basis MDCs from HCV-infected individuals are able to prime naive T cells. Additionally, reduced numbers of peripheral blood MDC have been observed in HCV-infected individuals and may play a role in the defective response to vaccine. Canaday et al. specifically focused on analysis of peptide–MHC complex formation and presentation, the culmination of uptake, degradation and trafficking of antigen. They found that this specific antigen-presenting cell function is preserved in the setting of chronic HCV infection.

**DC in liver microenvironment**

As the liver is the primary site for HCV replication, DC changes in peripheral blood may or may not reflect what is happening locally at the site of infection. Several studies demonstrated enrichment of DC in the liver compartment compared with peripheral blood. Galle et al. employed electron microscopy, immunohistochemistry and immunofluorescence to show that DC are indeed enriched in the livers of HCV-infected individuals. Wertheimer et al. also showed a clear enrichment of DC in the intrahepatic compartment compared with the peripheral circulation. To investigate the contribution of intrahepatic PDC and MDC to local immune responses during HCV infection, Lai et al. developed methods to isolate and characterize MDC and PDC from human liver. The MDC from HCV-infected liver demonstrated greater expression of MHC class II, CD86 and CD123, that were more efficient stimulators of allogeneic T cells and secreted less IL-10. In contrast, PDC were present at lower frequencies in HCV-infected liver and expressed higher levels of the regulatory receptor BDCA-2. In HCV-infected liver, the combination of enhanced MDC function and a reduced number of PDC might contribute to viral persistence in the face of persistent inflammation. Nattermann et al. demonstrated that chronic HCV infection, associated with intrahepatic DC enrichment, migration of DC is markedly affected by interaction of HCV E2 with CD81. A two-photon confocal microscopic analysis revealed that DC and T lymphocytes were rapidly recruited within human liver slices undergoing an ex vivo HCV infection. However, liver MDC enrichment is not unique to HCV infection, as similar trends were seen in non-HCV-infected liver disease, such as hepatitis B virus infection.

**HCV on DC development and function**

Several studies have suggested that DC can be infected with HCV, but the role of HCV in DC development and function is still elusive. Virologically, HCV first attaches itself to the host cell surface by means of weak interactions with glycosylaminoglycans or the low-density lipoprotein receptor. Once bound and concentrated on the cell surface, virions are able to interact with entry receptors such as CD81 and SR-BI with high affinity. The virus–receptor complex then translocates to the tight junctions where claudin and occludin act as cofactors and induce receptor-mediated endocytosis. Barth et al. used HCV-like particles (HCV-LPs) to study the interaction of HCV with human DC. The iDC exhibited an envelope-specific and saturable binding of HCV-LPs,
indicating receptor-mediated DC–HCV-LP interaction. They revealed that HCV-LPs were rapidly taken up by DC in a temperature-dependent manner, and C-type lectins such as mannose receptor or DC-SIGN (DC-specific intercellular adhesion molecule 3-grabbing non-integrin) were not sufficient for mediating HCV-LP binding. Lambotin et al. suggested that HCV cell entry factors, which are crucial for viral uptake in hepatocytes, do not support the cell culture-produced HCV (HCVcc) uptake in DC subsets. HCVcc acquisition by DC subsets does not depend on the C-type lectin DC-SIGN, but is partially mediated by HCVcc E2 protein interaction at the cell surface.

To date, the mechanisms whereby HCV affects DC function remain largely elusive. It is possible that HCV proteins play a role in suppressing protective immunity through interactions with host immune cells, such as DC. Indeed, the HCV core protein has been reported to impair the function of DC. The HCV core protein was able to selectively inhibit TLR4-induced IL-12 production after interacting with the gC1q receptor on the surface of MDDC by activating the phosphatidyl inositol 3-kinase pathway, leading to reduced T helper type 1 (Th1) cell responses. Consistent with these studies, Liang et al. had an inhibitory effect on DC maturation; however, transfection of iDC with in vitro transcribed core RNA and treated with maturation factors. Neither core nor NS3 has an inhibitory effect on DC maturation; however, transfection of iDC with in vitro transcribed core RNA appeared to result in changes compatible with maturation confirmed by a DC-specific membrane array. The effects of core on maturation of iDC were confirmed with a significant increase in surface expression of CD83 and HLA-DR, a reduction of phagocytosis, as well as an increase in proliferation naïve patients with chronic HCV infection had a reduced frequency of circulating PDC as the result of increased apoptosis and showed diminished IFN-α production after stimulation with TLR9 ligands. The HCV core protein reduced TLR9-triggered IFN-α and increased TNF-α and IL-10 production in peripheral blood mononuclear cells (PBMCs) but not in isolated PDC, suggesting that HCV core induces PDC defects. The addition of rTNF-α and IL-10 induced apoptosis and inhibited IFN-α production and inhibited IFN-α production in PDC. Neutralization of TNF-α or IL-10 prevented HCV core-induced inhibition of IFN-α production. Antisense-blocking antibody, but not anti-TLR4-blocking antibody, prevented the HCV core-induced inhibition of IFN-α production. These results suggest that HCV interferes with antiviral immunity through TLR2-mediated monocyte activation triggered by the HCV core protein to induce cytokines, which in turn lead to PDC apoptosis and inhibit IFN-α production. These mechanisms may contribute to viral escape by HCV from immune responses. Consistent with these studies, Liang et al. treated freshly purified human MDC and PDC with HCV JFH1 strain (HCV genotype 2a). They found that HCV up-regulated MDC maturation marker (CD83, CD86 and CD40) expression and did not inhibit TLR3 ligand [poly(I:C)]-induced MDC maturation whereas HCV JFH1 inhibited the ability of poly(I:C)-treated MDC to activate naive CD4+ T cells. The HCV JFH1 also inhibited TLR7 ligand (R848)-induced PDC CD40 expression, and this was associated with an impaired ability to activate naive CD4+ T cells. Parallel experiments with recombinant HCV proteins indicated that HCV core protein may be responsible for a portion of the activity.

It has recently been shown that TLR7 may be implicated in anti-HCV immunity, HCV encodes G/U-rich ssRNA TLR7 ligands that induce immune activation of PBMCs and PDC. Studies suggested that a TLR7-dependent impairment of co-stimulatory molecule expression caused by HCV persistence may affect DC activity in non-responder patients. Exploitation of the MHC class I antigen-processing pathway by HCV core, impairs the ability of DC to stimulate CD8+ T cells and may contribute to the persistence of HCV infection. However, Landi et al.’s results show that HCV core does not have an inhibitory effect on human DC maturation, and could be a target for the immune system. To evaluate the effects of core and NS3 proteins on DC, they transfected monocyte-derived iDC with in vitro transcribed HCV core or NS3 RNA and treated with maturation factors. Neither core nor NS3 had an inhibitory effect on DC maturation; however, transfection of iDC with in vitro transcribed core RNA appeared to result in changes compatible with maturation confirmed by a DC-specific membrane array. The effects of core on maturation of iDC were confirmed with a significant increase in surface expression of CD83 and HLA-DR, a reduction of phagocytosis, as well as an increase in proliferation naïve patients with chronic HCV infection had a reduced frequency of circulating PDC as the result of increased apoptosis and showed diminished IFN-α production after stimulation with TLR9 ligands.

