Development of a dendritic cell vaccine encoding multiple cytotoxic T lymphocyte epitopes targeting hepatitis C virus

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Abstract. The aim of the present study was to develop a dendritic cell (DC) vaccine encoding hepatitis C virus (HCV) multiple cytotoxic T lymphocyte (CTL) epitopes that can stimulate T cell responses in vitro, and can be used for immunization in vivo. DCs were infected with recombinant replication-defective adenoviruses (Ads) expressing 2 HCV sequences fused with green fluorescent protein (GFP) and FLAG tags. One sequence (sequence 1) contained the HCV CTL epitopes, NS4B 1793-1801 and P7 774-782, as well as the HCV Th epitope, NS3 1248-1261. A second sequence (sequence 2) was the positive epitope control which contained HCV core 35-44, core 132-140 and NS3 1248-1261. The efficiency of infection was detected by flow cytometry and the expression of HCV epitopes in the DCs was confirmed by RT-PCR and western blot analysis. Ad infection significantly enhanced DC maturation and interleukin (IL)-12p70 production, resulting in T cell proliferation and increased interferon-γ secretion. The CTLs stimulated by Ad-infected DCs specifically killed Huh7.5 human hepatoma cells. The recombinant Ad-expressing multiple CTL HCV epitopes effectively infected the DCs in vitro and promoted T cell antiviral immune responses, thereby laying the foundation for the development of anti-HCV DC vaccines.

Introduction

Infection with hepatitis C virus (HCV) has become a global health issue with a prevalence of 170 million individuals infected worldwide (1). The use of pegylated interferon together with ribavirin remains the standard of care treatment for non-genotype 1-infected patients. For genotype 1-infected patients, who represent the majority of cases, current standard treatment consists of triple therapy; pegylated interferon, ribavirin and a first generation protease inhibitor (telaprevir or boceprevir). Although triple therapy represents a major advance by increasing the possibility of viral eradication, it also presents new challenges which include the need for strict compliance, risk of additional side-effects, the development of resistant variants and drug-drug interactions (2,3). No vaccine is currently available to prevent hepatitis C. Therefore, the development of effective preventive and therapeutic vaccines for HCV is mandatory (4).

HCV contains a single-stranded, positive-sense RNA genome of approximately 9,600 nucleotides, encoding structural (core, E1, E2 and p7) and non-structural (NS2, NS3, NS4a, NS4b, NS5a and NS5b) proteins (5). The outcome of HCV infection appears to be determined by a complex interplay between immunological factors, host genetics and viral escape mutations. Thus, a vaccine must be effective against different HCV genotypes crossing multiple viral quasispecies, elicit broad T cell responses and sustain long-lived memory CD4+ and CD8+ T cell responses (6). Major histocompatibility complex (MHC) class I is directly associated with antigen presentation to CD8+ T cells and the induction of effective cross-genotype CD8+ T cell responses has the potential to limit the escape of HCV from immune responses (7). Therefore, a robust multi-specific and cross-genotype CD8+ T cell response to different viral epitopes is required for a successful response against HCV infection.

Peripheral blood dendritic cells (DCs) from HCV-infected patients have been shown to exhibit reduced function compared with DCs from healthy subjects (8,9). These defects in DCs correlate with an impairment of the effector function of HCV-specific CD8+ T cells. HCV-specific tetramer-positive T cells are found in peripheral blood mononuclear cells.
Materials and methods

