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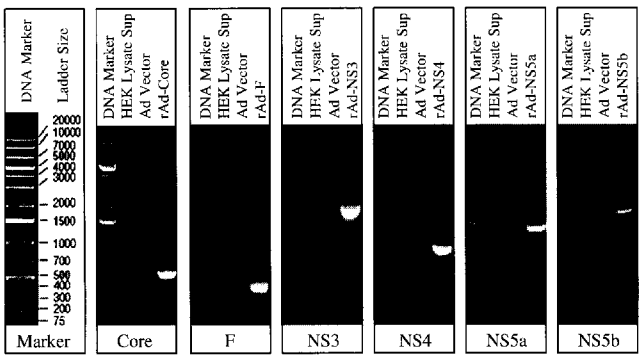
(54) Title: METHODS OF INDUCING AN IMMUNE RESPONSE TO HEPATITIS C VIRUS

Figure 1A: PCR Amplification of DNA purified from adenovirus vector stock using HCV specific primers



(57) Abstract: The present disclosure provides methods for inducing an immune response to hepatitis C virus (HCV) in an individual. The present disclosure provides methods for treating an HCV infection in an individual.

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METHODS OF INDUCING AN IMMUNE RESPONSE TO HEPATITIS C VIRUS

CROSS-REFERENCE

[0001] This application claims the benefit of U.S. Provisional Patent Application No. 62/103,919, filed January 15, 2015, which application is incorporated herein by reference in its entirety.

INTRODUCTION

[0002] Adenoviruses are diverse group of DNA viruses, which include both primate and non-primate adenoviruses, and are classified under family Adenoviridae. Adenoviruses are 90–100 nm size, non-enveloped double stranded DNA viruses, which have an icosahedral nucleo-capsid. Human adenoviruses have been classified in to 51 serotypes and 6 sub groups (A-F) on the basis of neutralization with specific anti-sera.

[0003] Recombinant adenoviruses carrying a foreign transgene are intensively being tested both as prophylactic and therapeutic vaccine for a number of pathogens in human clinical trials. They have proven to be safe, efficient and excellent vehicles for transferring vaccine antigens and eliciting immune responses against the transgene antigen in many non-human animal and human clinical testing.

[0004] Hepatitis C virus (HCV) infection is the most common chronic blood borne infection in the United States. Although the numbers of new infections have declined, the burden of chronic infection is substantial, with Centers for Disease Control estimates of 3.9 million (1.8%) infected persons in the United States. Chronic liver disease is the tenth leading cause of death among adults in the United States, and accounts for approximately 25,000 deaths annually, or approximately 1% of all deaths. Studies indicate that 40% of chronic liver disease is HCV-related, resulting in an estimated 8,000-10,000 deaths each year. HCV-associated end-stage liver disease is the most frequent indication for liver transplantation among adults.

Literature

U.S. Patent No. 8,871,515; U.S. Patent No. 8,142,794

SUMMARY

[0005] The present disclosure provides methods for inducing an immune response to hepatitis C virus (HCV) in an individual. The present disclosure provides methods for treating an HCV infection in an individual.

[0006] The present disclosure provides a method of inducing an immune response in an individual to an HCV protein, the method comprising administering to the individual an effective amount of an immunogenic composition comprising an adenoviral nucleic acid or an adenovirus polypeptide. In some cases, the adenoviral nucleic acid or adenovirus polypeptide is administered via an oral, intranasal, subcutaneous, transdermal, intratracheal, rectal, intramuscular or parenteral route of administration. In some cases, the adenoviral nucleic acid or adenovirus polypeptide is administered multiple times. In some cases, the immune response comprises a humoral and/or a cellular immune response. In some cases, the adenoviral nucleic acid is a full-length adenovirus nucleic acid or an adenovirus nucleic acid comprising a deletion. In some cases, the adenoviral nucleic acid does not encode a non-adenovirus polypeptide. In some cases, the adenoviral nucleic acid comprises a nucleotide sequence encoding one or more HCV polypeptides. In some cases, the adenoviral nucleic acid comprises a nucleotide sequence encoding an antigen associated with a pathogen other than HCV or comprises a nucleotide sequence encoding a cancer-associated antigen. In some cases, the adenoviral nucleic acid comprises a nucleotide sequence associated with an immunostimulatory or immunomodulatory sequence. In some cases, the method comprises simultaneously administering a non-recombinant adenovirus. In some cases, the method comprises administering a structural or a non-structural HCV polypeptide or a nucleic acid comprising a nucleotide sequence encoding the structural or non-structural HCV polypeptide. In some cases, the HCV polypeptide is one or more of E1, E2, F, core, P7, NS2, NS3, NS4 and NS5. In some cases, the structural or non-structural HCV antigen is administered before the adenovirus nucleic acid or the adenovirus polypeptide. In some cases, the structural or non-structural HCV antigen is administered after the adenovirus nucleic acid or the adenovirus polypeptide. I some cases, the immunogenic composition comprises an adjuvant. In some cases, the composition comprises a cytokine and/or an antibody.

[0007] The present disclosure provides a method of inducing an immune response in an individual to a hepatitis C virus antigen, the method comprising: a) obtaining dendritic cells (DCs) from the individual; b) genetically modifying the DCs to express one or more adenoviral proteins; and c) administering the genetically modified DCs to the individual.

[0008] The present disclosure provides a method of inducing an immune response in an individual to a hepatitis C virus antigen, the method comprising: a) obtaining DCs from the individual; b) infecting the DCs with replication competent adenovirus or replication-defective adenovirus; and c) administering the infected DCs to the individual.

[0009] The present disclosure provides a method of inducing an immune response in an individual to a hepatitis C virus antigen, the method comprising: a) obtaining DCs from the individual; b) introducing one or more adenoviral proteins, or nucleic acids encoding one or more adenoviral

proteins, into the DCs, thereby generating adenoviral protein-expressing DCs; and c) administering the adenoviral protein-expressing DCs to the individual.

[0010] The present disclosure provides a method of treating a hepatitis C virus (HCV) infection in an individual, the method comprising inducing an immune response to one or more HCV antigens in the individual, wherein said inducing comprises a method as disclosed above or elsewhere herein. In some cases, the method comprises administering to the individual an effective amount of at least a second therapeutic agent that treats an HCV infection. In some cases, the HCV-infected individual is a treatment-naïve individual. In some cases, the HCV-infected individual failed a prior treatment for HCV infection. The HCV can be an HCV of any genotype or subtype.

BRIEF DESCRIPTION OF THE DRAWINGS

[0011] **FIGS. 1A and 1B. FIG. 1A** shows an electronic picture of agarose electrophoresis of PCR amplified products of genetic material prepared from various adenoviral vector stocks using HCV gene specific primers. This confirms that the adenoviral vector stocks used in experiments to immunize mice were free from contamination of adenoviral vectors encoding HCV antigens. First panel shows the DNA ladder, followed by agarose gel electrophoresis of PCR products obtained with HCV Core, F, NS3, NS4, NS5a and NS5b specific primers. HEK lysate supernatant and rAd-HCV were taken as negative and positive controls. **Figure 1B** shows the detection of cross-reactive binding of anti-core and anti-NS3 antibodies to mouse quadriceps muscles upon immunization with adenoviral vector. Mice were immunized with 2×10^7 pfu replication deficient adenoviruses containing HCV-core, NS3 or empty adenoviral vector (Ad) intramuscularly. Twelve, 24 and 48 hours after immunization, quadricep muscle cells were isolated and cut into thin slices. Immunohistochemistry was done to confirm expression of core and NS3 protein and cross-reactive binding of anti-core and anti-NS3 Mabs. Samples from PBS control mice were obtained 12 hours after inoculation and were stained with anti-core antibody (A, left panel) and anti-NS3 antibody (A, right panel). Samples from mice immunized with adenoviral vector alone (Ad), recombinant adenoviral vectors containing core (rAd-Core) or NS3 (rAd-NS3) antigens and stained with anti-core and anti-NS3 antibody after 12 hours (B), 24 hours (C) and 48 hours (D). At each time point, rAd-core and rAd-NS3 immunized mice were stained with anti-core or anti-NS3 antibody, respectively, as positive controls.

[0012] **FIGS. 2A-C** demonstrate cross-reactive cellular immune responses generated against HCV core protein and a pool of 5 high homology peptides (Core Pool: 5, 14, 16, 17 & 27) in mice immunized with adenoviral vector (Ad) in the absence or presence of toll-like receptor agonist poly I:C (Ad+IC) or resiquimod (Ad+RQ) as adjuvants. Groups of five C57bl/6 female mice were immunized twice (at 14 days interval) with $2x10^7$ pfu/mouse adenoviral vector

intramuscularly in quadriceps muscles in a total volume of 150 microlitre/mouse. A recombinant Ad vector expressing NS3 coding region (rAd-NS3) was also used to demonstrate adenoviral vector inducing cross-reactive immunity. PBS immunized mice were used as negative controls. Eight days after second immunization, mice were euthanized and spleen and inguinal lymph nodes were collected. Enriched T cells ($4x10^5$/well) from spleens and lymph nodes were cultured with irradiated syngeneic spleen cells as APCs ($4x10^5$/well) and recombinant HCV core protein or HCV core derived synthetic peptides pool (5 μg/ml) for four days. Culture supernatants were collected for cytokine analysis and proliferation of T cells was examined by ^3H thymidine incorporation assay. All data represent mean ± standard deviations of triplicate wells. The experiments were repeated 3-5 times and representative data is presented. **Fig. 2A:** proliferation of spleen or lymph nodes derived T cells upon stimulation with recombinant core protein antigen or peptide pool. **Fig. 2B** and **2C**: induction of IFN-γ and IL-10 (picogram/ml) in *ex vivo* antigen stimulated T cell culture supernatants.

[0013] **FIGS. 3A-C** show cross-reactive cellular immune responses against HCV NS3 protein and a pool of 5 high homology peptides (NS3 Pool: 5, 6, 8, 15 & 17) in mice immunized with adenoviral vector (Ad) in the absence or presence of toll-like receptor agonists [poly I:C (Ad+IC) or resiquimod (Ad+RQ)], rAd-NS3 or PBS. The immunization and *ex vivo* T cell culture protocols were similar as described in figure 2. **Fig. 3A:** proliferation of spleen and lymph node T cells upon stimulation with HCV-NS3 protein or peptides pool at 5 μg/ml concentration. **Fig. 3B** and **3C:** induction of IFN-γ and IL-10 (picogram/ml) in *ex vivo* antigen stimulated T cell culture supernatants.

[0014] **FIGS. 4A-C** show cross-reactive cellular immune responses against HCV NS5 protein and a pool of 5 high homology peptides (NS5a Pool: 6, 24 and NS5b pool: 5, 19 & 27) in mice immunized with adenoviral vector (Ad) in the absence or presence of toll-like receptor agonists [poly I:C (Ad+IC) or resiquimod (Ad+RQ)], rAd-NS3 or PBS. The immunization and *ex vivo* T cell culture protocols were similar to figure 2. **Fig. 4A:** proliferation of spleen or lymph node T cells upon stimulation with HCV-NS5 protein antigen or peptide pool at 5 μg/ml concentration. **Fig. 4B** and **4C:** induction of IFN-γ and IL-10 (picogram/ml) in *ex vivo* antigen stimulated T cell culture supernatants.