Beyond HCV core protein and NS3, NS4 also suppressed T-cell responses as a result of the effect on monocytes or DC. The DCs produce high levels of type I IFN in response to double-stranded RNA generated upon viral replication. However, HCV suppresses this response via the NS3–NS4A viral protein, which blocks IFN regulatory factor 3-mediated induction of type I IFN. In Brady et al.’s study, supernatants from NS4-stimulated monocytes inhibited LPS-induced maturation of DC and sup-
pressed their capacity to stimulate proliferation and IFN-γ production by allospecific T cells. Their data suggested that HCV subverts cellular immunity by inducing IL-10 and inhibiting IL-12 production by monocytes, which in turn inhibits the activation of DC that drive the differentiation of Th1 cells. Takaki et al. also found that HCV non-structural proteins, particularly NS4, change the iDC phenotype and reduce antigen-specific T-cell stimulatory function with Th1 cytokine reductions. HCV NS5 was also shown to impair PDC function with several other in vivo studies indicating decreased numbers and impaired function of PDC in chronically HCV-infected patients.

Over-expression of HCV core, NS3, NS5A or NS5B proteins induced apoptosis in mature DC. Likewise, individual HCV proteins, Core, NS3, NS4, NS5 as well as fused polyprotein (Core–NS3–NS4) were found to impair functions of both iDC and mDC by regulating the expression of co-stimulatory and antigen presentation molecules, strikingly reducing IL-12 secretion, inducing the expression of FasL to mediate apoptosis, interfering with allo-stimulatory capacity, inhibiting TLR signalling and expression of FasL to mediate apoptosis, interfering with

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Further indications that HCV affects DC function came directly from studies using the cell culture-produced HCV (HCVcc). Culture with HCVcc demonstrated inhibition of maturation of MDCD induced by a cocktail of cytokines (IL-1β, TNF, IL-6, prostaglandin E2) while enhancing the production of IL-10. In addition, DC exposed to HCVcc were impaired in their ability to stimu-
late antigen-specific T-cell responses. Similar experiments performed by Shinya and Rehermann proved that HCVcc inhibited TLR-9 mediated IFN-α production by PBMC and PDC. In contrast to its effect on PDC, HCVcc did not inhibit TLR3-mediated and TLR4-mediated maturation and IL-12, IL-6, IL-10, IFN-γ and TNF-α production by MDCs and MDDCs. Likewise, HCVcc altered the capacity of either MDCs nor MDDCs to induce CD4 T-cell proliferation. Gondois-Rey et al. also proved that HCVcc and HCV-LPs but not HCV core or envelope glycoprotein E2 inhibit PDC-associated production of IFN-α stimulated via TLR9.

**DC-based vaccine against HCV infection**

Several approaches involving DC-based vaccines were developed as early-stage attempts to manage/cure HCV infection, some of them being developed at the experimental level while some advanced towards the translational level. The DC-based HCV vaccine development is summarized in Table 2.

Moriya et al. employed the anthrax toxin fusion protein containing the HCV-core epitope as a vehicle for antigen loading on DC, and reported that immunization with the fusion protein-treated DC induced HCV-core-specific cytotoxic lymphocytes (CTL) in mice. Later, they immunized mice with DC transduced with recombinant adeno-virus expressing HCV-core protein effectively induced HCV-core-specific CTL. Hence, adeno-virus-transduced DC may be a promising candidate for a CTL-based vaccine against HCV infection.

Racanelli et al. present a system to induce cellular immunity and to study the immunological implications of time-delayed DC apoptosis and antigen reprocessing in vivo. They generated a self-replicating cytopathic pestivirus RNA to enhance production and presentation of HCV antigens and to induce apoptosis in DC 24–48 hr after transfection. Replicon-transfected H-2b DC used to immunize HLA-A2 transgenic mice induced protection upon challenge with a vaccinia virus expressing HCV antigens. Induction of cell death enhanced the immunogenicity of DC-associated antigen. Transfer of cellular material from vaccine DC to endogenous antigen-presenting cells was visualized in lymph nodes and spleen, and cross-primed CD8+ T cells were characterized.

Dendritic cells pulsed with HCV-LPs stimulated HCV core-specific CD4+ T cells, indicating that uptake of HCV-LPs by DC leads to antigen processing and presentation on MHC class II molecules. The HCV-LP-derived antigens were efficiently cross-presented to HCV core-specific CD8+ T cells. These findings demonstrate that HCV-LPs represent a novel model system to study HCV-DC interaction allowing definition of the molecular mechanisms of HCV uptake, DC activation, and antigen presentation to T cells. Furthermore, HCV-LP may be a potent vaccine candidate for the induction of antiviral cellular immune responses in humans.

By using recombinant adeno-viral vectors, DC expressing HCV NS3 or core proteins expressed several inflammatory cytokine mRNAs, had a normal phenotype, and effectively stimulated allogeneic T cells, as well as T cells specific for another foreign antigen (tetanus toxoid). These findings are important for the rational design of cellular-vaccine approaches for the immunotherapy of chronic HCV. Zabaleta et al. proved that immunization with DC transfected with an adeno-virus encoding NS3 protein, from HCV (AdNS3), induced multi-epitopic CD4 Th1 and CD8 T-cell responses in different mouse strains. Moreover, immunization with AdNS3-transfected DC did not induce anti-adeno-viral antibodies, as compared with direct immunization with AdNS3, but elicited T-cell responses even in the presence of pre-existing anti-adeno-viral antibodies. Finally, responses induced by this protocol down-regulated the expression of HCV RNA in the liver. By using recombinant adeno-associated virus (rAAV) vectors, DC expressing core (49–180) can generate significant antigen-specific CTL. The researchers believe that direct manipulation of professional antigen-
presenting DC may provide new clinical treatments through the forced feeding of antigens into DC coupled with their stimulation and manipulation towards an effective Th1 response, and AAV-loading appears to naturally stimulate a Th1 response in vitro. By using lentiviral vectors, Jirmo et al. demonstrated the high capability of lentiviral vectors to transfer whole sets of HCV structural or non-structural gene clusters in vitro into monocytes before their differentiation into DC. Notably, gene delivery of the HCV-NS cluster into monocytes resulted in its persistent expression in differentiated DC leading to potent stimulation of CD4+ and CD8+ allogeneic and autologous responses. Hence, lentiviral-mediated expression of the multi-antigenic HCV-NS cluster in monocytes subsequently differentiated into DC is a novel potential anti-HCV vaccine modality. Gehring et al. generated immune responses against HCV by DC containing NS5 protein-coated microparticles. They revealed that it was essential to use microbeads as carriers to achieve efficient uptake of the immunogen by DCs because intravenous injection of soluble NS5 protein did not induce detectable T-cell responses as demonstrated in the tumour challenge experiments and Th1-type cytokine secretion.