Construction and production of recombinant Ad encoding multiple CTL HCV epitopes. In the current study, DCs were infected with recombinant replication-defective Ads expressing 2 HCV sequences fused with green fluorescent protein (GFP) and FLAG tags. Sequence 1 contained the HCV CTL epitopes, NS4B 1793-1801 and NS3 1248-1261. Sequence 2 contained the HCV CTL epitope, NS4B 1793-1801 and P7 774-782. The GFP gene and the FLAG tag were inserted into the pTrack plasmid containing the human cytomegalovirus (CMV) promoter. The multiple CTL HCV epitopes vector and supercoiled pAdEasy-1 were co-transformed into E. coli DH10B competent cells. The resulting AdHCV plasmid was propagated and purified according to the AdEasy protocol. Ad vectors containing sequence 1 were termed Ad1, while the Ad vector containing sequence 2 was termed Ad2. The control AdGFP vector containing the GFP gene but without the FLAG tag was used as a control. The Ad vectors were propagated and purified and replicated into the 293T cells. Viral particles were harvested from freeze-thaw lysates. After 2 rounds of purification by CsCl ultracentrifugation, the Ad vector titers were determined by plaque assay on the 293T cells. Ad vectors were stored in small aliquots at -80°C and thawed immediately before use and kept on ice prior to dilution and addition to the cells.

Generation of immature DCs (imDCs). Informed consent was obtained from all donors prior to participation in the study. The study protocol was approved by the Human Ethics Committee of Tangdu Hospital, Xi’an, China and was carried out in conformity with the guidelines of the Helsinki declaration. PBMCs obtained from the peripheral blood of healthy adults were isolated by Ficoll-Hypaque (Sigma, St. Louis, MO, USA) density gradient separation. CD14+ monocytes were isolated using CD14 isolation beads (Miltenyi Biotec, Bergisch Gladbach, Germany). Purity was assessed by staining with a FITC anti-CD14 antibody (BD Biosciences, San Jose, CA, USA) and was routinely found to be >95%. To generate imDCs, the purified cells were adjusted to 1x10⁶ cells/ml and cultured in serum-free X-VIVO15 medium (04-744Q; Lonza, Basel, Switzerland) in the presence of recombinant granulocyte-macrophage colony-stimulating factor (GM-CSF; 100 ng/ml) and interleukin-4 (IL-4; 100 ng/ml) (both from PeproTech, Rocky Hill, NJ, USA) in 6-well plates at 37°C in a 5% CO₂ atmosphere for 5 days.

Ad infection of imDCs and induction of DC maturation. For Ad infection, 5x10⁵ imDCs/ml were resuspended in fresh X-VIVO15 medium and seeded into 24-well plates. Recombinant Ads (Ad1, Ad2 and AdGFP) were added to the imDCs at various multiplicities of infection (MOIs) ranging from 50 to 1,000. Following incubation for 4 h, the cells were washed with PBS and further cultured in X-VIVO15 medium as previously described. In some experiments, the control mature DCs (mDCs) were generated by the addition of a maturation cocktail to the imDC for the final 2 days. The maturation cocktail consisted of 1,000 U/ml IL-1β, IL-6 and tumor necrosis factor (TNF)-α, as well as 1 μg/ml prostaglandin E2 (PGE2) (all from PeproTech). The Ad-infected cells were evaluated by fluorescence microscopy and flow cytometry.

Flow cytometric analysis. DCs on day 8 (mDCs) and after infection with Ads for 48 h (Ad1-mDCs, Ad2-mDCs and AdGFP-mDCs) were assessed for cell surface phenotypes by flow cytometry. Briefly, the DCs were washed and resuspended in PBS and incubated with various fluorochrome-conjugated monoclonal antibodies in 5 ml FACS tubes at 4°C for 30 min in the dark. Fluorescein isothiocyanate (FITC)-conjugated antibodies against CD80, phycoerythrin (PE)-conjugated antibodies against CD83, peridinin chlorophyll protein complex (PerCP)-conjugated antibodies against CD86 and allopho-
cocyian (APC)-conjugated antibodies against HLA-DR (BD Biosciences) were used. The cells were then washed twice and fixed in PBS containing 1% formaldehyde. The phenotype of the cells was analyzed by flow cytometry using a BD FACS Calibur.