[0015] **FIGS. 5A-H** show cross-reactive antibody (IgG and IgG1) responses against HCV core, NS3, NS4 and NS5 proteins in serum of mice immunized with adenoviral vector (Ad) in the absence or presence of toll-like receptor agonists [poly I:C (Ad+IC) or resiquimod (Ad+RQ)], rAd-NS3 or PBS as described in figure 2. Sera from mice of the same group were pooled. For the detection of antibodies against HCV core, NS3, NS4 or NS5, 96-well plates were coated with specific recombinant HCV protein (1 μg/ml in 1xPBS) and incubated overnight at 4°C. Plates

were washed and blocked with 1% BSA followed by addition of diluted pooled serum in duplicate. The plates were incubated for 2 hours, washed with 1xPBST, and added with secondary antibody (anti-mouse IgG AP labeled) (Southern Biotech, Alabama, USA) and color was developed with PNPP substrate. Absorbance was read using a FluoStar ELISA Reader. Graphs A-H represent the absorbance value at different dilution of serum. Means $\pm$ SD of triplicate values are shown.

[0016] **FIG. 6A** shows HCV specific and cross-reactive proliferation of spleen T cells obtained from mice immunized twice with adenoviral vector (Ad) via different routes (intramuscular, intranasal and oral) upon stimulation with HCV protein antigens (core, NS3, NS4 and NS5) or high homology peptide pools from respective HCV antigens.

[0017] **FIG 6B** shows amounts of IFN-γ production (picogram/ml) in culture supernatants from T cell proliferation assay. Solid dark bars represent Ad immunized mice and white bars PBS immunized mice. No bars in figure 6B represent cytokines below detection levels.

[0018] **FIG. 6C** shows antigen specific proliferation of spleen and/or lymph node T cells from mice immunized once intramuscularly (i.m.) or intranasally (i.n.) with different doses of Ad vector (Ad) or rAd-NS3, upon *in vitro* stimulation with various HCV protein antigens (core, NS3, NS4, NS5). I, II. Single intramuscular immunization with Ad or rAd-NS3 ($0.5x10^6$pfu/mouse); III, IV. Single intramuscular immunization with Ad or rAd-NS3 ($1.0x10^6$pfu/mouse); V. Single intramuscular immunization with Ad or rAd-NS3 ($2.0x10^7$pfu/mouse); and VI. Single intranasal immunization with Ad or rAd-NS3 ($1x10^7$pfu/mouse).

[0019] **FIGS. 7A-C. FIG. 7A** shows cross-reactive proliferation of spleen T cells obtained from adenoviral vector (Ad) immunized mice (twice intramuscularly), upon *in vitro* stimulation with individual HCV core peptides at 5 μg/ml (listed in TABLE 1; Figure 18) or core protein at 1 μg/ml. The immunization and *ex vivo* T cell culture protocols used were similar to figure 2. **FIG 7B** and **7C:** induction of IFN-γ and IL-10 (picogram/ml) in *ex vivo* HCV core antigen stimulated T cell culture supernatants. Solid dark bars represent Ad immunized mice and white bars represent PBS immunized mice.

[0020] **FIGS. 8A-C. FIG. 8A** shows cross-reactive proliferation of spleen T cells obtained from adenoviral vector (Ad) immunized mice (twice intramuscularly), upon *in vitro* stimulation with 5 μg/ml of individual HCV F peptides (listed in TABLE 2; Figure 19). The immunization and *ex vivo* T cell culture protocols were similar to figure 2. **FIG 8B** and **8C** show induction of IFN-γ and IL-10 (picogram/ml) in *ex vivo* HCV F peptides stimulated T cell culture supernatants. Solid dark bars represent Ad immunized mice and white bars show PBS immunized mice.

[0021] **FIGS. 9A-C. FIG. 9A** shows cross-reactive proliferation of spleen T cells obtained from adenoviral vector (Ad) immunized mice (twice intramuscularly), upon *in vitro* stimulation with 5 μg/ml of HCV NS3 peptides (listed in TABLE 3; Figure 20) or NS3 protein (1 μg/ml). The immunization and *ex vivo* T cell culture protocols were similar to figure 2. **FIG 9B** and **9C** show induction of IFN-γ and IL-10 (picogram/ml) in *ex vivo* HCV NS3 antigen stimulated T cell culture supernatants. Solid dark bars represent Ad immunized mice and white bars show PBS immunized mice.

[0022] **FIGS. 10A-C** show cross-reactive cellular immune responses against HCV NS4 protein or peptides in mice immunized with adenoviral vector (Ad) upon two intramuscular immunizations. **FIG. 10A**: splenic T cell proliferation upon stimulation with individual HCV NS4 peptides at 5 μg/ml (TABLE 4; Figure 21) or HCV NS4 protein antigen at 1 μg/ml concentrations. **FIG. 10B** and **10C:** IFN-γ and IL-10 concentration (picogram/ml) in spleen T cell culture supernatants from T cell proliferation.

[0023] **FIGS. 11A-C. FIG. 11A** shows cross-reactive proliferation of spleen T cells from adenoviral vector (Ad) immunized mice, upon *in vitro* stimulation with 5 μg/ml of individual HCV NS5a peptides (listed in TABLE 5a; Figure 22) or HCV NS5 protein antigen at 1 μg/ml. The immunization and *ex vivo* T cell culture protocols were similar to figure 2. **FIG 11B** and **11C:** induction of IFN-γ and IL-10 (picogram/ml) in *ex vivo* antigen stimulated T cell culture supernatants. Solid dark bars represent Ad immunized mice and white bars show PBS immunized mice.

[0024] **FIGS. 12A-C. FIG. 12A** shows cross-reactive proliferation of spleen T cells from adenoviral vector (Ad) immunized mice, upon *in vitro* stimulation with 5 μg/ml of HCV NS5B peptides (listed in TABLE 5B; Figure 23) or HCV NS5 protein antigen at 1 μg/ml. The immunization and *ex vivo* T cell culture protocols were similar to figure 2. **FIG 12B** and **12C:** induction of IFN-γ and IL-10 (picogram/ml) in *ex vivo* antigen stimulated T cell culture supernatants. Solid dark bars represent Ad immunized mice and white bars show PBS immunized mice.

[0025] **FIGS. 13A-D. FIG. 13A and 13B** show proliferation of spleen derived $CD4^+$ and $CD8^+$ T cells harvested from adenoviral vector (Ad) immunized mice in response to various HCV protein antigens by CFSE dilution assay. The immunization protocol was similar as described in figure 2. Spleen T cells were stained with CFSE, and incubated with irradiated syngeneic spleen cells as APCs and various HCV antigens (core, NS3, NS4 and NS5 at 5 μg/ml concentration) for 4 days. Loss of CFSE due to cell division was compared with the T cells incubated in the absence of added antigen and represented as histograms. Shift of peak of $CFSE^+$ T cells towards left was considered as indication of level of antigen specific T cell proliferation. **FIG. 13 C** and **D** show

intracellular cytokine (IFN-γ and IL-10) expression of $CD4^+$ and $CD8^+$ T cells obtained from Ad vector or PBS immunized mice and stimulated with various HCV proteins (Core, NS3, NS4 and NS5) for four days. The data represent the percentage of IFN-γ and IL-10 expressing $CD4^+$ T cells and $CD8^+$ T cells.

[0026] **FIGS 14A-D. 14A and B** show proliferation of $CD4^+$ and $CD8^+$ T cells harvested from Ad immunized mice in response to various HCV specific synthetic peptides by CFSE dilution assay. The immunization protocol used was similar to figure 2. Spleen T cells were stained with CFSE, and incubated with irradiated syngeneic spleen cells as APCs and various representative peptides (showing high homologies to Ad proteins) from HCV proteins Core (peptide #6), NS3 (peptide #8), NS4 (peptide #4) and NS5 (peptide #19) at 5 μg/ml. After 4 days of incubation, loss of CFSE due to cell division was compared with the T cells incubated in the absence of peptide antigen and represented as histograms. Shift in peak of $CFSE^+$ T cells towards left was considered as indication of antigen specific T cell proliferation. **FIG. 14C** and **D** show intracellular cytokine (IFN-γ and IL-10) expression of $CD4^+$ and $CD8^+$ T cells from spleens of mice immunized with Ad vector or PBS and cultured ex vivo with representative HCV derived synthetic peptides at 5 μg/ml concentration. The data represent the percentage of IFN-γ and IL-10 expressing $CD4^+$ T cells and $CD8^+$ T cells in response to various representative peptides of HCV proteins.

[0027] **FIG. 15** shows the cytotoxic activity of effector T cells harvested from Ad vector immunized mice against HCV peptides loaded CFSE stained EL4 targets. Spleen T cells harvested from Ad immunized mice were stimulated *in vitro* with the HCV protein antigens Core, NS3, NS4 or NS5 at 5 μg/ml concentration for 4 days. The target EL4 cells were incubated with corresponding HCV peptides (Core peptides: 2, 14, 17, 25, 27, 28, 32; NS3 peptides: 8, 10; NS4 peptides: 3, 4, 8; and NS5 peptides: 1a, 2a, 16a, 20a, 5b, 19b, 23b, 39b; or All:a mixture of the above peptides from core, NS3, NS4 and NS5) and peptide loaded EL4 cells were cultured with effectors at 10:1 (effectors: target) ratio for 4-5 hours. CFSE labeled live targets were quantified by flow cytometry and subtracted from background CFSE labeled targets to get numbers of killed targets. Empty (no peptide loaded) EL4 targets were used as a negative control.

[0028] **FIGS. 16A and 16B. FIG. 16A** shows the stimulation of cross-reactive HCV specific $CD4^+$ and $CD8^+$ T cell proliferative responses (by CFSE dilution assay) against a representative HCV antigen (NS5) upon immunization with adenoviral vector (Ad) infected bone marrow derived dendritic cells (DCs) . Group immunized with bone marrow DCs (treated with media) only served as control. **FIG 16B:** demonstrates increased expression of granzyme B on cross-reactive $CD8^+$ T cells in response to HCV NS5 antigen.

[0029] **FIGS. 17A and 17B. FIG. 17 A** shows that immunization of mice with adenoviral vector (Ad) leads to reduction in the titer of infectious vaccinia-HCV chimeric virus in mice. Groups of mice were immunized with Ad vector ($2x10^7$ pfu/mouse) (n = 11), Ad vector in presence of poly I:C adjuvant (n = 5), and PBS control (n = 4). Eight days after two intramuscular immunizations (14 days apart), mice were challenged intraperitoneally with infectious chimeric Vac-HCV (NS3-NS4-NS5) ($1x10^7$ PFU/mouse), and ovaries were harvested 5 days after challenge. Viral loads in each mouse ovary were determined by plaque assay using TK-1 cells. **FIG. 17 B** demonstrates that immunization of mice with adenoviral vector (Ad) leads to reduction in the titer of infectious vaccinia-HCV (Vac-Core-NS3) chimeric virus in mice but not of the wild-type-Vaccinia (WT-Vac). Groups (n=5) of mice were immunized with Ad vector or HEK cell lysate (control). Eight days after two intramuscular immunizations (14 days apart), mice were challenged with chimeric vaccinia (Vac-Core-NS3) or wild-type vaccinia (WT-Vac) ($1x10^7$ PFU/mouse) intraperitoneally, and ovaries were harvested 5 days after challenge. Viral loads in each mouse ovary were determined by plaque assay using TK-1 cells.