Because DC are essential for T-cell activation and viral clearance in HCV-infected patients is associated with a vigorous T-cell response, vaccination with HCV antigen-loaded DC may constitute an efficient and important antiviral therapy for HCV. Encke et al. proposed a new type of HCV vaccine based on ex vivo stimulated and matured DC loaded with HCV-specific antigens. This vaccine circumvents the impaired DC maturation and the down-regulated DC function of HCV-infected patients in vivo by giving the necessary maturation stimuli and the HCV antigens in a different setting and location ex vivo. Strong humoral and cellular immune responses were detected after HCV core DC vaccination. Furthermore, DC vaccination shows partial protection in a therapeutic and prophylactic model of HCV infection. In conclusion, mice immunized with HCV core-pulsed DC generated a specific antiviral response in a mouse HCV challenge model. The use of HCV-primed DC for vaccination in chronically infected patients as a prophylactic vaccine seems to

Table 2. Dendritic cell-based vaccine against hepatitis C virus infection

| Vaccine | Challenge inoculums | Outcome | Ref. |
|---------|---------------------|---------|------|
| DC treated with fusion protein | Anthrax toxin fusion protein containing the HCV-core epitope | HCV-core-specific CTLs in mice | 115 |
| DC transduced with recombinant adenovirus | Recombinant adenovirus expressing HCV-core protein | HCV-core-specific CTLs in mice | 116 |
| DC transfected with cytopathic Repl-HCVNS3 RNA | Recombinant pestivirus replicon encodes the complete HCV NS3 | Induce cross-priming of HCV-NS3-specific CD8+ T cells | 36 |
| DC pulsed with HCV-LPs | HCV-LP core, HCV-LP E2 | HCV core-specific CD4 and CD8 T cells | 55 |
| DC transfected with adenovirus | Adenovirus encoding NS3 protein, from HCV (AdNS3) | Multi-epitopic CD4 T helper cell 1 (Th1) and CD8 T-cell responses in different mouse strains | 117 |
| DC transfected with recombinant adeno-associated virus (rAAV) | rAAV expressing core (49–180) | Significant antigen-specific CTL | 118 |
| DC transfected with lentiviral vectors (LV) | LV expressing HCV structural or non-structural gene clusters | Potent stimulation of CD4 and CD8 T-cell allogenic and autologous responses | 32 |
| DC containing microparticles | NS5 protein-coated microparticles | Antigen-specific CTL activity in mice and significantly reduced the growth of NS5-expressing tumour cells in vivo | 119,120 |
| DC loaded with HCV-specific antigens | HCV core antigens | Strong humoral and cellular immune responses in mice | 121 |
| MDC with peptides | HCV core and NS3 and NS4 protein | Successfully trigger the generation of CTLs with high efficacy and duration and protection against tumor challenge | 122 |
| DC transduced with a short amphipathic peptide carrier, Pep1 | Recombinant HCV core or NS5 protein | | 34 |
| DC loaded with EDA-NS3 | Fusion protein EDA-NS3, poly(I:C) and anti-CD40 | Strong and long lasting NS3-specific CD4 and CD8 T cell responses, down-regulated intrahepatic expression of HCV-NS3 RNA | 123,124 |
| DC pulsed with lipopeptide | Lipopeptides contained a CD4+T cell epitope, a HLA-A2 restricted CTL epitope and the lipid Pam2Cys | Specific CD8+ T cell responses in HLA-A2 transgenic mice and six patients | 125,126 |

CTL, cytotoxic T lymphocyte; DC, dendritic cell; HCV, hepatitis C virus; HCV-LP, HCV-like particle; MDC, myeloid DC;
be a new promising modality for immunotherapy of HCV. Ito et al.\textsuperscript{122} started with the premise that self-DC could be used to deliver HCV antigens in vivo; hence, they pulsed MDC with peptides from structural (core) and non-structural (NS3 and NS4) HCV proteins to successfully trigger the generation of CTLs. Explored by Kuzushita et al.,\textsuperscript{34} DC were substantially transduced with recombinant HCV core or NS5 protein by using a protein delivery based on a short amphipathic peptide carrier, Pep1. This DC vaccine induced HCV-specific T-cell priming (Th1 type) with high efficacy and duration and protection against tumour challenge. All evidence suggesting that a vaccine consisting of HCV protein transfected DCs should be useful as both prophylactic and therapeutic vaccination against HCV.

Lasarte and colleagues reported that fusion of an antigen with the extra domain A from fibronectin (EDA) leads to antigen targeting TLR4-expressing DC, enhancing cross-presentation and immunogenicity.\textsuperscript{123} To test if EDA-NS3 might behave as an immunogen capable of eliciting robust anti-HCV responses, they prepared a fusion protein and tested its capacity to activate DC maturation in vitro and its immunogenicity in vivo. Their results suggested that EDA-NS3 combined with these adjuvants may be considered for the development of a vaccine against HCV infection.\textsuperscript{124}

Gowans et al. took the DC-based approach one step forward and performed a phase I clinical trial of self-derived DC immunotherapy in HCV-infected individuals who had failed conventional therapy. The lipopeptides they employed contained a single CD4\textsuperscript{+} Th-cell epitope, an HLA-A2-restricted cytotoxic T-cell epitope and the lipid Pam2Cys.\textsuperscript{125} Lipopeptides were able to induce specific CD8\textsuperscript{+} T-cell responses in HLA-A2 transgenic mice and consistently activated human MDDC from both healthy individuals and HCV-infected patients. Lipopeptide-pulsed human DC were also found to secrete the pro-inflammatory cytokine IL-12p70 and were able to activate antigen-specific IFN-\(\gamma\) production by autologous CD8\textsuperscript{+} T cells obtained from a patient with hepatitis C. These results show that DC from HCV-infected patients can be matured and antigen loaded with TLR2-targeting lipopeptides for effective presentation of CD8\textsuperscript{+} T-cell epitopes; the use of autologous lipopeptide-pulsed DC or direct lipopeptide vaccination may be successful approaches for the priming or boosting of anti-HCV CD8\textsuperscript{+} T-cell responses to aid in the clearance of the virus in chronically infected individuals.\textsuperscript{126} They examined the potential of autologous MDDC, presenting HCV-specific HLA A2.1-restricted cytotoxic T-cell epitopes, to influence the course of infection in six patients who failed conventional therapy. In this phase I dose escalation study, no patient showed a severe adverse reaction although all experienced transient minor adverse effects. Patients generated de novo responses, not only to peptides presented by the cellular vaccine but also to additional viral epitopes not represented in the lipopeptides, suggestive of epitope spreading. Despite this, no increases in ALT levels were observed. However, the responses were not sustained and failed to influence the viral load, the anti-HCV core antibody response and the level of circulating cytokines. They suggested that immunotherapy using autologous MDDC pulsed with lipopeptides was safe, but was unable to generate sustained responses or alter the outcome of the infection. Alternative dosing regimens or vaccination routes may need to be considered to achieve therapeutic benefit.\textsuperscript{33}

**Strategies to enhance the efforts of DC-based vaccine**

During the last decade, DC have been regarded as promising tools for the development of more effective therapeutic vaccines in cancer patients. For patients with late-stage disease, strategies that combine novel highly immunogenic DC-based vaccines and immunomodulatory antibodies may have a significant effect on enhancing therapeutic immunity by simultaneously enhancing the potency of beneficial immune arms and offsetting immunoregulatory pathways. These optimized therapeutic modalities include the following.