**ELISA detection of IL-12p70.** Following Ad infection, the DCs were cultured in 24-well plates for 48 h. Some wells with uninfected DCs were used as the control samples. For both infected and uninfected DCs, cytokine release into the supernatant was evaluated by enzyme-linked immunosorbent assay (ELISA) using an IL-12p70 ELISA detection kit (BD Biosciences).

**RNA isolation, cDNA synthesis and reverse transcription.** Twenty-four hours after infection, the DCs were harvested and total RNA was prepared from 1 or 2x10⁶ DCs using the RNeasy mini kit (74104; Qiagen, Hilden, Germany). Complementary DNA (cDNA) was synthesized using the cDNA synthesis kit (Fermentas/Thermo Scientific Molecular Biology, Pittsburgh, PA, USA) according to the manufacturer's instructions. The upstream primers for sequences 1 and 2 were 5'-ATGTCATGATGGCTTTCCAGGC-3' and 5'-ATGTCATTGTGGCACCAG-3', respectively, and the downstream primer (5'-CTACTTATCGTCATCCCTGT-3') was the same for both sequences. The primers were specific for the NS4B and P7 peptides. The conditions used for PCR amplification were as follows: 95°C for 5 min, 35 cycles at 95°C for 30 sec, 55°C for 30 sec, and 72°C for 45 sec followed by a final extension at 72°C for 10 min. Polymerase chain reaction products were visualized on 2% agarose gels.

**Western blot analysis.** Target protein expression was detected by western blot analysis. Total protein was extracted using RIPA reagent (PL005-PL008 PL035; Sangon Biotech, Shanghai, China). Following the removal of cell debris by centrifugation (13,200 x g at 10 min), 50 µg from each lystate sample were boiled for 5 min in sample buffer, separated using 15% SDS-PAGE and transferred onto PVDF membranes (Millipore). Non-specific reactivity was blocked in 5% non-fat milk in TBST for 1 h at room temperature. The membranes were then incubated with polyclonal mouse anti-FLAG antibody (f1804; Sigma) followed by goat anti-mouse IgG-HRP (sc-2031; Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA). Reactive protein was detected by enhanced chemiluminescence (ECL) (Millipore, Billerica, MA, USA).

**T cell proliferation assay.** T cell proliferation was assessed using the cell counting kit-8 (CCK-8). 2-(2-Methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulphophenyl)-2H-tetrazolium, monosodium salt (WST-8) produces a water-soluble formazan dye upon reduction in the presence of an electron carrier. The amount of formazan dye generated by the activity of dehydrogenases in the cells is directly proportional to the number of living cells (26). Proliferative responses were measured in triplicate in flat-bottom 96-well microtiter plates. Autologous non-adherent T cells (1x10⁷/well, obtained after removal of adherent T cells) were co-cultured with various concentrations of infected or uninfected DCs (10³-10⁶) in 200 µl of X-VIVO™ 15 medium at 37°C for 4 days. The assay included a blank control (medium only) and a negative control (T cells alone).

After the addition of 20 µl of WST-8/well for 4 h, the optical density (OD) was recorded at a wavelength of 450 nm according to the manufacturer's instructions. The stimulating index of proliferation was calculated as follows: stimulating index of proliferation = (mean experimental OD - mean blank control OD)/(mean negative control OD - mean blank control OD).