[0030] **FIG. 18** provides **TABLE 1**, which shows the score of amino acid sequence homology between adenoviral vector (Ad) proteins and peptide epitopes of HCV Core protein, and also number of epitope regions in Ad proteins, which show homology (>25) with the HCV Core peptides/epitopes (S. No. 1-45 correspond to SEQ ID NOs:1-45).

[0031] **FIG. 19** provides **TABLE 2**, which illustrates the score of amino acid sequence homology between Ad proteins and peptide epitopes of HCV frame shift protein (F), and also number of epitope regions in Ad proteins, which show homology (>25) with the HCV F peptides/epitopes (S. No. 1-16 correspond to SEQ ID NOs:46-61).

[0032] **FIG. 20** provides **TABLE 3**, which shows the score of amino acid sequence homology between Ad proteins and selected peptide epitopes of HCV NS3 protein, and also number of epitope regions in Ad proteins, which show homology (>25) with the HCV NS3 peptides/epitopes (S. No. 1-11 correspond to SEQ ID NOs:62-72).

[0033] **FIG. 21** provides **TABLE 4**, which shows the score of amino acid sequence homology between Ad proteins and peptide epitopes of HCV NS4 protein and also number of epitope regions in Ad proteins, which show homology with the HCV NS4 peptides/epitopes (first set of S. No. 1-4 correspond to SEQ ID NOs:73-76; second set of S. No. 1-16 correspond to SEQ ID NOs:77-92).

[0034] **FIGS. 22 and 23** provide **TABLE 5a** and **5b**, which show the score of amino acid sequence homology between Ad proteins and peptide epitopes of HCV NS5a and NS5b proteins, respectively. Tables 5a and 5b also summarize the number of epitope regions in Ad proteins, which show homology (>25) with the HCV NS5a and 5b peptides/epitopes (Table 5a: S. No. 1-

29 correspond to SEQ ID NOs:93-121; Table 5b: S. No. 1-39 correspond to SEQ ID NOs:121-160).

[0035] **FIG. 24** provides **TABLE 6**, which lists the Ad5 vector proteins whose amino acid sequences were aligned with HCV synthetic peptide sequence.

[0036] **FIGS. 25A-I** provides **TABLE 7**, which provides amino acid sequences of adenoviral proteins (SEQ ID NOs:161-187).

[0037] **FIG. 26** demonstrates cross-reactive T cell responses generated against HIV-gp120 and HCV core, NS3, NS4 and NS5 protein in mice immunized with recombinant adenoviral vector expressing HIV-nef antigen (rAd-nef) in the absence or presence of toll-like receptor agonist poly I:C (rAd-nef+Poly I:C) as adjuvant. Groups of five C57bl/6 male mice were immunized twice (at 14 days interval) with $2x10^7$ pfu/mouse adenoviral vector intranasally in each nostril (15ul/nostril) in a total volume of 30 microliter/mouse. PBS immunized mice were used as negative controls. Eight days after second immunization, mice were euthanized and spleen was collected. Enriched T cells ($4x10^5$/well) from spleens were cultured with irradiated syngeneic spleen cells as APCs ($4x10^5$/well) and recombinant HIV gp-120, and HCV core, NS3, NS4 and NS5 proteins for four days. Proliferation of T cells was examined by ^3H thymidine incorporation assay. All data represent mean $\pm$ standard deviations of triplicate wells. Proliferation of spleen derived T cells upon stimulation with recombinant HCV (core, NS3, NS4 and NS5) protein antigens and HIV-nef protein is shown as Avg$\pm$S.D.

[0038] **FIG. 27** demonstrates cross-reactive T cell responses generated against HCV core, NS3 and NS4 protein antigens in mice immunized with recombinant adenoviral vector expressing mycobacterial antigen 85B (rAd-Ag85B) or Ad vector. Groups of five C57bl/6 male mice were immunized twice (at 14 days interval) with $2x10^7$ pfu/mouse adenoviral vector intramuscularly in quadricep muscles in a total volume of 150 microlitre/mouse. Eight days after second immunization, mice were euthanized and spleen was collected. Enriched T cells ($4x10^5$/well) from spleens were cultured with irradiated syngeneic spleen cells as APCs ($4x10^5$/well) and recombinant HCV (core, NS3 and NS4) protein antigens for four days. Proliferation of T cells was examined by ^3H thymidine incorporation assay. All data represent mean $\pm$ standard deviations of triplicate wells. Proliferation of spleen derived T cells upon stimulation with recombinant HCV (core, NS3 andNS4) protein antigens and sonicated mycobacteria is shown as Avg$\pm$S.D.

[0039] **FIG. 28** demonstrates that priming of mice with adenoviral vector (Ad, $2x10^7$ pfu/mouse, intramuscular) and boosting with pool of HCV-NS3 peptides with heat-killed *Caulobacter crescentus* (HKCC) intranasally leads to reduction in the titer of infectious vaccinia-HCV (Vac-

Core-NS3) chimeric virus in mice. Groups (n=5) of female mice were immunized with Ad (i.m.) followed by a boost with a mixture of HCV NS3 peptides and HKCC (i.n) or PBS. Eight days after second immunization (14 days apart), mice were challenged with chimeric vaccinia (Vac-Core-NS3) ($1x10^7$ PFU/mouse) intraperitoneally, and ovaries were harvested 5 days after challenge. Viral loads in individual mouse ovaries were determined by plaque assay using TK-1 cells.

[0040] **FIG. 29** provides **TABLE 8**, which shows the score of amino acid sequence homology between peptide epitopes of HCV core protein and Chimp Ad25 proteins (S. No. 1-45 corresponds to SEQ ID NOs:1-45).

DEFINITIONS

[0041] The terms "Adenovirus" and "Adenoviral vector" as used herein include any and all viruses that may be categorized as an Adenovirus, including any Adenovirus that infects a human or an animal, including all groups, subgroups, and serotypes. Thus, as used herein, "Adenovirus" and "Adenovirus vector" refer to the virus itself or derivatives thereof and cover all serotypes and subtypes and both naturally occurring and recombinant forms. In one embodiment, such Adenoviruses infect human cells. Such Adenoviruses may be wildtype or may be modified in various ways known in the art or as disclosed herein. Such modifications include modifications to the Adenovirus genome that is packaged in the particle. Such modifications include deletions known in the art, such as deletions in one or more of the E1a, E1b, E2a, E2b, E3, or E4 coding regions.

[0042] By "HCV" herein is meant any one of a number of different genotypes and isolates of hepatitis C virus. Representative HCV genotypes and isolates include: H77, the "Chiron" isolate, J6, Con1, isolate 1, BK, EC1, EC10, HC-J2, HC-J5; HC-J6, HC-J7, HC-J8, HC-JT, HCT18, HCT27, HCV-476, HCV-KF, "Hunan", "Japanese", "Taiwan", TH, type 1, type 1a, H77 type 1b, type 1c, type 1d, type 1e, type 1f, type 10, type 2, type 2a, type 2b, type 2c, type 2d, type 2f, type 3, type 3a, type 3b, type 3g, type 4, type 4a, type 4c, type 4d, type 4f, type 4h, type 4k, type 5, type 5a, type 6 and type 6a.

[0043] As used herein, "subject" or "individual" or "patient" refers to any subject for whom or which therapy is desired, and generally refers to the recipient of the therapy to be practiced according to the invention. The subject can be any vertebrate, but will typically be a mammal. If a mammal, the subject will in many embodiments be a human, but may also be a domestic livestock, a field animal such as a horse, laboratory subject or pet animal.

[0044] The term "effective amount" or "therapeutically effective amount" means a dosage sufficient to provide for treatment for the disease state being treated or to otherwise provide the desired effect (*e.g.*, reduction of viral load). The precise dosage will vary according to a variety of factors such as subject-dependent variables (*e.g.*, age, immune system health, *etc.*), the disease (*e.g.*, the particular viral strain), and the treatment being effected. In the case of treatment of HCV infection, an "effective amount" can be considered that amount sufficient to reduce the HCV viral load in a subject, as described in more detail below.

[0045] The terms "treat," "treating," "treatment" and the like are used interchangeably herein and mean obtaining a desired pharmacological and/or physiological effect. The effect may be prophylactic in terms of completely or partially preventing a disease or symptom thereof and/or may be therapeutic in terms of partially or completely curing a disease and/or adverse effect attributed the disease. "Treating" as used herein covers treating a disease in a vertebrate, e.g., a mammal, e.g., a human, and includes: (a) preventing the disease from occurring in a subject which may be predisposed to the disease but has not yet been diagnosed as having it; (b) inhibiting the disease, i.e. arresting its development; or (c) relieving the disease, i.e. causing regression of the disease.

[0046] Before the present invention is further described, it is to be understood that this invention is not limited to particular embodiments described, as such may, of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting, since the scope of the present invention will be limited only by the appended claims.

[0047] Where a range of values is provided, it is understood that each intervening value, to the tenth of the unit of the lower limit unless the context clearly dictates otherwise, between the upper and lower limit of that range and any other stated or intervening value in that stated range, is encompassed within the invention. The upper and lower limits of these smaller ranges may independently be included in the smaller ranges, and are also encompassed within the invention, subject to any specifically excluded limit in the stated range. Where the stated range includes one or both of the limits, ranges excluding either or both of those included limits are also included in the invention.

[0048] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although any methods and materials similar or equivalent to those described herein can also be used in the practice or testing of the present invention, the preferred methods and materials are now described. All publications mentioned herein are incorporated herein by reference to

disclose and describe the methods and/or materials in connection with which the publications are cited.

[0049] It must be noted that as used herein and in the appended claims, the singular forms "a," "an," and "the" include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to "an adenoviral nucleic acid" includes a plurality of such nucleic acids and reference to "the HCV polypeptide" includes reference to one or more HCV polypeptides and equivalents thereof known to those skilled in the art, and so forth. It is further noted that the claims may be drafted to exclude any optional element. As such, this statement is intended to serve as antecedent basis for use of such exclusive terminology as "solely," "only" and the like in connection with the recitation of claim elements, or use of a "negative" limitation.

[0050] It is appreciated that certain features of the invention, which are, for clarity, described in the context of separate embodiments, may also be provided in combination in a single embodiment. Conversely, various features of the invention, which are, for brevity, described in the context of a single embodiment, may also be provided separately or in any suitable sub-combination. All combinations of the embodiments pertaining to the invention are specifically embraced by the present invention and are disclosed herein just as if each and every combination was individually and explicitly disclosed. In addition, all sub-combinations of the various embodiments and elements thereof are also specifically embraced by the present invention and are disclosed herein just as if each and every such sub-combination was individually and explicitly disclosed herein.

[0051] The publications discussed herein are provided solely for their disclosure prior to the filing date of the present application. Nothing herein is to be construed as an admission that the present invention is not entitled to antedate such publication by virtue of prior invention. Further, the dates of publication provided may be different from the actual publication dates which may need to be independently confirmed.

DETAILED DESCRIPTION

[0052] The present disclosure provides a method of inducing an immune response to one or more hepatitis C virus (HCV) antigens in an individual in need thereof. The method generally involves administering to the individual an immunogenic adenovirus composition, where the adenovirus nucleic acid or adenovirus polypeptide present in the immunogenic adenovirus composition induces an immune response in the individual to one or more HCV antigens. In some cases, the adenovirus a wild-type adenovirus. In some cases, the adenovirus is a recombinant adenovirus. The present disclosure provides a method of treating an HCV infection in an individual, the method comprising inducing an immune response to one or more HCV antigens in the individual.