**Adjuvant**

Glucopyranosyl lipid A (GLA) is a new synthetic nontoxic analogue of lipopolysaccharide. Pantel et al.\textsuperscript{127} studied DC directly from vaccinated mice. Within 4 hr, GLA caused DC to up-regulate CD86 and CD40 and produce cytokines including IL-12p70 in vivo. Importantly, DC removed from mice 4 hr after vaccination became immunogenic, capable of inducing T-cell immunity upon injection into naive mice. These data indicate that a synthetic and clinically feasible TLR4 agonist rapidly stimulates full maturation of DCs in vivo, allowing for adaptive immunity to develop many weeks to months later. Relative to several other TLR agonists, Longhi et al.\textsuperscript{128} found polyinosinic : polycytidylic acid (poly I:C) to be the most effective adjuvant for Th1 CD4\textsuperscript{+} T-cell responses to a DC-targeted HIV gag protein vaccine in mice. Spranger et al.\textsuperscript{129} described a new method for preparation of human DCs that secrete bioactive IL-12p70 using synthetic immunostimulatory compounds as TLR7/8 agonists R848 or CL075. Maturation mixtures included the TLR7/8 agonists, combined with the TLR3 agonist poly I:C, yielded 3 days mature DC that secreted high levels of IL-12p70, showed strong chemotaxis to CCR7 ligands, and had a positive co-stimulatory potential. They also had excellent capacity to activate natural killer cells, effectively polarized CD4\textsuperscript{+} and CD8\textsuperscript{+} T cells to secrete IFN-\(\gamma\) and to induce T-cell-mediated cytotoxic function. Thereby,
mature DCs prepared within 3 days using such maturation mixtures displayed optimal functions required for vaccine development.

Synthetic oligodeoxynucleotides (ODNs) containing unmethylated CpG motifs trigger cells that express TLR9 (including human PDCs and B cells) to mount an innate immune response characterized by the production of Th1 and pro-inflammatory cytokines. When used as vaccine adjuvants, CpG ODNs improve the function of professional antigen-presenting cells and boost the generation of humoral and cellular vaccine-specific immune responses. Preclinical studies indicate that CpG ODNs improve the activity of vaccines targeting infectious diseases and cancer. Clinical trials demonstrate that CpG ODNs have a good safety profile and increase the immunogenicity of co-administered vaccines. Rizza et al. predicted that IFN-α itself, as well as IFN-α-conditioned DC, can represent valuable components in the coming years of new and clinically effective protocols of therapeutic vaccination in patients with cancer and some chronic infectious diseases, whose immune suppression status can be restored by a selective use of these cytokines targeted to DCs and specific T-cell subsets under different experimental conditions.

Blockade of regulatory/suppressive pathways

In chronic HCV infection, virus-specific dysfunctional CD8 T cells often over-express various inhibitory receptors. Programmed cell death 1 (PD-1) was the first among these inhibitory receptors that were identified to be over-expressed in functionally impaired T cells. The roles of other inhibitory receptors such as cytotoxic T lymphocyte-associated antigen-4 (CTLA-4) and T-cell immunoglobulin and mucin domain-containing molecule 3 (Tim-3) have also been demonstrated in T-cell dysfunctions that occur in patients with chronic HCV infection. Blocking these inhibitory receptors in vitro restores the functions of HCV-specific CD8 T cells and allows enhanced proliferation, cytolytic activity and cytokine production. Therefore, the blockade of the inhibitory receptors is considered as a novel strategy for the treatment of chronic HCV infection. Recently, Zhang et al. demonstrated that up-regulation of PD-1 and suppressor of cytokine signalling-1 (SOCS-1) correlates with IL-12 inhibition by HCV core protein and that blockade of PD-1 or SOCS-1 signalling may improve TLR-mediated signal transducer and activator of transcription 1 (STAT-1) activation and IL-12 production in monocytes/macrophages. Blocking PD-1 or silencing SOCS-1 gene expression also decreases Tim-3 expression and enhances IL-12 secretion and STAT-1 phosphorylation. These findings suggest that Tim-3 plays a crucial role in negative regulation of innate immune responses, through cross-talk with PD-1 and SOCS-1 and limiting STAT-1 phosphorylation, and may be a novel target for immunotherapy to HCV infection.

The high levels of IL-10 present in chronic HCV infection have been suggested as responsible for the poor antiviral cellular immune responses found in these patients. To overcome the immunosuppressive effect of IL-10 on antigen-presenting cells such as DC, Diaz-Valdes et al. developed peptide inhibitors of IL-10 to restore DC functions and concomitantly induce efficient antiviral immune responses. The results suggest that IL-10-inhibiting peptides may have important applications to enhance anti-HCV immune responses by restoring the immunostimulatory capabilities of DC.

Regulatory T cells (Treg cells) suppress autoreactive immune responses and limit the efficacy of vaccines, however, it remains a challenge to selectively eliminate or inhibit Treg cells. The zinc-finger A20, a negative regulator of the TLR and TNF receptor signalling pathways, was found to play a crucial part in controlling the maturation, cytokine production and immunostimulatory potency of DC. A20-silenced DC showed spontaneous and enhanced expression of co-stimulatory molecules and pro-inflammatory cytokines and had different effects on T-cell subsets: they inhibited Treg cells and hyperactivated tumour-infiltrating cytotoxic T lymphocytes and T helper cells that produced IL-6 and TNF-α and were refractory to Treg-cell-mediated suppression. Mechanistic studies revealed that A20 regulated DC production of retinoic acid and pro-inflammatory cytokines, inhibiting the expression of gut-homing receptors on T and B cells. Their work provided a strategy for the development of an efficient vaccination.

Optimal of antigen loading

When compared with other cell types, DC are not easily transduced by adenoviruses, requiring high multiplicities of infection to obtain expression of antigen in most cells. Pereboev et al. have reported that CFm40L, an adapter molecule combining the coxsackie-adenovirus receptor fused to the ecto-domain of CD40L by way of a trimerization motif, was able to efficiently target adenoviruses to DC. Moreover, direct immunization with adenoviral particles coated with this adapter molecule was able to induce stronger immune responses than uncoated adenoviral particles. In their studies, targeting of an adenovirus encoding HCV NS3 protein (AdNS3) to DC with CFm40L strongly enhanced NS3 presentation in vitro, activating IFN-γ-producing T cells. Immunization of mice with these DC promoted strong CD4 and CD8 T-cell responses against HCV NS3. CFh40L, a similar adapter molecule containing human CD40L, enhanced transduction and maturation of human MDDC from patients with chronic HCV infection and healthy donors revealed similar maturation levels. DC transduced with AdNS3 and the
adapter molecule CFm/h40L exhibit enhanced immunostimulatory functions, induced robust anti-HCV NS3 immunity in animals, and can induce antiviral immune responses in subjects with chronic HCV infection. This strategy may serve as therapeutic vaccination for patients with chronic hepatitis C.\textsuperscript{31}

To determine whether T-cell responses induced by the protein vaccines could be enhanced after boosting with a viral vector, non-human primates were boosted with a replication defective, recombinant New York vaccinia virus (NYVAC)-HIV Gag/Pol/Nef vector. Boosting with recombinant NYVAC strongly enhances IFN-\gamma-producing T cells following priming with DEC-HIV Gag p24 or HIV Gag p24 plus Poly I/CLC. The NYVAC boosting generates multifunctional CD4\textsuperscript{+} and CD8\textsuperscript{+} cytokine-producing T cells with a similar breadth to those elicited by protein priming. Hence, a robust, broad, durable and polyfunctional CD4\textsuperscript{+} and CD8\textsuperscript{+} T-cell response is generated by boosting a relatively low frequency of cross-primed CD8\textsuperscript{+} T cells induced by a protein vaccine with a single immunization with NYVAC-HIV Gag/Pol/Nef.\textsuperscript{139} These studies can be extended to other diseases to verify whether heterologous prime-boost immunization with vectors and protein vaccines is a logical vaccine approach to optimize both humoral and cellular immunity.