**Cytotoxicity assay and IFN-γ ELISA.** The release of lactate dehydrogenase (LDH) was measured using a CytoTox 96 Non-Radioactive Cytotoxicity Assay kit (Promega, Madison, WI, USA). Effector T cells were incubated with Ad-infected DCs at a ratio of 10:1 for 7 days at 37°C with 5% CO₂. T cells alone, T cells incubated with uninfected DCs and T cells incubated with GFP-DCs were used as the negative controls. Hub7.5 cells electrotansfected with FL-J6/JFH transcripts were used as target cells (27,28). Hub7.5 cells were electroporated at 230 V, 950 µF using 4 mm electroporation cuvettes, and then cultured in opti-MEM for 2 days. A total of 1x10⁴ target cells in a volume of 100 µl of phenol red-free DMEM were plated into individual wells of a 96-well U-bottom plate in triplicate. Effector cells were added at an effector:target ratio (E:T) of 25:1, 50:1 and 100:1. The cell mixture was incubated for 4 h at 37°C. The spontaneous release by target and effector cells was determined by incubation of the respective populations alone. Following incubation, the conditioned medium was collected and centrifuged at 250 x g for 4 min. A sample (50 µl) of each supernatant was transferred to separate wells of a 96-well plate and substrate solution (50 µl) was added. After 30 min of incubation at room temperature, the absorbance was measured at 490 nm using a microplate reader. Maximum release was determined by cell lysis with 1% Triton X-100. The results were calculated as the means of triplicate assays, and the percentage of specific lysis was calculated according to the following formula: cytotoxicity (%) = [((experimental - effector spontaneous - target spontaneous)/(target maximum - target spontaneous))] x 100%. To quantify cytokine production, 100 µl of each supernatant from the cytotoxicity assay were collected and evaluated using an IFN-γ ELISA detection kit (BD Biosciences) according to the manufacturer's instructions.

**Statistical analysis.** All data were analyzed using SPSS 13.0 software. One-way analysis of variance (ANOVA) was used for multiple group comparison. A P-value <0.05 was considered to indicate a statistically significant difference.

**Results**

**Production of high titer recombinant Ads expressing multiple CTL HCV epitopes.** Recombinant Ads encoding multiple CTL epitopes from HCV were constructed. GFP was used as a positive marker for epitope expression. The titers of the viral stocks were determined by a plaque assay using 293T cells, and were 1.68x10⁹, 1.74x10⁸, and 1.56x10⁷ pfu/ml for AdGFP, Ad1 and Ad2, respectively.

**Analysis of Ad transfection efficiency by flow cytometric assessment of GFP.** CD14⁺ monocytes were cultured in the presence of GM-CSF and IL-4 for 5 days and then incubated with a maturation mix or transfected with Ad at different
MOIs, and the cultures were further incubated until day 7. Upon examination by fluorescence microscopy, cells initially showed identical morphological changes from CD14+ monocytes (day 1) to imDCs (day 5) as well as mDCs (day 7) (Fig. 1). The formation of veils and clusters was evident during the differentiation of monocytes into highly mobile imDCs. After maturation, there were no morphological differences between the mDCs and Ad1-DCs, as shown by microscopic examination. Flow cytometry was used to determine the efficiency of Ad infection by GFP expression. The infection efficiencies were 11.4, 38.8, 76.8, 85.7, 88.3 and 93.8%, at an MOI of 50, 100, 250, 500, 750 and 1,000, respectively.

Identification of multiple CTL HCV epitopes in DCs. The expression of multiple CTL HCV epitopes in DCs was measured by RT-PCR and western blot analysis. Twenty-four hours after infection with Ad1 and Ad2, the DCs were harvested and total RNA was extracted for RT-PCR analysis to detect HCV epitopes. A 150-bp fragment corresponding to the expected size is shown in Fig. 3A. Forty-eight hours after the Ad infection of DCs, western blot analysis was performed to assess recombinant protein expression. As CMCE was fused with FLAG, anti-FLAG was used as the primary antibody. A 10-kDa protein was detected in the Ad1- and Ad2-infected DCs, but not in the uninfected mDCs, indicating that sequence 1 and 2 target proteins were successfully expressed in DCs (Fig. 3B).