METHODS OF INDUCING AN IMMUNE RESPONSE TO HCV

[0053] The present disclosure provides a method of inducing an immune response to one or more HCV antigens in an individual in need thereof. The method generally involves administering to the individual an immunogenic adenovirus composition, where the adenovirus nucleic acid or adenovirus polypeptide present in the immunogenic adenovirus composition induces an immune response in the individual to one or more HCV antigens. In some cases, the adenovirus a wild-type adenovirus. In some cases, the adenovirus is a recombinant adenovirus. In some embodiments, the individual is not infected with HCV.

[0054] As noted above, a method of the present disclosure for inducing an immune response to one or more HCV antigens involves administering to an individual in need thereof an effective amount of an adenovirus composition. An "adenovirus composition" can include an adenovirus nucleic acid or an adenovirus polypeptide.

[0055] In some cases, the adenovirus composition used in a method of the present disclosure for inducing an immune response in an individual to one or more HCV antigens comprises an adenovirus polypeptide. In some cases, the adenovirus polypeptide is a fusion protein; e.g., a fusion protein comprising an adenovirus polypeptide and a non-adenovirus polypeptide, where the non-adenovirus polypeptide is referred to as a "fusion partner." Suitable fusion partners include, e.g., carrier polypeptides (e.g., keyhole limpet hemocyanin; hepatitis B virus core antigen; and the like); a polypeptide associated with (or produced by) a bacterial pathogen; a polypeptide associated with (or produced by) a viral pathogen; a tumor-associated antigen; and the like.

[0056] In some cases, an adenovirus suitable for use in a method of the present disclosure is a recombinant adenovirus. In some cases, recombinant adenovirus does not include any non-adenovirus sequences. In some cases, recombinant adenovirus lacks sequences present in a naturally-occurring adenovirus; e.g., the recombinant adenovirus comprises a deletion relative to a naturally-occurring adenovirus. In some cases, recombinant adenovirus lacks sequences present in a naturally-occurring adenovirus; and includes non-adenovirus sequences.

[0057] In some cases, recombinant adenovirus suitable for use in a method of the present disclosure lacks sequences present in a naturally-occurring adenovirus; e.g., the recombinant adenovirus comprises a deletion relative to a naturally-occurring adenovirus. A recombinant adenovirus can lack from 1 nucleotide (nt) to 1 kb relative to a naturally-occurring adenovirus. A recombinant adenovirus suitable for use in a method of the present disclosure can have a deletion of an all or part of an adenovirus gene. For example, a recombinant adenovirus suitable for use in a method of the present disclosure can be deleted for all or part of the adenovirus E3 gene. As another

example, a recombinant adenovirus suitable for use in a method of the present disclosure can be deleted for all or part of the adenovirus E1 gene. As another example, a recombinant adenovirus suitable for use in a method of the present disclosure can be deleted for all or part of the adenovirus E1 gene and can be deleted for all or part of the adenovirus E3 gene. In some cases, an adenovirus composition for use in a method of the present disclosure is a replication-defective adenovirus.

[0058] Adenovirus in an immunogenic adenovirus composition for use in a method of the present disclosure can be from any of a variety of sources. Suitable sources include, but are not limited to, bovine adenovirus, a canine adenovirus, non-human primate adenovirus, gorilla adenovirus, a chicken adenovirus, a porcine adenovirus, a swine adenovirus, an adeno associated virus-dependent adenovirus, human adenovirus, and any serotype or subtype of an adenovirus. For example, where the adenovirus is a human adenovirus, any of 57 different serotypes from 7 subtypes of human adenoviruses can be used. As another example, where the adenovirus is a Chimp adenovirus, any of a variety of different serotypes from three different groups (B,C,E) of Chimp adenoviruses can be used. In some cases, adenovirus vector in an immunogenic adenovirus composition for use in a method of the present disclosure can be purified or partially purified. In some cases, an immunogenic adenovirus composition for use in a method of the present disclosure comprises adenovirus from different species and/or adenovirus of more than one serotype. In some cases, subtypes and serotypes of different species of adenoviruses can be used sequentially for repeated immunizations to avoid generation of neutralizing immunity.

[0059] In some cases, an adenovirus suitable for use in a method of the present disclosure is mutated or genetically engineered.

[0060] In some cases, chimeric adenovirus containing one or more sections from two or more different adenoviruses (from interspecies or intraspecies Ad viruses) can be used. Adenoviruses can also be constructed by modifying the backbone of one of the adenoviruses (e.g., one or more hypervariable or conserved regions, capsid, host cell receptor binding domains, knob, shaft, hexon and/or fiber proteins). One or more of these regions may be deleted, mutated, replaced by a linker or replaced by another region of a different virus or adenovirus. The fiber, knob or shaft can also be modified to give specific targeting ability to adenovirus vectors (e.g., dendritic cell (DC) targeting, hepatocyte targeting, smooth muscle targeting, fibroblast targeting etc.).

[0061] In some cases, an adenovirus can be modified to facilitate uptake and/or target into cells or tissues of interest.

[0062] Adenovirus vectors that are modified and optimized can allow for a significant reduction in therapeutic or prophylactic dose resulting in reduced toxicity.

[0063] In some cases, the adenovirus used in a method of the present disclosure is a recombinant adenovirus expressing an immunostimulatory or an immunomodulatory sequence that are heterologous to the virus e.g., TLR agonists (such as polyI:C, CpG, flagellin), double stranded RNA adjuvants, NOD like receptor agonists, RIG-I agonists, T helper peptide epitopes/proteins (e.g., Tetanus toxoid, HBV core (human or woodchuck), Heat shock proteins), cytokines (e.g., IL-2, IL-12, GM-CSF, IL-15, IFN-g etc.), chemokines, adapter proteins involved in innate immune signaling (e.g., Ewing's Sarcoma related transcript-2, EAT-2), peptide mimetics, costimulatory molecules (e.g., CD40L), antibodies to coinhibitory molecules (e.g., anti-CTLA-4, anti-PD-1);

[0064] In some cases, the adenovirus used in a method of the present disclosure does not include nucleotide sequences encoding non-adenoviral proteins. In some cases, the adenovirus used in a method of the present disclosure includes nucleotide sequences encoding an HCV peptide listed in Tables 1-5, or a fragment of an HCV peptide listed in Tables 1-5.

[0065] Recombinant vectors can be produced, which vectors comprise nucleotide sequences encoding one or more adenoviral proteins or fragments thereof. In some cases, a method of the present disclosure for inducing an immune response in an individual to one or more HCV antigens comprises administering to the individual a composition comprising a recombinant bacterial, yeast, or viral vector comprising nucleotide sequences encoding one or more adenoviral proteins. A recombinant vector can be a viral vector such as adeno-associated virus, lentivirus, herpes virus, poxvirus, canarypox virus, vesicular stomatitis virus, alpha virus, measles virus, papaya mosaic virus, cytomegalovoirus, modified vaccinia Ankara virus MVA, polio virus, Marba virus etc.), bacterial vector vaccines (such as Salmonella, Shigella, E. coli, Lactococcus lactis, Listeria sp., Lactobacillus sp.), fungal vectors (such as heat killed recombinant Saccharomyces yeast), plant viruses, virus-like particles (VLPs), virosomes, synthetic vaccine particles, synthetic biomimetic supramolecular biovectors, depathogenized viral/bacterial strains (such as NIBRG14 from H5N1). The vector could be in the form of live wild-type, non-replicative, mutated, modified, defective or attenuated. The vectors could be from human, animal, plant or prokaryote origin and in any effective amount.

[0066] In some cases, the recombinant bacterial, yeast or viral vectors can include, in addition to adenoviral nucleotide sequences, an immunostimulatory or an immunomodulatory sequence that are heterologous to the virus e.g., TLR agonists (such as polyI:C, CpG, flagellin), double stranded RNA adjuvants, NOD like receptor agonists, RIG-I agonists, T helper peptide epitopes/proteins (e.g., Tetanus toxoid, HBV core (human or woodchuck), Heat shock proteins), cytokines (e.g., IL-2, IL-12, GM-CSF, IL-15, IFN-γ, etc.), chemokines, adapter proteins involved in innate immune signaling (e.g., Ewing's Sarcoma related transcript-2, EAT-2), peptide

mimetics, costimulatory molecules (e.g., CD40L), antibodies to coinhibitory molecules (e.g., anti-CTLA-4, anti-PD-1).

[0067] In some cases, a prime-boost immunization regimen is carried out. The prime boost regimen can include the following in any order: where a first immunogenic composition includes structural HCV antigens (E1 and/or E2) as recombinant protein, glycoprotein, polypeptide, fusion protein, DNA or recombinant vector (viral, bacterial or fungal) to induce cross reactive neutralizing antibodies; and a second immunogenic composition includes adenovirus (Ad) or recombinant adenovirus (rAd) containing various HCV antigens to induce cellular and humoral immune responses against multiple structural (e.g., core, F p7) and non structural antigens (e.g., NS2, NS3, NS4A, NS4B, NS5A, NS5B or combinations thereof) of HCV. The nucleotide sequences encoding the antigens can be from different genotypes (1-7) or subtypes of HCV. The prime boost regimens can include recombinant proteins, synthetic proteins, or peptide antigens along with adenovirus or recombinant adenovirus, with or without adjuvants.

[0068] Thus, in some cases, a method of the present disclosure of inducing an immune response in an individual to one or more HCV antigens comprises: a) administering, at a first time, to the individual HCV structural antigens E1 and/or E2; and b) administering, at a second time, to the individual an adenovirus, as described above. In some cases, a method of the present disclosure of inducing an immune response in an individual to one or more HCV antigens comprises: a) administering, at a first time, to the individual an adenovirus, as described above; b) administering, at a second time, to the individual HCV structural antigens E1 and/or E2. The first time and the second time can be separated from one another by 1 hour to 1 year, e.g., from 1 hour to12 hours, from 12 hours to 24 hours, from 2 days to 1 week, from 1 week to 1 month, from 1 month to 3 months, from 3 months to 6 months, or from 6 months to 1 year, or more than 1 year.

[0069] In some cases, a prime-boost immunization schedule is followed. For example, in some cases, a first immunogenic composition (prime) includes nonstructural antigens as recombinant protein, DNA recombinant vector (viral, bacterial or fungal) and the second immunogenic composition (boost) includes Ad or rAd containing various HCV antigens (from different genotypes and subtypes) to induce cellular and humoral immune responses against multiple structural (core, F, E1, E2, p7) and non structural antigens (NS2, NS3, NS4, NS5 or NS3-NS5) of HCV and vice versa. The prime boost regimen can include polypeptide antigens along with Ad or rAd, with or without adjuvants. The prime boost regimen can include more than one serotype of Ad, chimeric, modified and recombinant Ad.

[0070] Thus, in some cases, a method of the present disclosure of inducing an immune response in an individual to one or more HCV antigens comprises: a) administering, at a first time, to the

individual an HCV non-structural antigen; and b) administering, at a second time, to the individual an adenovirus, as described above. In some cases, a method of the present disclosure of inducing an immune response in an individual to one or more HCV antigens comprises: a) administering, at a first time, to the individual an adenovirus, as described above; b) administering, at a second time, to the individual an HCV non-structural antigen. The first time and the second time can be separated from one another by 1 hours to 1 year, e.g., from 1 hour to12 hours, from 12 hours to 24 hours, from 2 days to 1 week, from 1 week to 1 month, from 1 month to 3 months, from 3 months to 6 months, or from 6 months to 1 year, or more than 1 year.