Despite initially encouraging data from preclinical and clinical studies, the efficacy of human adenoviral vector serotype 5 (AdHu5) was hampered by a strong pre-existing anti-vector immunity among vaccinated macaques, in which transgene-specific T cells homed to different organs in the presence of anti-vector immunity.\textsuperscript{140} Listeria monocyctogenes is known to induce strong cellular immune responses. Listeria monocyctogenes induces multiple effector mechanisms, including antigen presentation via MHC class I and II pathways as well as induction of innate immune responses.\textsuperscript{141} As L. monocyctogenes is a ubiquitous bacterium, anti-L. monocyctogenes immune responses are likely to be present among the majority of individuals. Sciaranghella \textit{et al.}\textsuperscript{142} constructed a live-attenuated L. monocyctogenes vector, which encodes SIVmac239 gag. The novel, live-attenuated L. monocyctogenes vector may be an attractive platform for oral vaccine delivery.

Conclusions

Although HCV leads to impairment of both MDC and PDC according to many researchers, the mechanisms how HCV affects DC function remains elusive.\textsuperscript{55} Further research is needed in regard to the mechanisms of HCV-induced DC impairment and the correlation between DC function and HCV persistence. Dendritic cell-based vaccination/therapeutic approaches are safe and promising in terms of their propensity to establish anti-HCV adaptive immune responses. However, possible side-effects of DC-based therapeutic vaccine should be carefully evaluated, especially those possibly inducing a strong T-cell-mediated immunity, because of the dual role of virus-specific cytotoxic T cells mediating both viral clearance and tissue damage. Nevertheless, the achievements in this field of studies brought us the hope of opening new routes to the prevention and treatment of HCV infection. Prospects of a DC-based vaccine against HCV infection include employment of adjuvants, the blockage of negative regulatory signal and enhancement of positive regulatory signals, so as to improve the vaccine immune response against HCV infection, reduce HCV viral load, and hinder progression of chronic liver disease.

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References

1. Shepard CW, Finelli L, Alter MJ. Global epidemiology of hepatitis C virus infection. \textit{Lancet Infect Dis} 2005; 5:558–67.
2. Alter MJ. Epidemiology of hepatitis C virus infection. \textit{World J Gastroenterol} 2007; 13:2436–41.
3. Ghany MG, Strader DB, Thomas DL, Seeff LB. Diagnosis, management, and treatment of hepatitis C. an update. Hepatology 2009; 49:1335–74.
4. Mansy MP, McHutchinson JG, Gordon SC, et al. Peginterferon alpha-2b plus ribavirin compared with interferon alfa-2b plus ribavirin for initial treatment of chronic hepatitis C: a randomised trial. \textit{Lancet} 2001; 358:958–65.
5. Fried MW, Shiffman ML, Reddy KR, et al. Peginterferon alfa-2a plus ribavirin for chronic hepatitis C virus infection. \textit{N Engl J Med} 2002; 347:975–82.
6. Hadjiyannakis SI, Sette H Jr, Morgan TB, et al. Peginterferon-alpha2a and ribavirin combination therapy in chronic hepatitis C: a randomized study of treatment duration and ribavirin dose. \textit{Ann Intern Med} 2004; 140:346–55.
7. Manns MP, Wedemeyer H, Gomberg M. Treating viral hepatitis C: efficacy, side effects, and complications. \textit{Gut} 2006; 55:1350–9.
8. Thompson AE, McHutchinson JG. Antiviral resistance and specifically targeted therapy for HCV (STAT-C). \textit{J Viral Hepat} 2009; 16:377–87.
9. Sharma SD. Hepatitis C virus: molecular biology and current therapeutic options. \textit{Indian J Med Res} 2010; 131:17–34.
10. Torrell J, Johnson D, Wedemeyer H. Progress in the development of preventive and therapeutic vaccines for hepatitis C virus. \textit{J Hepatol} 2011; 54:1273–85.
11. Lauer GM, Barnes E, Lucas M, et al. High resolution analysis of cellular immune responses in resolved and persistent hepatitis C virus infection. \textit{Gastroenterology} 2004; 127:924–36.
12. Schulte zur Wiesch J, Lauer GM, Day CL, et al. Broad repertoire of the CD4\textsuperscript{+} T cell response in spontaneously controlled hepatitis C virus infection includes dominant and highly promiscuous epitopes. \textit{J Immunol} 2005; 175:3603–13.
13. Smyk-Pearson S, Tsetter IA, Lenette D, Suzuki AW, Lewinskiin ZM, Rosen HR. Differential antigenic hierarchy associated with spontaneous recovery from hepatitis C virus infection: implications for vaccine design. \textit{J Infect Dis} 2006; 194:454–63.
14. Petka JM, Zeisel MB, Blaser E, et al. Rapid induction of virus-neutralizing antibodies and viral clearance in a single-source outbreak of hepatitis C. \textit{Proc Natl Acad Sci U S A} 2007; 104:6023–30.
15. Yu CI, Chuang BL. A new insight into hepatitis C vaccine development. \textit{J Biomed Biotechnol} 2010;2010:547850.
Y. Zhou et al.

16 Vajdy M, Selby M, Medina-Selby A et al. Hepatitis C virus polyprotein vaccine formu-
lations capable of inducing broad antibody and cellular immune responses. J Gen Virol 2006; 87( Pt 8):2253–62.

17 O’Hagan DT, Singh M, Tong C et al. Cationic microparticles are a potent delivery 
system for a hepatitis B DNA Vaccine. Vaccine 2004; 23:672–80.

18 Habesberger F, Baumert TF, Stoll-Keller F. G1-5005, a yeast vector vaccine expressing 
an NS3–core fusion protein for chronic HCV infection. Curr Opin Mol Ther 2009; 
11:456–62.

19 Drane D, Maraskovsky E, Gibson R et al. Priming of CD4+ and CD8+ T cell responses 
using a HCV core ISCOMATRIX vaccine: a phase I study in healthy volunteers. Hum 
Vaccine 2009; 5:315–7.

20 Kladis CS, Wedemeyer H, Berg T et al. Therapeutic vaccination of chronic hepatitis C 
nonresponder patients with the peptide vaccine IC41. Gastroenterology 2008; 
134:1385–95.

21 Yutani S, Komatsu N, Shichijo S et al. Phase I clinical study of a peptide vaccination 
for hepatitis C virus-infected patients with different human leukocyte antigen-class I-A 
alleles. Cancer Sci 2009; 100:1935–42.