Assessment of DC phenotype by examining cell surface activation markers. The activation of DCs by Ad treatment was determined by comparing the expression level of DC activation markers (normally expressed at low levels in imDCs) on cytokine-treated control DCs and Ad-treated DCs using fluorescence activated cell sorter (FACS) analysis. The following markers were upregulated upon DC activation: MHC class II (HLA-DR molecules), co-receptor molecules, such as CD80 and CD86, and the maturation marker, CD83 (Fig. 4). The expression of CD80, CD83, CD86 and HLA-DR in imDCs was very low (data not shown). Following infection with Ad1, the DCs had higher expression rates of CD80, CD83, CD86 and HLA-DR (76.87, 87.75, 97.51 and 97.85%, respectively) compared with the uninfected mDCs (48.29, 60.89, 91.23 and 92.30%, respectively). Ad2-DC and AdGFP showed a similar expression of these cell surface markers. The upregulation of activation markers indicates that imDCs have undergone functional maturation and can effectively present antigen to T cells and secrete cytokines, such as IL-12p70.

IL-12p70 secretion by Ad-infected DCs and IFN-γ production by T cells co-cultured with Ad-infected DCs. IL-12p70 production by DCs was evaluated by ELISA before and after Ad infection. At an Ad MOI of 250, the infection efficiency was approximately 75%. The results revealed that the levels of IL-12p70 were significantly higher after 24 h of infection compared with the uninfected DCs (P<0.05) (Fig. 5A). Thus, after Ad infection, IL-12p70 secretion increased slightly in the DCs infected with Ad1 compared with the DCs infected with Ad2, although the difference was not statistically significant. IL-12p70 secretion by Ad1-DCs and Ad2-DCs was more
than that of GFP-DCs and mDCs. The cells activated by DCs infected with Ad1 or Ad2 vectors were incubated with Huh7.5 cells that had been electrotransfected with FL-J6/JFH transcripts. Supernatants were harvested to assess IFN-γ production. IFN-γ secretion by Ad1-DC-T was higher than that by other groups (Fig. 5B).

Figure 3. Expression of multiple cytotoxic T lymphocyte (CTL) hepatitis C virus (HCV) epitopes in dendritic cells (DCs). (A) PCR amplification of specific sequences. Lanes 1 and 2, sequence 1; lane 3, DNA marker; lanes 4 and 5, sequence 2; lane 6, negative control. (B) Western blot analysis of HCV epitope expression. Expression of HCV epitopes fused with FLAG in Ad1-DCs and Ad2-DCs using anti-FLAG antibody. Lane 1, Ad1-DCs; lane 2, Ad2-DCs; lane 3, mDCs.

Figure 4. Cell surface markers of dendritic cells (DCs) measured by flow cytometry. This figure illustrates that the DCs infected with adenovirus (Ad) (Ad1-DC, Ad2-DC and AdGFP) express similar levels of CD80, CD83, CD86 and HLA-DR, compared with CD14+ monocyte-induced mature DCs. The results indicated that infected DCs exhibited a mature phenotypic change toward antigen presentation.
Stimulation of autologous T cells by Ad-infected DCs. Autologous T cell stimulation using increasingly limited numbers of DCs is considered to be a hallmark to determine the function of DCs as professional APCs. The ability of Ad1-DCs, Ad2-DCs, GFP-DCs and mDCs to stimulate autologous T cell proliferation was determined by WTS-8 assay using different DC:T cell ratios (1:100, 1:20 and 1:10). Both Ad1-DCs and Ad2-DCs stimulated autologous T cell proliferation to a much higher degree than the GFP-DCs and uninfected mDCs, although there was no statistically significant difference between Ad1-DCs and Ad2-DCs in the stimulation of T cell proliferation (Table I).