[0071] In some cases, a prime-boost immunization schedule is followed. For example, in some cases, a first immunogenic composition comprising Ad or rAd is administered at a first time; and second immunogenic composition, comprising a non-adenoviral vector encoding one or more HCV antigens, is administered at a second time. Examples of non-adenoviral vectors include but are not limited to adeno-associated virus, lentivirus, retroviruses, herpes virus, poxviruses, vesicular stomatitis virus, alpha virus, measles virus, plant viruses, alpha virus, insect virus, equine virus, papaya mosaic virus, cytomegalovoirus, vaccinia, modified vaccinia Ankara virus (MVA), polio virus, Marba virus etc.), bacterial vector vaccines (such as Salmonella, Shigella, *E. coli*, *Lactococcus lactis*, Listeria sp., Lactobacillus sp.), fungal vectors (such as heat killed recombinant Saccharomyces yeast), plant viruses, virus-like particles (VLPs), virosomes, DNA vector, synthetic vaccine particles, synthetic biomimetic supramolecular biovectors, depathogenized viral/bacterial strains (such as NIBRG14 from H5N1).

[0072] In some cases, a first immunogenic composition comprising a non-adenoviral vector encoding one or more HCV antigens is administered at a first time; and a second immunogenic composition comprising Ad or rAd is administered at a second time.

[0073] In some cases, a method of the present disclosure comprises administering adenovirus as an adenoviral virion. In some cases, a method of the present disclosure comprises administering adenovirus or adenoviral proteins in a dendritic cell. Thus, e.g., in some cases, a method of the present disclosure for inducing an immune response in an individual to one or more HCV antigens comprises: a) obtaining dendritic cells (DCs) from the individual; b) genetically modifying the DCs to express one or more adenoviral proteins; and c) administering the genetically modified DCs to the individual. In some cases, a method of the present disclosure for inducing an immune response in an individual to one or more HCV antigens comprises: a) obtaining DCs from the individual; b) infecting the DCs with replication competent adenovirus or replication-defective adenovirus; and c) administering the infected DCs to the individual. In some cases, a method of the present disclosure for inducing an immune response in an individual to one or more HCV antigens comprises: a) obtaining DCs from the individual; b) introducing

one or more adenoviral proteins, or RNA encoding one or more adenoviral proteins, into the DCs, thereby generating adenoviral protein-expressing DCs; and c) administering the adenoviral protein-expressing DCs to the individual.

[0074] In some cases, adenoviral proteins are fused with carriers such as keyhole limpet hemocyanin (KLH), hepatitis B virus core, etc. In some cases, a method of the present disclosure for inducing an immune response in an individual to one or more HCV antigens comprises administering to the individual a composition comprising an adenoviral protein fused to a carrier, where suitable carriers include KLH, HBV core antigen, and the like.

[0075] As noted above, a method of the present disclosure comprises administering to an individual in need thereof an effective amount of an adenovirus composition. In some cases, an effective amount of an adenovirus composition is an amount that, when administered in one or more doses to an individual in need thereof, induces an immune response to more than one HCV antigen. In some cases, an effective amount of an adenovirus composition is an amount that, when administered in one or more doses to an individual in need thereof, induces an immune response to an HCV core antigen and at least one of HCV F, HCV NS3, HCV NS4, and HCV NS5. In some cases, an effective amount of an adenovirus composition is an amount that, when administered in one or more doses to an individual in need thereof, induces an immune response to an HCV NS3 antigen and at least one of HCV F, HCV core, HCV NS4, and HCV NS5. In some cases, an effective amount of an adenovirus composition is an amount that, when administered in one or more doses to an individual in need thereof, induces an immune response to HCV core antigen, HCV F, HCV NS3, HCV NS4, and HCV NS5. In some cases, an effective amount of an adenovirus composition is an amount that, when administered in one or more doses to an individual in need thereof, induces an immune response to HCV core antigen, HCV F, HCV NS3, and HCV NS4. In some cases, an effective amount of an adenovirus composition is an amount that, when administered in one or more doses to an individual in need thereof, induces an immune response to HCV core antigen, HCV NS3, HCV NS4, and HCV NS5. In some cases, the immune response is a humoral immune response. In some cases, the immune response is a cellular immune response.

[0076] In some cases, an effective amount of an adenovirus composition is an amount that, when administered in one or more doses to an individual in need thereof, induces neutralizing and/or non-neutralizing antibody to HCV in the individual. In some cases, an effective amount of an adenovirus composition is an amount that, when administered in one or more doses to an individual in need thereof, induces neutralizing antibody to HCV in the individual.

[0077] In some cases, an effective amount of an adenovirus composition is an amount that, when administered in one or more doses to an individual in need thereof, induces a cytotoxic T

lymphocyte (CTL) response to HCV in the individual. In some cases, an effective amount of an adenovirus composition is an amount that, when administered in one or more doses to an individual in need thereof, induces a helper T lymphocyte (TH) response to HCV in the individual.

[0078] In some cases, an effective amount of an adenovirus composition is an amount that, when administered in one or more doses to an individual in need thereof, induces neutralizing and/or non-neutralizing antibody to HCV in the individual and induces a T helper and/or CTL response to HCV in the individual.

[0079] In some cases, an effective amount of an adenovirus composition is an amount that, when administered in one or more doses to an individual in need thereof, induces an effector immune response to HCV in the individual.

[0080] In some cases, a method of the present disclosure comprises multiple administration of an adenoviral nucleic acid or adenoviral polypeptide. For example, in some cases, a sequential immunization schedule is used, where a first composition comprising a first adenovirus nucleic acid from adenovirus of a first species or serotype is administered; and a second composition comprising a second adenovirus nucleic acid from adenovirus of a second species or serotype is administered from 1 day to 1 year (or more than 1 year) after the first composition is administered. For example, in some cases, a sequential immunization schedule is used, where a first composition comprising a first adenovirus nucleic acid from adenovirus of a first species or serotype is administered; and a second composition comprising a second adenovirus nucleic acid from adenovirus of a second species or serotype is administered from 1 day to 7 days, from 1 week to 2 weeks, from 2 weeks to 4 weeks, from 1 month to 6 months, or from 6 months to 1 year, or more than 1 year, after the first composition is administered.

Adenoviral proteins

[0081] In some cases, an adenovirus composition comprises an adenovirus polypeptide, or a nucleic acid comprising a nucleotide sequence encoding an adenovirus polypeptide, where the adenovirus polypeptide(s) are depicted in Figures 25A-25I (Table 7). In some cases, the adenoviral polypeptides comprise amino acid sequences having at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the amino acid sequence depicted in Table 7. In some cases, the adenovirus composition comprises all of the adenovirus polypeptides, or a nucleic acid comprising a nucleotide sequence encoding same, depicted in Table 7. In some cases, the adenovirus composition comprises 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 21, 22, 23, 24, 25, or 26 of the adenovirus polypeptides, or a nucleic acid comprising a nucleotide sequence encoding same, depicted in Table 7. In some cases, the adenoviral polypeptides comprise amino acid sequences having at

least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 21, 22, 23, 24, 25, or 26 of the adenovirus polypeptides depicted in Table 7.

Adjuvants

[0082] In some cases, an adenovirus composition is administered to an individual in need thereof, where the adenovirus composition comprises an adjuvant. Exemplary adjuvants include, but are not limited to: (1) oil-in-water emulsion formulations (with or without other specific immunostimulating agents such as muramyl peptides (see below) or bacterial cell wall components), such as for example (a) MF59™ (WO 90/14837; Chapter 10 in Vaccine design: the subunit and adjuvant approach, eds. Powell & Newman, Plenum Press 1995), containing 5% Squalene, 0.5% Tween 80, and 0.5% Span 85 (optionally containing MTP-PE) formulated into submicron particles using a microfluidizer, (b) SAF, containing 10% Squalane, 0.4% Tween 80, 5% pluronic-blocked polymer L121, and thr-MDP either microfluidized into a submicron emulsion or vortexed to generate a larger particle size emulsion, and (c) RIBI™ adjuvant system (RAS), (Ribi Immunochem, Hamilton, Mont.) containing 2% Squalene, 0.2% Tween 80, and one or more bacterial cell wall components such as monophosphorylipid A (MPL), trehalose dimycolate (TDM), and cell wall skeleton (CWS), e.g., MPL+CWS (Detox™); (2) saponin adjuvants, such as QS21 or Stimulon™(Cambridge Bioscience, Worcester, Mass.) may be used or particles generated therefrom such as ISCOMs (immunostimulating complexes), which ISCOMS may be devoid of additional detergent e.g. WO 00/07621; (3) Complete Freund's Adjuvant (CFA) and Incomplete Freund's Adjuvant (IFA); (4) cytokines, such as interleukins (e.g. IL-1, IL-2, IL-4, IL-5, IL-6, IL-7, IL-12, IL-15, IL-28, etc.) (WO99/44636), etc.), interferons (e.g. gamma interferon), macrophage colony stimulating factor (M-CSF), tumor necrosis factor (TNF), colony-stimulating factors (e.g., GM-CSF), etc.; (5) monophosphoryl lipid A (MPL) or 3-O-deacylated MPL (3dMPL) e.g. GB-2220221, EP-A-0689454, optionally in the substantial absence of alum when used with pneumococcal saccharides e.g. WO 00/56358; (6) combinations of 3dMPL with, for example, QS21 and/or oil-in-water emulsions e.g. EP-A-0835318, EP-A-0735898, EP-A-0761231; (7) oligonucleotides comprising CpG motifs (Krieg Vaccine 2000, 19, 618-622; WO 96/02555, WO 98/16247, WO 98/18810, WO 98/40100, WO 98/55495, WO 98/37919 and WO 98/52581), i.e., oligonucleotides containing at least one CG dinucleotide, where the cytosine is unmethylated; (8) a polyoxyethylene ether or a polyoxyethylene ester e.g. WO 99/52549; (9) a polyoxyethylene sorbitan ester surfactant in combination with an octoxynol (WO 01/21207) or a polyoxyethylene alkyl ether or ester surfactant in combination with at least one additional non-ionic surfactant such as an octoxynol (WO 01/21152); (10) a saponin and an immunostimulatory oligonucleotide (e.g. a CpG

oligonucleotide) (WO 00/62800); (11) an immunostimulant and a particle of metal salt e.g. WO 00/23105; (12) a saponin and an oil-in-water emulsion e.g. WO 99/11241; (13) a saponin (e.g. QS21)+3dMPL+IM2 (optionally including a sterol) e.g. WO 98/57659; (14) alphaGalCer and its derivatives; (16) toll-like receptor (TLR) agonists, NOD-like receptor (NLR) agonists, RIG-I agonists, agonists for C-type lectin receptors and other pathogen recognition receptor (PRR) agonists e.g., CpG ODNs, ISS-ODNs, rinatolimod, polyI:C and its derivatives, flagellin, ampligen, imidazoquinalines (e.g., imiquimod, resiquimod), muramyl dipeptides; (17) other substances that act as immunostimulating agents to enhance the efficacy of the composition. Muramyl peptides include N-acetyl-muramyl-L-threonyl-D-isoglutamine (thr-MDP), N-25 acetyl-normuramyl-L-alanyl-D-isoglutamine (nor-MDP), N-acetylmuramyl-L-alanyl-D-isoglutarninyl-L-alanine-2-(1'-2'-dipalmitoyl-sn-glycero-3-hydroxyphosphoryloxy)-ethylamine MTP-PE), etc. Adjuvants suitable for administration to a human included in some cases. In some cases, the adjuvant is aluminum hydroxide. In some cases, the adjuvant is aluminum potassium sulfate. In some cases, the adjuvant is aluminum phosphate. In some cases, the adjuvant is aluminum hydroxyphosphate sulfate. In some cases, the adjuvant is aluminum sulfate.