22 Alvarez-Lajonchere L, Shoukry NH, Gra B et al. Immunogenicity of CIBG-230, a ther-
apoetetic DNA vaccine preparation, in HCV-chronically infected individuals in a Phase 
I clinical trial. J Viral Hepat 2009; 16:156–7.

23 Lang Kuhu KA, Toporowski R, Ginsberg AA et al. Peripheral immunization induces 
functional intrahepatic hepatitis C specific immunity following selective retention of 
vaccine-specific CD8 T cells by the liver. Hum Vaccin 2011; 7:1326–335.

24 Alvarez-Lajonchere L, Duenas-Carrera S. Advances in DNA immunization against 
hepatitis C virus infection. Hum Vaccin 2009; 5:586–71.

25 Lang KA, Yan J, Draghia-Akli R, Khan A, Weiner DB. Strong HCV NS3- and NS4A- 
specific cellular immune responses induced in mice and rhesus macaques by a novel 
HCV genotype 1a/1b consensus DNA vaccine. Vaccine 2008; 26:6225–33.

26 Ahlen G, Nystrom J, Pultz L, Frivald L, Hultgren C, Salberg M. In vivo clearance of 
d Hepatitis C virus nonstructural 3/4A-expressing hepatocytes by DNA vaccine-primed cyto-
toxic T lymphocytes. J Infect Dis 2005; 192:2112–6.

27 Habesberger F, Honnet G, Bain C et al. A peptide vaccine is safe, induces T-cell 
responses, and decreases viral load in patients with chronic hepatitis C. Gastroen-
terology 2011;141:899–914.

28 Fournillier A, Gerossier E, Evlashev A et al. An accelerated vaccine schedule with a 
poly-antigenic hepatitis C virus MVA-based candidate vaccine induces potent, long 
lasting and in vivo cross-reactive T cell responses. Vaccine 2007; 25:7339–53.

29 Capone S, Meola A, Ercole BB et al. Reduced numbers and impaired ability of mye-
loid dendritic cells correlate with impaired hepatitis C virus infection. J Hepatol 2009; 
50:1326–37.

30 Fattori E, Zampaglione I, Arcuri M et al. Efficient immunization of rhesus macaques 
with an HCV candidate vaccine by heterologous priming-boosting with novel adenov-
viral vectors based on different serotypes. Gastro Ther 2006; 13:1088–96.

31 Echeverria I, Perea-bova A, Silva I et al. Enhanced T cell responses against hepatitis C 
virus by ex vivo targeting of adventitial dendritic cells to hepatic cords. Hepatology 2011; 
53:2621–37.

32 Kanto T, Hayashi N, Takahara T et al. Impaired allostimulatory properties of dendritic 
cells generated from chronic hepatitis C virus-infected individuals. J Infect Dis 2006; 
194:1359–64.

33 Kanto T, Hayashi N, Takahara T et al. Impaired allostimulatory capacity of peripheral 
human dendritic cells from chronic hepatitis C virus-infected individuals. J Immunol 
2009; 182:601–7.

34 Kuzushita N, Gregory SH, Monti NA, Carlson R, Gehring S, Wands JR. Vaccination 
with protein-transduced dendritic cells elicits a sustained response to hepatitis C viral 
proteins. J Transl Med 2009; 7:109.

35 Tawab A, Fan Y, Read EJ, Kurlander RJ. Effect of ex vivo culture duration on pheno-
type and cytokine production by mature dendritic cells derived from peripheral blood 
monocytes. Transfus 2009; 49:536–47.

36 Anguille S, Smith EL, Cools N, Gossens B, Beneman ZN, Van Tendeloo VF. Short-
term cultured, interleukin-15 differentiated dendritic cells have potent immunostimula-
tory properties. J Transl Med 2009; 7:109.

37 Li R, Woltman AM, Janssen HL, Boonstra A. Modulation of dendritic cell function by 
persistent viruses. J Leukoc Biol 2009; 85:205–14.

38 Ryan EJ, O’Farrell C. The affect of chronic hepatitis C infection on dendritic 
cell function: a summary of the experimental evidence. J Viral Hepat 2011; 18:601–7.

39 Auffermann-Gretzinger S, Kreffl EB, Levy S. Impaired dendritic cell maturation 
in patients with chronic, but not resolved, hepatitis C virus infection. Blood 2001; 
97:3171–8.

40 Della Bella S, Crosignani A, Riva A, Perrella A, Atripaldi L, Bellopede P et al. Reduced 
numbers and impaired ability of mye-
loid and plasmacytoid dendritic cells to polarize T helper cells in chronic hepatitis C 
virus infection. J Infect Dis 2004; 190:1919–26.

41 Della Bella S, Crosignani A, Riva A, Perrella A, Atripaldi L, Bellopede P et al. Reduced numbers and impaired ability of mye-
loid and plasmacytoid dendritic cells to polarize T helper cells in chronic hepatitis C 
virus infection. J Infect Dis 2004; 190:1919–26.

42 Tsay S, Martin W, Shoukry NH, Gra B, Bhardwaj N, Hwang WY, Lin JH, Giovino A, 
Glennon B, Benhamou C. Current approaches in dendritic cell generation and future 
implications for cancer immunotherapy. Cancer Immunol Immunotherap 2007; 56:1513–37.

43 Etkin E, Williams PE, Kynaston H, Rowbottom AW. Human mucocyte isolation 
methods influence cytokine production from in vitro generated dendritic cells. Immu-
nology 2005; 114:204–12.

44 Kanto T, Inoue M, Miyatake H et al. Impaired allostimulatory properties of dendritic 
cells in chronic hepatitis C virus infection. J Immunol 2001; 167:797–7.

45 Figgot CG, de Vries JJ, Lenterhuis WF, Meijer CJ. Dendritic cell immunotherapy: map-
ing the way. Nat Med 2004; 10:475–80.

46 Dauer M, Obermaier R, Herten J et al. Mature dendritic cells derived from human 
monocytes within 48 hours: a novel strategy for dendritic cell differentiation from 
blood precursors. J Immunol 2003; 170:4089–96.

47 Kurlander RJ, Bailey DR, Clark DS et al. Improved allometabolising capacity of peripheral 
human dendritic cells from chronic hepatitis C virus-infected individuals. J Immunol 
1999; 162:5844–91.

48 Kanto T, Hayashi N, Takahara T et al. Impaired allometabolising capacity of peripheral 
human dendritic cells from recovered from hepatitis C virus-infected individuals. Nat 
Med 2003; 9:1019–25.

49 Katto T, Inoue M, Miyatake H et al. Reduced numbers and impaired ability of mye-
lid and plasmacytoid dendritic cells to polarize T helper cells in chronic hepatitis C 
virus infection. J Infect Dis 2004; 190:1919–26.

50 Della Bella S, Crosignani A, Riva A, Perrella A, Atripaldi L, Bellopede P et al. Reduced numbers and impaired ability of mye-
lid and plasmacytoid dendritic cells to polarize T helper cells in chronic hepatitis C 
virus infection. J Infect Dis 2004; 190:1919–26.