Cytotoxicity assays. The functional capability of the CTLs responding to Ad-infected DCs was tested by determining whether they could specifically lyse target cells. The effector T cells were plated in 96-well plates in medium containing IL-2 (20 ng/ml). The DCs were added at a 1:10 ratio, and the cells were co-cultured at 37°C in 5% CO₂ for 7 days. The cytotoxic activity was assayed using Huh7.5 cells electrotransfected with FL-J6/JFH transcripts as the targets. The results indicated that Ad1-DCs and Ad2-DCs specifically induced high CTL activity against Huh7.5 cells, whereas the GFP-DC-T, DC-T and T cells alone had little or no cytotoxicity against the target cells (Fig. 6). The percentages of lysed Huh7.5 cells mediated by Ad1-DC-T (36%) and Ad2-DC-T (30%) were much higher than those released by the GFP-DC-T, DC-T and T cells alone (P<0.05). No significant differences were detected between Ad1-DC-T and Ad2-DC-T in terms of target cell lysis (P>0.05).

Discussion

DCs are professional antigen-presenting cells with potent immunostimulatory capabilities. Due to their pivotal role in antigen processing and antiviral immunity, the use of ex vivo genetically manipulated DCs to augment the immune response is an attractive approach for immunotherapy (30). Previous studies have demonstrated that DC-based vaccines stimulate T cell immunity against viral infection and tumor growth (10). However, the efficacy of the DC vaccine correlates with the
level of antigen expressed by the DCs, which greatly depends on the efficiency of gene transfer. The use of viral vectors is a more promising strategy for antigen loading, as viral vectors efficiently express full-length endogenous antigen in DCs. Expression of full-length antigen allows the DCs to present a wide variety of both MHC class I and II epitopes. Consistent with the data presented in this study, other studies have shown that DCs infected with adenovirus effectively elicit a strong and specific CTL response (30), and the safety and efficiency of Ad-based DC vaccines have been documented in clinical trials (31). The modification by adenoviruses is only transient, thereby reducing the risk of insertional mutagenesis exerted by retroviral infection. Although Ad vectors have seen their share of setbacks in recent years, they remain viable tools for the prevention or treatment of a multitude of diseases (32).

In our study, Ad1-DCs and Ad2-DCs stimulated higher T lymphocyte proliferation than GFP-DCs and uninfected mDCs. However, there was no significant difference between Ad1-DCs and Ad2-DCs in promoting auto-T cell proliferation (Table I). As a positive control, sequence 2 included the HCV CTL epitopes, core 35-44, core 131-141, as well as the HCV Th epitope, NS3 1248-1261. HCV core 35-44 and core 131-141 have been demonstrated to induce an HCV-specific CTL response (19). The Th epitope, NS3 1248-1261 has been associated with viral clearance in acute hepatitis C infection, and has found to be present in patients with a diverse HLA background (17). Our study demonstrates that HCV epitopes are expressed in DCs and can be presented to T cells, promoting T cell proliferation.

IL-12 is produced primarily by antigen-presenting cells and plays a critical role in host defense through its ability to stimulate both innate and adaptive immune effector cells. IL-12 has been found to stimulate natural killer (NK) cells to proliferate, produce IFN-γ and exhibit potent cytotoxicity. CD4+ T cells, upon IL-12 stimulation, undergo differentiation to become Th1 effectors at the expense of Th2 differentiation. IL-12 can also directly activate CD8+ T cells and enhance their cytolytic potential (33). The production of IL-12 is critical for the induction of Th1 immunity directed towards the elimination of intracellular pathogens and viruses. The core protein of HCV seems to have a suppressive action on IL-12 production at the transcriptional level. A specific Th1 cell defect correlates with insufficient Th and CTL responses, and lower production of type 1 cytokine (IL-2, IFN-γ, lymphokine-activated killer cells). Taken together, these factors may be responsible for the observed failure to resolve HCV infections (34). The enhanced IL-12 release by infected DCs compared with uninfected DCs and GFP-DCs demonstrates that HCV epitopes promote the maturation and function of DCs in an antigen-specific manner. IL-12 stimulates the proliferation of activated CD8+ T cells.