[0083] Further exemplary suitable adjuvants include, but are not limited to: cholera toxin B subunit, BCG, *Pseudomonas aeruginosa* exoprotein A, tocopherol, HBV core, E. coli heat labile toxins (such as LT-A, LT-B), Pertussis toxin, Diphtheria toxoid, tetanus toxoid, Aluminium salt-based adjuvants (such as Alum, Aluminum phosphate, Aluminum sulphate, Alhydrogel), Calcium phosphate, kaolin, monophosphoryl lipid A (MPL^R) and its derivatives, glucoppyranosyl lipid A, synthetic lipid A, Lipid A mimetics, Vitamin E, Depovax^{TM}, Saponins (Quil-A, AS01, AS02 (squalene+MPL+QS-21)), AS03, AS04 (alum+MPL^R), Tomatin, Protolin, RC-529, Pluronic^{TM}, Monatides, Matrix-M, OM-174, Lipovac, IC-31, bacterial/mycobacterial peptides (such as KLK), polyphosphagene and its derivatives, Gellan, nucleotides (mono, di, poly), $Gram^+$ and $Gram^-$ non-pathogenic bacteria such as *Caulobacter crescentus* in live, inactivated and/or heat-killed form, etc.

[0084] An adenovirus composition can include one or more mucoadhesives such as sodium alginate, starch, lectins, thiolated polymers, GelVac^{TM}, sodium carboxymethylcellulose, hydroxylpropyl methylcellulose, carbomers, cetyl trimethyl ammonium bromide.

[0085] An adenovirus composition can include one or more additional adjuvant formulations such as oil-in-water emulsions, water-in-oil emulsions, nanoemulsions, particulate delivery systems, liposomes, microspheres, biodegradable microspheres, patches virosomes, proteoliposomes, proteasomes, Immunostimulatory complexes (ISCOMs, ISCOMATRIX), microparticles, nanoparticles, polymeric micro/nano particles, polymeric lamellar substrate particles (PLSP), microparticle resins, synthetic/biodegradable and biocompatible semisynthetic or natural

polymers (such as PLG, PLGA, PLA, polycaprolactone, silicone polymer, polyesters, poly-dimethyl siloxane, sodium polystyrene sulphonate, polystyrene benzyl trimethyl ammonium chloride, polystyrene divinyl benzene resin, polyphosphazene, poly-[di-(carboxylactophenoxy)phosphazene] (PCPP), poly-(methylmethacrylate), dextran, polyvinylpyrrolidone, hyaluronic acid and derivatives, chitosan and its derivatives, polysaccharides, lipopolysaccharides, polycationic compound(s) (such as Poly-amino acids, poly-(γ-glutamic acid), poly-arginine-HCl, poly-L-lysine, polypeptides, biopolymers), cationic dimethyldioctadecyl ammonium (DDA), alpha-galactosyl ceramide and its derivatives, archaeal lipids and derivatives, lactanes, gallen, glycerolipids, cochleates, etc.

[0086] An adenovirus composition can include one or more additional adjuvant formulations such as oil-in-water emulsions or water-in-oil emulsions including edible oils (such as olive oil, mustard oil, vegetable oil, mineral oil etc.).

[0087] An adenovirus composition can include one or more additional surfactants and detergents (e.g., non-ionic detergents) (such as Tween-80, Polysorbate 80, Span 85, Stearyl tyrosine etc.).

[0088] In some cases, an adenovirus composition is administered to an individual in need thereof, where the adenovirus composition comprises one or more chemokines, one or more costimulatory molecules (e.g., CD40L, 4-BBL, anti-CD40 Mab), or one or more antibodies to coinhibitory molecules (e.g., anti-CTLA-4, anti-PD-1).

Non-adenovirus polypeptides

[0089] As noted above, in some embodiments, an adenovirus used in a method of the present disclosure does not include a nucleotide sequence encoding a polypeptide other than an adenovirus polypeptide. In other instances, an adenovirus used in a method of the present disclosure includes a nucleotide sequence encoding a polypeptide other than an adenovirus polypeptide. In some cases, an adenovirus used in a method of the present disclosure includes a nucleotide sequence encoding a polypeptide other than an adenovirus polypeptide, where the non-adenovirus polypeptide is an HCV polypeptide. In some cases, an adenovirus used in a method of the present disclosure includes a nucleotide sequence encoding a polypeptide other than an adenovirus polypeptide, where the non-adenovirus polypeptide is from a pathogen other than HCV. In some cases, an adenovirus used in a method of the present disclosure includes a nucleotide sequence encoding a polypeptide such as an antigen from a pathogen (e.g., a pathogen other than HCV), or a tumor-associate antigen. The recombinant virus may comprise a plurality of antigen-encoding nucleotide sequences. The plurality of the antigen-encoding nucleotide sequences may be multiple copies of the same antigen-encoding sequence or multiple antigen sequences that differ from each other. The plurality of antigen-encoding nucleotide sequences may be derived from a single pathogen or cancer, different strains/serotypes of a pathogen or

cancer or different kinds of pathogens or cancer. In some cases, the antigen sequences may include sequences one of which induces T cell responses against a pathogenic antigen(s) and/or another induces B cell (humoral) responses against another antigen.

[0090] In some cases, adenovirus including a nucleotide sequence encoding a polypeptide other than adenovirus polypeptide can be mixed together with another adenovirus or a non-adenoviral vector expressing different nucleotide sequence encoding for a second antigen.

[0091] Suitable antigens include, but are not limited to, an antigen derived from a pathogenic microorganism; a tumor-associated antigen; and the like. Antigens derived from a pathogenic microorganism include antigens derived from a virus, a bacterium, a fungus, a protozoan, or a helminth.

[0092] In some cases, an adenovirus composition is administered to an individual in need thereof, where the adenovirus composition comprises one or more inactivated/non-pathogenic/commensal gram positive or gram-negative bacteria, virus or their antigens.

Antigens other than an adenoviral antigen

[0093] In some cases, an adenovirus used in a method of the present disclosure includes a nucleotide sequence encoding a polypeptide other than an adenovirus polypeptide, where the non-adenovirus antigen is a polypeptide, e.g., a full-length protein or a portion of an antigenic protein that contains an immunodominant antigen, a neutralizing antigen, or epitopes of a pathogenic antigen other than an HCV polypeptide. A suitable antigen can be any type of antigen known in the art. Antigens can be in variety of forms as described below.

[0094] A recombinant adenovirus vector can be constructed by one of ordinary skill in the art following procedures known in the literature. The amino acid sequence of an antigen encoded by the recombinant adenovirus vector may be natural, mutated, truncated, modified, optimized, or inactivated, and can from the same or different strains of the pathogen(s) or can be from the same cancer cell or multiple different cancer cells.

Antigens from pathogenic bacteria

[0095] In some cases, an adenovirus used in a method of the present disclosure includes a nucleotide sequence encoding an antigen derived from or associated with a pathogenic bacterium. In some cases, an adenovirus used in a method of the present disclosure includes a nucleotide sequence encoding one or more bacterial antigens, e.g., 1, 2, 3, 4, 5, or more bacterial antigens, from one or more bacteria.

[0096] Non-limiting examples of pathogenic bacteria include Mycobacteria, Streptococcus, Staphylococcus, Pseudomonas, Salmonella, Neisseria, and Listeria. In some cases, the bacteria is

Neisseria gonorrhea, *M. tuberculosis*, *M. leprae*, *Listeria monocytogenes*, *Streptococcus pneumoniae*, *S. pyogenes*, *S. agalactiae*, *S. viridans*, *S. faecalis*, or *S. bovis*.

[0097] Other examples of bacteria contemplated include, but are not limited to, Gram positive bacteria (e.g., Listeria, Bacillus such as *Bacillus anthracis*, Erysipelothrix species), Gram negative bacteria (e.g., Bartonella, Brucella, Campylobacter, Enterobacter, Escherichia, Francisella, Hemophilus, Klebsiella, Morganella, Proteus, Providencia, Pseudomonas, Salmonella, Serratia, Shigella, Vibrio, and Yersinia species), spirochete bacteria (e.g., Borrelia species including Borrelia burgdorferi that causes Lyme disease), anaerobic bacteria (e.g., Actinomyces and Clostridium species), Gram positive and negative coccal bacteria, Enterococcus species, Streptococcus species, Pneumococcus species, Staphylococcus species, Neisseria species.

[0098] Additional non-limiting examples of specific infectious bacteria include *Helicobacter pyloris*, *Borelia burgdorferi*, *Legionella pneumophila*, *Mycobacteria avium*, *M. intracellulare*, *M. kansaii*, *M. gordonae*, *M. africanum*, *Staphylococcus aureus*, *Neisseria meningitidis*, *Haemophilus influenzae*, *Bacillus anthracis*, *Corynebacterium diphtheriae*, *Erysipelothrix rhusiopathiae*, *Clostridium perfringens*, *Clostridium tetani*, *Enterobacter aerogenes*, *Klebsiella pneumoniae*, *Pasturella multocida*, *Fusobacterium nucleatum*, *Streptobacillus moniliformis*, *Treponema pallidium*, *Treponema pertenue*, *Leptospira*, *Rickettsia*, and *Actinomyces israelli*.

[0099] An antigen can be derived from any of the aforementioned bacteria.

[00100] Non-limiting examples of suitable bacterial antigens include pertussis toxin, filamentous hemagglutinin, pertactin, FIM2, FIM3, adenylate cyclase and other pertussis bacterial antigen components; diphtheria bacterial antigens such as diphtheria toxin or toxoid and other diphtheria bacterial antigen components; tetanus bacterial antigens such as tetanus toxin or toxoid and other tetanus bacterial antigen components; streptococcal bacterial antigens such as M proteins and other streptococcal bacterial antigen components; gram-negative bacilli bacterial antigens such as lipopolysaccharides and other gram-negative bacterial antigen components, Mycobacterium tuberculosis bacterial antigens such as mycolic acid, heat shock protein 65 (HSP65), the 30 kDa major secreted protein, antigen 85A and 85B and other mycobacterial antigen components; Helicobacter pylori bacterial antigen components; pneumococcal bacterial antigens such as pneumolysin, pneumococcal capsular polysaccharides and other pneumococcal bacterial antigen components; haemophilus influenza bacterial antigens such as capsular polysaccharides and other haemophilus influenza bacterial antigen components; anthrax bacterial antigens such as anthrax protective antigen and other anthrax bacterial antigen components; rickettsiae bacterial antigens such as rompA and other rickettsiae bacterial antigen component. Also included with the bacterial antigens described herein are any other bacterial, mycobacterial, mycoplasmal, rickettsial, or chlamydial antigens.

[00101] A bacterial antigen can be purified (e.g., at least 50% pure, at least 60% pure, at least 70% pure, at least 80% pure, at least 90% pure, at least 95% pure, at least 98% pure, or at least 99% pure, or more than 99% pure). A bacterial antigen can be an extract from a bacterial cell. A bacterial antigen can be synthetically produced, e.g., by recombinant means.

Fungal antigens

[00102] In some cases, an adenovirus used in a method of the present disclosure includes a nucleotide sequence encoding one or more fungal antigens, e.g., 1, 2, 3, 4, 5, or more fungal antigens, from one or more fungi.