51 Tsay S, Martin W, Shoukry NH, Gra B, Bhardwaj N, Hwang WY, Lin JH, Giovino A, 
Glennon B, Benhamou C. Current approaches in dendritic cell generation and future 
implications for cancer immunotherapy. Cancer Immunol Immunotherap 2007; 56:1513–37.

52 Kurlander RJ, Bailey DR, Clark DS et al. Improved allometabolising capacity of peripheral 
human dendritic cells from chronic hepatitis C virus-infected individuals. J Immunol 
1999; 162:5844–91.

53 Katto T, Inoue M, Miyatake H et al. Reduced numbers and impaired ability of mye-
lid and plasmacytoid dendritic cells to polarize T helper cells in chronic hepatitis C 
virus infection. J Infect Dis 2004; 190:1919–26.

54 Kuroda T, Takahara T et al. Impaired allometabolising capacity of peripheral 
human dendritic cells from recovered from hepatitis C virus-infected individuals. Nat 
Med 2003; 9:1019–25.
DC vaccine against HCV

68 Mengehold JA, Golden-Mason L, Castellano N, Im KA, Dillon SM, Wilson CC, Rosen HR. Impaired plasmacytoid dendritic cell maturation and differential chemotaxis in chronic hepatitis C virus: associations with antiviral treatment outcomes. J Infect Dis 2009; 199:964–73.

69 Dolganiuc A, Paek E, Kodyk Y, Thomas J, Szabo G. Myeloid dendritic cells of patients with chronic HCV infection induce proliferation of regulatory T lymphocytes. Gastroenterology 2008; 135:2119–27.

70 Uhelenheimer A, Gerlich FT, Jung MC et al. Plasmacytoid dendritic cells in acute and chronic hepatitis C virus infection. Hepatology 2005; 41:643–51.

71 Saito K, Ari-Goughoulte M, Truscott SM et al. Hepatitis C virus inhibits cell surface expression of HLA-DR, prevents dendritic cell maturation, and induces interleukin-10 production. J Viral Hepat 2008; 15:320–8.

72 Anthony DD, Younkers NL, Post AB, Assad R, Heimel FP, Lederman MM, Lehmann PV, Valdez H. Selective impairments in dendritic cell-associated function distinguish hepatitis C virus and HIV infection. J Immunol 2004; 172:4970–6.

73 Rollier C, Drexhage JA, Verstrepen BE, Verschoor EJ, Bontrop RE, Koopman G, Heeke A. Antigen-presenting cells at the vicinity of focal and confluent necrosis. Hepatology 2009; 50:117–25.

74 Tanimoto K, Akbar SM, Michitaka K, Horiike N, Onji M. Antigen-presenting cells at the vicinity of focal and confluent necrosis. Hepatology 2009; 50:117–25.

75 Piccioli D, Tavarini S, Nuti S et al. Comparable functions of plasmacytoid and monocytes-derived dendritic cells in chronic hepatitis C patients and healthy donors. J Hepatol 2010; 52:61–7.

76 Langman 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.

77 Larson M, Babcock E, Grakoui A, Shounky N, Laurer G, Rice C, Walker B, Bharelwaj N. Lack of protective and functional impairment in dendritic cells from chimpanzees chronically infected with hepatitis C virus. J Viral Hepat 2008; 15:611–64.

78 Langman 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.

79 Piccioli D, Tavarini S, Nuti S et al. Differential effects of hepatitis C virus JFH1 on human myeloid and plasmacytoid dendritic cells. J Virol 2009; 83:569–77.

80 Liang H, Russell RS, Younkers NL, McDonald D, Rodriguez R, Harding CV, Anthony DD. Differential effects of hepatitis C virus IFN on human myeloid and plasmacytoid dendritic cells. J Virol 2009; 83:569–77.

81 Zhang YL, Guo YJ, Bin L, Sun SH. Hepatitis C virus-specific single-stranded RNA induces innate immunity via Toll-like receptor 7. J Hepatol 2009; 51:29–38.

82 Simone O, Tortorella C, Zaccaro B, Napoli N, Antonaci S. Impairment of TLR7-dependent signaling in dendritic cells from chronic hepatitis C virus (HCV)-infected non-responders to interferon/ribavirin therapy. J Clin Immunol 2010; 30:556–65.

83 O’Brine J, Mitchell J, Farazaneh F, Harrison PM. Inhibition of major histocompatibility complex Class I antigen presentation by hepatitis C virus core protein in myeloid dendritic cells. Virolology 2009; 389:1–7.

84 Lunde A, Yu H, Rubik LA, van Drunen Littel-van der Hark S. Human dendritic cells expressing hepatitis C virus core protein display transcriptional and functional changes consistent with maturation. J Virol 2013; 87:1800–13.

85 Li W, Li J, Tyrrell DL, Agrawal B. Expression of hepatitis C virus-derived core or NS3 antigens in human dendritic cells leads to induction of pro-inflammatory cytokines and normal T-cell stimulation capabilities. J Gen Virol 2006; 87(1):61–72.

86 Rodrigue-Gervais IG, Bigley H, Jouan L, Sauve D, Sekaly RP, Willems B, Lamarre D. Dendritic cell inhibition is connected to exhaustion of CD8+ T cell polyfunctionality during chronic hepatitis C virus infection. J Immunol 2010; 184:3314–44.

87 Diebold SS, Montoya M, Unger H et al. Viral infection switches non-plasmacytoid dendritic cells into high interferon producers. Nature 2003; 424:324–8.

88 Feyr E, Li K, Wang C, Sampter R Jr, Ibeda M, Lemon SM, Gale M Jr. Regulation of interferon-regulatory factor-3 by the hepatitis C virus genome protease. Science 2003; 300:1145–8.

89 Brady MT, MacDonald AJ, Rowan AG, Mills KH. Hepatitis C virus non-structural protein 4 suppresses Th1 responses by stimulating IL-10 production from monocytes. Eur J Immunol 2003; 33:3448–57.

90 Takai A, Tatsuikawa M, Iwasa Y et al. Hepatitis C virus NS5 protein impairs the Th1 polarization of immature dendritic cells. J Virol 2010; 87:1555–62.

91 Aminj D, Abdel-Haq NJ, Faizul M, Kamal M, Mosadegh V. Decreased interferon-alpha production and impaired regulatory function of plasmacytoid dendritic cells induced by the hepatitis C virus NS 5 protein. Microb Cell Biol 2006; 52:499–507.

92 Savoshian S, Abraham JD, Thumann C, Kieny MP, Schuster C. Hepatitis C virus core, NS3, NS5A, NS5B proteins induce apoptosis in mature dendritic cells. J Med Virol 2005; 75:402–11.

93 Kriulisadas DR, Alen JS, Han H, Kumar R, Agrawal B. Immunomodulation by hepatitis C virus-derived proteins targeting human dendritic cells by multiple mechanisms. J Immunother 2010; 22:491–502.

94 Shen T, Chen X, Chen Y, Xu Q, Lu F, Liu S. Increased PD-L1 expression and PD-L1/CD80 ratio on dendritic cells were associated with impaired dendritic cell function in HCV infection. J Med Virol 2010; 82:1152–9.