The T cell functional assays further proved that DCs correctly presented specific HCV epitopes to T cells following Ad infection, leading to the T cell production of IFN-γ, inhibiting HCV replication and effectively killing Huh7.5 cells transfected with FL-J6/JFH transcripts. In another study, FL-J6/JFH (35) plasmids encoding the full-length HCV chimeric genome were transcribed into HCV RNA in vitro, and the resulting RNA was transfected into Huh7.5 cells by electroporation. In our previous study (27), we showed that high levels of virus were present in the supernatant of Huh7.5 transfected cells, and the elevated expression of HCV NS5A proteins was observed in the transfected cells by indirect immunofluorescence staining. Large numbers of enveloped or non-enveloped virus-like particles (VLPs) were also observed in the transfected Huh7.5 cells by transmission electron microscopy (27). Therefore, in the current study, Huh7.5 cells transfected with FL-J6/JFH transcripts were employed as target cells to simulate the natural HCV infection of human hepatocytes.

Upon T cell receptor engagement with peptide/MHC class I complexes, CD8+ T cells vigorously expand and exhibit multiple effector functions, including cytotoxicity mediated by perforin-granzyme and Fas/FasL pathways and the production of cytokines (e.g., IFN-γ). HCV-specific CD8+ T cells exert strong antiviral effects through 2 distinct mechanisms. The first requires direct cell-cell contact resulting in cytolysis, and the second is a non-cytolytic inhibition mediated by IFN-γ. Of note, studies on acute HCV infection in humans and chimpanzees have indicated the presence of both effector functions in HCV-specific CD8+ T cells. For example, several studies have shown a correlation between the appearance of virus-specific CD8+ T cells responses in the peripheral blood and liver, and the onset of liver disease, indicating cytolytic effector functions (36,37). In addition, certain studies have demonstrated that activated HCV-specific CD8+ T cells inhibit viral replication by both cytokine-mediated and direct cytolysis effects (38,39). A number of studies on chimpanzees and acutely infected humans have supported the idea that an effective HCV vaccine must be able to induce strong HCV-specific cytotoxic IFN-γ+ T cells which are able to target the virus (40). IFN-γ inhibits protein synthesis and RNA replication of subgenomic and genomic HCV replicons (41). IFN-γ enhances the immune lysis of HCV-infected cells and inhibit hepatic fibrosis by an effect on TGF-β and on HCV-induced carcinogenesis (34). Thus, a successful prophylactic or therapeutic dendritic cell vaccine must elicit HCV-specific CD8+ T cells with a strong ability to produce IFN-γ.

Several studies have been published indicating progress with therapeutic HCV vaccines (42-44). For example, IC41 is a synthetic peptide vaccine containing 7 relevant HCV T cell epitopes and Th1/Tc1 adjuvant poly-L-arginines (42), and CIGB-230 is a mixture of pDKE2, a plasmid expressing HCV structural antigens, and a recombinant HCV core protein (43); both of these vaccine candidates have recently been shown to be safe, and are currently being used in a clinical trial. A phase I clinical trial has recently been completed which assessed dendritic cell immunotherapy in HCV-infected individuals using autologous mdDCs presenting HCV-specific HLA A2.1-restricted CTL epitopes pulsed with lipopeptides. The vaccine was found to be safe, but was unable to generate sustained responses or alter the outcome of HCV infection (44). These vaccines, although still in their early developmental stages, show a bright future for HCV vaccine development.

In conclusion, in this study, we successfully constructed recombinant Ads encoding multiple CTL HCV epitopes, although more epitopes may be required to eliminate the infection. The recombinant Ads effectively infected DCs and induced DC maturation and the production of IL-12. Ad-infected DCs stimulated T lymphocyte proliferation, IFN-γ production and induced specific T cell cytotoxicity against HCV-expressing hepatocytes. This preliminary in vitro study
lays the foundations for further development of anti-HCV DC vaccines. We believe that a DC-based vaccine against HCV infection combined with adjuvants may block negative and thus attenuate the progression of chronic liver disease (45).

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