[00103] Fungal antigens include, but are not limited to, e.g., candida fungal antigen components; histoplasma fungal antigens such as heat shock protein 60 (HSP60) and other histoplasma fungal antigen components; cryptococcal fungal antigens such as capsular polysaccharides and other cryptococcal fungal antigen components; coccidioides fungal antigens such as spherule antigens and other coccidioides fungal antigen components; and tinea fungal antigens such as trichophytin and other coccidioides fungal antigen components.

[00104] Fungal antigens can be obtained from Candida spp. including *C. albicans*, Aspergillus spp., Cryptococcus spp. including *C. neoformans*, Blastomyces sp., Pneumocytes spp., or Coccidioides spp.

Parasite antigens

[00105] In some cases, an adenovirus used in a method of the present disclosure includes a nucleotide sequence encoding a parasite antigen. Parasites include protozoan parasites and helminths. In some cases, an adenovirus used in a method of the present disclosure includes a nucleotide sequence encoding one or more parasitic antigens, e.g., 1, 2, 3, 4, 5, or more parasitic antigens, from one or more parasites.

[00106] Examples of parasites include Plasmodium spp., *Toxoplasma gondii*, Babesia spp., *Trichinella spiralis*, *Entamoeba histolytica*, *Giardia lamblia*, *Enterocytozoon bieneusi*, Naegleria, Acanthamoeba, *Trypanosoma rhodesiense* and *Trypanosoma gambiense*, Isospora spp., Cryptosporidium spp, Eimeria spp., Neospora spp., Sarcocystis spp., and Schistosoma spp.

[00107] Parasite antigens can be derived from Plasmodium spp. (such as RTS, S, TRAP, MSP-1, MSP-3, RAP1, RAP2 etc.), Toxoplasma spp. including *T. gondii* (such as SAG2, SAG3, Tg34), Entamoeba spp. including *E. histolytica*, Schistosoma spp., *Trypanosoma cruzi* Cryptosporidium spp., Angiostrongylus spp., Ancyclostoma spp., Wuchereria spp., Brugia spp., Giardia spp., Leishmania spp., Pneumonocystis spp., Enterobius spp., Ascaris spp., Trichuris spp., Trichomonas spp., Necator spp., Onchocerca spp., Dracanculus spp., Trichinella spp., Strongyloides spp., Opisthorchis spp., Paragonimus spp., Fasciola spp., or Taenia spp.

Protozoan antigens

[00108] In some cases, an adenovirus used in a method of the present disclosure includes a nucleotide sequence encoding a protozoan antigen. A protozoan antigen can be derived from any protozoan parasite, including, but not limited to, Giardia; a plasmodium species (e.g., *Plasmodium falciparum*); *Toxoplasma gondii*; a cryptosporidium; a Trichomonas species; a trypanosome (e.g., *Trypanosoma cruzi*); or Leishmania.

[00109] Protozoan antigens include, but are not limited to, e.g., plasmodium falciparum antigens such as merozoite surface antigens, sporozoite surface antigens, circumsporozoite antigens, gametocyte/gamete surface antigens, blood-stage antigen pf 155/RESA and other plasmodial antigen components; toxoplasma antigens such as SAG-1, p30 and other toxoplasmal antigen components; schistosomae antigens such as glutathione-S-transferase, paramyosin, and other schistosomal antigen components; leishmania major and other leishmanial antigens such as gp63, lipophosphoglycan and its associated protein and other leishmanial antigen components; and *Trypanosoma cruzi* antigens such as the 75-77 kDa antigen, the 56 kDa antigen and other trypanosomal antigen components.

Helminth antigens

[00110] In some cases, an adenovirus used in a method of the present disclosure includes a nucleotide sequence encoding a helminth antigen. Helminth antigens include antigens derived from flatworms, thorny-headed worms, and roundworms (nematodes).

Viral antigens

[00111] In some cases, an adenovirus used in a method of the present disclosure includes a nucleotide sequence encoding a one or more viral antigens, e.g., 1, 2, 3, 4, 5, or more viral antigens, from one or more viruses. In some cases, the viral antigen is an HCV antigen. In other embodiments, the antigen is other than an HCV antigen.

[00112] Viruses that can be the source of the viral antigen(s) include, but are not limited to, herpes viruses (HSV-1, HSV-2, VZV, EBV, CMV, HHV-6, HHV-8), influenza viruses (Flu A, B), hepatitis viruses (HepA, HepB, HepC, HepE), human immunodeficiency viruses (HIV-1, HIV-2), respiratory syncytial viruses, measles viruses, rhinoviruses, adenoviruses, SARS viruses, papillomaviruses, orthopoxviruses, West Nile viruses, and a dengue viruses. Viruses that can be the source of the viral antigen(s) include members of the Flaviviridae family of viruses. Viruses that can be the source of the viral antigen(s) include a flavivirus selected from the group consisting of dengue, Kunjin, Japanese encephalitits, West Nile, and yellow fever virus. Viruses that can be the source of the viral antigen(s) include lymphocytic choriomenignitis virus, hepatitis B virus, Epstein Barr virus, and human immunodeficiency virus. Viruses that can be the

source of the viral antigen(s) include, but are not limited to: Retroviridae (e.g. human immunodeficiency viruses, such as HIV-1, also referred to as LAV or HTLV-III/LAV, or HIV-III; and other isolates, such as HIV-LP; Picornaviridae (e.g. polio viruses, hepatitis A virus; enteroviruses, human Coxsackie viruses, rhinoviruses, echoviruses); Calciviridae (e.g. strains that cause gastroenteritis); Togaviridae (e.g. equine encephalitis viruses, rubella viruses); Flaviridae (e.g. dengue viruses, encephalitis viruses, yellow fever viruses); Coronaviridae (e.g. coronaviruses); Rhabdoviridae (e.g. vesicular stomatitis viruses, rabies viruses); Filoviridae (e.g. ebola-like viruses, Marburg viruses); Paramyxoviridae (e.g. parainfluenza viruses, mumps virus, measles virus, respiratory syncytial virus); Orthomyxoviridae (e.g. influenza viruses); Bungaviridae (e.g. Hantaan viruses, bunga viruses, phleboviruses and Nairo viruses); Arenaviridae (hemorrhagic fever viruses); Reoviridae (e.g. reoviruses, orbiviurses and rotaviruses); Bornaviridae; Hepadnaviridae (Hepatitis B virus); Parvoviridae (parvoviruses); Papovaviridae (papilloma viruses, polyoma viruses); Adenoviridae (e.g., adenoviruses); Herpesviridae (herpes simplex virus (HSV) 1 and 2), varicella zoster virus, cytomegalovirus (CMV), herpes virus; Poxviridae (variola viruses, vaccinia viruses, pox viruses); and Iridoviridae (e.g. African swine fever virus); and unclassified viruses (e.g. the etiological agents of Spongiform encephalopathies, the agent of delta hepatitis, thought to be a defective satellite of hepatitis B virus), the agents of non-A, non-B hepatitis (class 1, internally transmitted; class 2, parenterally transmitted, i.e., Hepatitis C); Norwalk and related viruses, and astroviruses.

[00113] Suitable viral antigens include antigens from the herpesvirus family, including proteins derived from herpes simplex virus (HSV) types 1 and 2, such as HSV-1 and HSV-2 glycoproteins gB, gD and gH; antigens derived from varicella zoster virus (VZV), Epstein-Barr virus (EBV) and cytomegalovirus (CMV) including CMV gB and gH; and antigens derived from other human herpesviruses such as HHV6 and HHV7. (See, e.g. Chee et al., Cytomegaloviruses (J. K. McDougall, ed., Springer-Verlag 1990) pp. 125-169, for a review of the protein coding content of cytomegalovirus; McGeoch et al., J. Gen. Virol. (1988) 69:1531-1574, for a discussion of the various HSV-1 encoded proteins; U.S. Pat. No. 5,171,568 for a discussion of HSV-1 and HSV-2 gB and gD proteins and the genes encoding therefor; Baer et al., Nature (1984) 310:207-211, for the identification of protein coding sequences in an EBV genome; and Davison and Scott, J. Gen. Virol. (1986) 67:1759-1816, for a review of VZV.)

[00114] Suitable viral antigens include antigens from the hepatitis family of viruses, including hepatitis A virus (HAV), hepatitis B virus (HBV), hepatitis C virus (HCV), the delta hepatitis virus (HDV), hepatitis E virus (HEV) and hepatitis G virus (HGV), can also be conveniently used in the techniques described herein. By way of example, the viral genomic sequence of HCV is known, as are methods for obtaining the sequence. See, e.g., International Publication Nos.

WO 89/04669; WO 90/11089; and WO 90/14436. The HCV genome encodes several viral proteins, including E1 (also known as E) and E2 (also known as E2/NSI) and an N-terminal nucleocapsid protein (termed "core") (see, Houghton et al., Hepatology (1991) 14:381-388, for a discussion of HCV proteins, including E1 and E2). Each of these proteins, as well as antigenic fragments thereof, will find use in the present composition and methods.

[00115] Suitable viral antigens include the 6-antigen from HDV (see, e.g., U.S. Pat. No. 5,378,814). Additionally, antigens derived from HBV, such as the core antigen, the surface antigen, sAg, as well as the presurface sequences, pre-S1 and pre-S2 (formerly called pre-S), as well as combinations of the above, such as sAg/pre-S1, sAg/pre-S2, sAg/pre-S1/pre-S2, and pre-S1/pre-S2, are suitable. See, e.g., "HBV Vaccines--from the laboratory to license: a case study" in Mackett, M. and Williamson, J. D., Human Vaccines and Vaccination, pp. 159-176, for a discussion of HBV structure; and U.S. Pat. Nos. 4,722,840, 5,098,704, 5,324,513; Beames et al., J. Virol. (1995) 69:6833-6838, Birnbaum et al., J. Virol. (1990) 64:3319-3330; and Zhou et al., J. Virol. (1991) 65:5457-5464.

[00116] Suitable viral antigens include antigens from members of filoviruses [e.g., Zaire, Sudan, Ivory Coast Ebola viruses, Marburg virus antigens such as structural proteins (membrane form of glycoproteins, soluble glycoproteins, NP, matrix proteins (VP24, VP40)) and nonstructural proteins (VP30, VP35)]. In some embodiments, the antigenic protein may be mutated so that it is less toxic to cells.

[00117] Suitable viral antigens include, but are not limited to, proteins from members of the families Picornaviridae (e.g., polioviruses, etc.); Caliciviridae; Togaviridae (e.g., rubella virus, dengue virus, etc.); Flaviviridae; Coronaviridae; Reoviridae; Birnaviridae; Rhabdoviridae (e.g., rabies virus, etc.); Filoviridae; Paramyxoviridae (e.g., mumps virus, measles virus, respiratory syncytial virus, etc.); Orthomyxoviridae (e.g., influenza virus types A, B and C, etc.); Bunyaviridae; Arenaviridae; Retroviradae (e.g., HTLV-I; HTLV-II; HIV-1 (also known as HTLV-III, LAV, ARV, hTLR, etc.)), including but not limited to antigens from the isolates HIV-IIIb, HIV-SF2, HIV-LAV, HIV-LAI, HIV-MN); HIV-1-CM235, HIV-1-US4; HIV-2; simian immunodeficiency virus (SIV) among others. Additionally, antigens may also be derived from human papillomavirus (HPV) and the tick-borne encephalitis viruses. See, e.g. Virology, 3rd Edition (W. K. Joklik ed. 1988); Fundamental Virology, 2nd Edition (B. N. Fields and D. M. Knipe, eds. 1991), for a description of these and other viruses.