95 Shima M, Rehermann B. Cell culture-produced hepatitis C virus impairs plasmacytoid dendritic cell function. Hepatology 2008; 47:385–95.

96 Goudie-Rey F, Dental C, Haltin P, Baumert TF, Olive D, Hirsch I. Hepatitis C virus is a weak inducer of interferon alpha in plasmacytoid dendritic cells in comparison with influenza and human herpesvirus type-1. PLoS ONE 2009; 4:e9319.

97 Moriya O, Matsui M, Osorio M et al. Induction of hepatitis C virus-specific cytotoxic T lymphocytes in mice by immunization with dendritic cells treated with an anthrax toxin fusion protein. Vaccine 2001; 20:799–814.

98 Matsui M, Moriya O, Abe Y, Naito Y, Mihara M, Akaike T. Induction of hepatitis C virus-specific cytotoxic T lymphocytes in mice by immunization with dendritic cells transduced with replication-defective recombinant adenovirus. Vaccine 2002; 21:211–20.

99 Zabelta A, Luepke D, Arrillaga L et al. Vaccination against hepatitis C virus with dendritic cells transduced with an adenovirus encoding NS5 protein. Mol Ther 2008; 16:210–7.
Y. Zhou et al.

118 Liu Y, Zhou W, You C et al. An autoimmune domain-reduced HCV core gene remains effective in stimulating anti-core cytotoxic T lymphocyte activity. Vaccine 2006; 24:1615–24.

119 Gehring S, Gregory SH, Wintermeyer P, Aloman C, Wands JR. Generation of immune responses against hepatitis C virus by dendritic cells containing NS5 protein-coated microparticles. Clin Vaccine Immunol 2005; 12:362–9.

120 Wintermeyer P, Gehring S, Eken A, Wands JR. Generation of cellular immune responses to HCV NS5 protein through in vivo activation of dendritic cells. J Viral Hepat 2010; 17:705–13.

121 Encke J, Findeklee J, Grieb J, Pfaff E, Stremmel W. Prophylactic and therapeutic vaccination with dendritic cells against hepatitis C virus infection. Clin Exp Immunol 2005; 142:362–9.

122 Ito A, Kanto T, Kuzushita N et al. Generation of hepatitis C virus-specific cytotoxic T lymphocytes from healthy individuals with peptide-pulsed dendritic cells. J Gastroenterol Hepatol 2001; 16:509–16.

123 Lasarte JJ, Casares N, Gorraiz M et al. The extra domain A from fibronectin targets antigens to TLR4-expressing cells and induces cytotoxic T cell responses in vivo. J Immunol 2007; 178:748–56.

124 Mansilla C, Gorraiz M, Martinez M et al. Immunization against hepatitis C virus with a fusion protein containing the extra domain A from fibronectin and the hepatitis C virus NS5 protein. J Hepatol 2009; 51:520–7.

125 Jones KL, Brown LE, Eriksson EM et al. Human dendritic cells pulsed with specific lipopeptides stimulate autologous antigen-specific T cells without the addition of exogenous maturation factors. J Viral Hepat 2008; 15:761–72.

126 Chua BY, Eriksson EM, Brown LE, Zeng W, Gowans EL, Torres J, Jackson DC. A self-adjuvanting lipopeptide-based vaccine candidate for the treatment of hepatitis C virus infection. Vaccine 2008; 26:4866–75.

127 Pantel A, Choung C, Dandumudi D et al. A new synthetic TLR4 agonist, GLA, allows dendritic cells targeted with antigenic doci to Th1 T-cell immunity in vivo. Eur J Immunol 2011; 41:101–109.

128 Longhi MP, Trumpfthaler C, Idoyaga J et al. Dendritic cells require a systemic type I interferon response to mature and induce CD4+ Th1 immunity with poly IC as adjuvant. J Exp Med 2009; 206:1589–602.

129 Spranger S, Jarovevic M, Burdik M et al. Generation of Th1-polarizing dendritic cells using the TLR7/8 agonist CL075. J Immunol 2010; 185:738–47.

130 Bode C, Zhao G, Steinhagen F, Kinjo T, Klinman DM. CpG DNA as a vaccine adjuvant. Expert Rev Vaccines 2011; 10:499–511.

131 Rizza P, Capone I, Moretti F, Proietti E, Belandelli F. IFN-alpha as a vaccine adjuvant: recent insights into the mechanisms and perspectives for its clinical use. Expert Rev Vaccines 2011; 10:487–98.

132 Lee J, Suh WI, Shin EC. T-cell dysfunction and inhibitory receptors in hepatitis C virus infection. Immune Netw 2010; 10:120–3.

133 Zhang Y, Ma CJ, Li G et al. Cross-talk between programmed death-1 and suppressor of cytokine signaling-1 in inhibition of IL-12 production by monocytes/macrophages in hepatitis C virus infection. J Immunol 2011; 186:3093–103.

134 Zhang Y, Ma CJ, Wang JM, Ji Xi, Wu XY, Jia ZS, Moorman JP, Yao ZQ. Tim-3 negatively regulates IL-12 expression by monocytes in HCV infection. PLoS ONE 2011; 6:e19664.

135 Díaz-Yalde N, Mantesa L, Belsue V et al. Improved dendritic cell-based immunization against hepatitis C virus using peptide inhibitors of interleukin 10. Hepatology 2011; 53:23–31.

136 Song XT, Evel-Kahler K, Shen L, Rollins L, Huang XF, Chen SY. A20 is an antigen presentation attenuator, and its inhibition overcomes regulatory T cell-mediated suppression. Nat Med 2008; 14:254–65.

137 Hong R, Song XT, Rollins L, Berry L, Huang XF, Chen SY. Mucosal and systemic anti-HIV immunity controlled by A20 in mouse dendritic cells. J Clin Invest 2011; 121:793–91.

138 Pereboev AV, Nagle JM, Shakhmatov MA, Tiziozi PL, Matthews QL, Kawakami Y, Curiel DT, Blackwell JL. Enhanced gene transfer to mouse dendritic cells using adenoviral vectors coated with a novel adapter molecule. Mol Ther 2004; 9:712–20.

139 Flynn BJ, Kastenmuller K, Wille-Reece U et al. Immunization with HIV Gag targeted to dendritic cells followed by recombinant New York vaccinia virus induces robust T-cell immunity in nonhuman primates. Proc Natl Acad Sci U S A 2011; 108:7315–6.

140 McCoy K, Tatsis N, Korioth-Schmitz B et al. Effect of preexisting immunity to adenovirus human serotype 5 antigens on the immune responses of nonhuman primates to vaccine regimens based on human- or chimpanzee-derived adenovirus vectors. J Virol 2007; 81:6594–604.

141 Wallecha A, Carroll KD, Maciag PC, Rivera S, Shahabi V, Paterson Y. Multiple effectors mechanisms induced by recombinant Listeria monocytogenes anticancer immunotherapy. Adv Appl Microbiol 2009; 66:1–27.

142 Sciaranghella G, Lakaheic SK, Ayash-Rashkovsky M et al. A live attenuated Listeria monocytogenes vaccine vector expressing HIV Gag is safe and immunogenic in macaques and can be administered repeatedly. Vaccine 2011; 29:476–86.