[00118] Suitable viral antigens include the gp120 or gp140 envelope proteins from any of the above HIV isolates, including members of the various genetic subtypes of HIV, are known and reported (see, e.g., Myers et al., Los Alamos Database, Los Alamos National Laboratory, Los Alamos, N. Mex. (1992); Myers et al., Human Retroviruses and Aids, 1990, Los Alamos, N.

Mex.: Los Alamos National Laboratory; and Modrow et al., J. Virol. (1987) 61:570-578, for a comparison of the envelope sequences of a variety of HIV isolates) and antigens derived from any of these isolates will find use in the present methods. Suitable viral antigens include proteins derived from any of the various HIV isolates, including any of the various envelope proteins such as gp160 and gp41, gag antigens such as p24gag and p55gag, as well as proteins derived from the pol and tat regions.

[00119] Suitable viral antigens include antigens of influenza virus. Specifically, the envelope glycoproteins HA and NA of influenza A can be used. Numerous HA subtypes of influenza A have been identified (Kawaoka et al., Virology (1990) 179:759-767; Webster et al., "Antigenic variation among type A influenza viruses," p. 127-168. In: P. Palese and D. W. Kingsbury (ed.), Genetics of influenza viruses. Springer-Verlag, New York). Conserved antigens of influenza such as nucleoprotein, M2 and M1 can also be used in vaccine compositions. Thus, proteins derived from any of these isolates can also be used in the compositions and methods described herein.

Cancer-associated antigens

[00120] In some cases, an adenovirus used in a method of the present disclosure includes a nucleotide sequence encoding a cancer-associated antigen. Cancer-associated antigens can be derived from the cell surface, cytoplasm, nucleus, organelles and the like of cells of tumor tissue. In some cases, an adenovirus used in a method of the present disclosure includes a nucleotide sequence encoding one or more cancer antigens, e.g., 1, 2, 3, 4, 5, or more cancer antigens, from one or more cancers.

[00121] Examples of cancer-associated antigens include, without limitation, antigens associated with hematological cancers such as leukemias and lymphomas, neurological tumors such as astrocytomas or glioblastomas, melanoma, breast cancer, lung cancer, head and neck cancer, gastrointestinal tumors such as gastric or colon cancer, liver cancer, pancreatic cancer, genitourinary tumors such cervix, uterus, ovarian cancer, vaginal cancer, testicular cancer, prostate cancer or penile cancer, bone tumors, vascular tumors, or cancers of the lip, nasopharynx, pharynx and oral cavity, esophagus, rectum, gall bladder, biliary tree, larynx, lung and bronchus, bladder, kidney, brain and other parts of the nervous system, thyroid, Hodgkin's disease, non-Hodgkin's lymphoma, multiple myeloma and leukemia.

[00122] Cancer-associated antigens include, e.g., mutated oncogenes; viral proteins associated with tumors; and tumor mucins and glycolipids. The antigens may be viral proteins associated with tumors. Certain antigens may be characteristic of tumors (one subset being proteins not usually expressed by a tumor precursor cell), or may be a protein which is normally expressed in a tumor precursor cell, but having a mutation characteristic of a tumor. Other antigens include

mutant variant(s) of the normal protein having an altered activity or subcellular distribution, e.g., mutations of genes giving rise to tumor antigens.

[00123] Specific non-limiting examples of suitable tumor antigens include: CEA, prostate specific antigen (PSA), HER-2/neu, BAGE, GAGE, MAGE 1-4, 6 and 12, MUC (Mucin) (e.g., MUC-1, MUC-2, etc.), GM2 and GD2 gangliosides, ras, myc, tyrosinase, MART (melanoma antigen), Pmel 17(gp100), GnT-V intron V sequence (N-acetylglucoaminyltransferase V intron V sequence), Prostate Ca psm, PRAME (melanoma antigen), β-catenin, MUM-1-B (melanoma ubiquitous mutated gene product), GAGE (melanoma antigen) 1, BAGE (melanoma antigen) 2-10, c-ERB2 (Her2/neu), EBNA (Epstein-Barr Virus nuclear antigen) 1-6, gp75, human papilloma virus (HPV) E6 and E7, p53, lung resistance protein (LRP), Bcl-2, and Ki-67.

[00124] Suitable cancer-associated antigens include, e.g., Melan-A/MART-1, Dipeptidyl peptidase IV (DPPIV), adenosine deaminase-binding protein (ADAbp), cyclophilin b, Colorectal associated antigen (CRC)-C017-1A/GA733, Carcinoembryonic Antigen (CEA) and its immunogenic epitopes CAP-1 and CAP-2, etv6, aml1, Prostate Specific Antigen (PSA) and its immunogenic epitopes PSA-1, PSA-2, and PSA-3, prostate-specific membrane antigen (PSMA), T-cell receptor/CD3-zeta chain, MAGE-family of tumor antigens (e.g., MAGE-A1, MAGE-A2, MAGE-A3, MAGE-A4, MAGE-A5, MAGE-A6, MAGE-A7, MAGE-A8, MAGE-A9, MAGE-A10, MAGE-A11, MAGE-A12, MAGE-Xp2 (MAGE-B2), MAGE-Xp3 (MAGE-B3), MAGE-Xp4 (MAGE-B4), MAGE-C1, MAGE-C2, MAGE-C3, MAGE-C4, MAGE-05), GAGE-family of tumor antigens (e.g., GAGE-1, GAGE-2, GAGE-3, GAGE-4, GAGE-5, GAGE-6, GAGE-7, GAGE-8, GAGE-9), BAGE, RAGE, LAGE-1, NAG, GnT-V, MUM-1, CDK4, tyrosinase, p53, MUC family, HER2/neu, p21ras, RCAS1, α-fetoprotein, E-cadherin, α-catenin, β-catenin and γ-catenin, p120ctn, gp100.sup.Pmel117, PRAME, NY-ESO-1, brain glycogen phosphorylase, SSX-1, SSX-2 (HOM-MEL-40), SSX-1, SSX-4, SSX-5, SCP-1, CT-7, cdc27, adenomatous polyposis coli protein (APC), fodrin, P1A, Connexin 37, Ig-idiotype, p15, gp75, GM2 and GD2 gangliosides, viral products such as human papilloma virus proteins, Smad family of tumor antigens, 1mp-1, EBV-encoded nuclear antigen (EBNA)-1, or c-erbB-2.

Compositions comprising an adenovirus nucleic acid and a non-adenoviral polypeptide

[00125] In some cases, an adenovirus composition for use in a method of the present disclosure comprises an adenovirus nucleic acid, as described above, and a non-adenoviral polypeptide. In some cases, the polypeptide is an antigen. For example, in some cases, the antigen is an antigen from a pathogen, a tumor-associated antigen, etc., as described above.

[00126] An antigen can be a whole cell extract, a cell lysates, a whole cell, a whole live cell, a whole inactivated cell, a whole irradiated cell, etc. Antigens may be crude, purified, or recombinant form. In some cases, an antigen is at least 50% pure, at least 60% pure, at least 70%

pure, at least 80% pure, at least 90% pure, at least 95% pure, at least 98% pure, or at least 99% pure, or more than 99% pure.

[00127] An adenovirus composition can comprise a single type of antigen. An adenovirus composition can include 2 or more different antigens. Adenovirus composition can include 2, 3, 4, 5, 6, or more than 6, different antigens. Where an adenovirus composition includes more than one antigen, the more than one antigen can be from the same pathogenic organism, or from the same cancer cell. Where an adenovirus composition includes more than one antigen, the more than one antigen can be from two or more different pathogenic organisms, or from two or more different cancer cells or two or more different types of cancers.

[00128] An antigen can be in the form of a protein, a lipopolysaccharide, a lipoprotein, a proteoglycan, glycoproteins, glycosaminoglycans, a fragment of a protein (e.g., less than full-length protein), etc.

[00129] Suitable antigens include, e.g., peptides, modified peptides, conformationally-constrained synthetic peptides, lipopeptides, monolipopeptides, dilipopeptides, peptides conjugated or fused to proteins as antigens. See, e.g., U.S. Patent No. 8,198,400. Suitable antigens include, e.g., proteins, purified or recombinant proteins, recombinant fusion proteins, proteins and peptides conjugated to toll-like receptor (TLR) agonists, glycoproteins, glycolipoproteins, polysaccharides, polysaccharide conjugates, lipids, glycolipids and carbohydrates.

[00130] An antigen or antigenic composition can be obtained from live viruses, dead viruses, attenuated viruses, bacteria, fungi, protozoa, helminths, etc.

[00131] In some cases, an adenovirus composition containing antigens other than an adenoviral antigen, or comprising an adenovirus encoding a polypeptide antigen other than an adenovirus polypeptide, can comprise one or more of an adjuvant, a surfactant, a detergent, and a mucoadhesive, where suitable adjuvants, surfactants, detergents, and mucoadhesives are described elsewhere herein.

[00132] In some cases, an adenovirus composition containing antigens other than an adenoviral antigen, or comprising an adenovirus encoding a polypeptide antigen other than an adenovirus polypeptide, can comprise an immunostimulatory or an immunomodulatory agent, where suitable immunostimulatory and immunomodulatory agents are described elsewhere herein. In some cases, an adenovirus encoding a polypeptide antigen other than an adenovirus polypeptide can comprise a nucleotide sequence encoding an immunostimulatory polypeptide or an immunomodulatory polypeptide, where suitable immunostimulatory and immunomodulatory polypeptides are described elsewhere herein.

[00133] In some cases, a mixture of different recombinant adenovirus vectors containing antigen and/or immunostimulatory sequences and/or immunomodulatory sequences can be mixed together for administration or administered at different sites simultaneously or sequentially.

[00134] In certain embodiments, an adenovirus composition containing antigens other than an adenoviral antigen, or comprising an adenovirus encoding a polypeptide antigen other than an adenovirus polypeptide, can be used in a prime-boost immunization regimen simultaneously or sequentially.

METHODS OF TREATING AN HCV INFECTION

[00135] The present disclosure provides a method of treating an HCV infection in an individual, the method comprising inducing or enhancing an immune response to one or more HCV antigens in the individual. Methods of inducing an immune response to an HCV infection are those described above. The method generally involves administering to the individual an immunogenic adenovirus composition, where the adenovirus nucleic acid or adenovirus polypeptide present in the immunogenic adenovirus composition induces an immune response in the individual to one or more HCV antigens. In some cases, the adenovirus is a wild-type adenovirus. In some cases, the adenovirus is a recombinant adenovirus.

[00136] In some cases, an effective amount of an adenovirus composition is an amount that, when administered in one or more doses to an individual in need thereof, reduces HCV viral load in the individual. In some cases, an effective amount of an adenovirus composition is an amount that, when administered in one or more doses to an individual in need thereof, is effective to achieve a 1.5-log, a 2-log, a 2.5-log, a 3-log, a 3.5-log, a 4-log, a 4.5-log, or a 5-log reduction in viral titer in the serum of the individual.

[00137] In some cases, an effective amount of an adenovirus composition is an amount that, when administered in one or more doses to an individual in need thereof, is effective to reduce a serum level of HCV in the individual. For example, in some embodiments, an effective amount of an adenovirus composition is an amount that, when administered in one or more doses to an individual in need thereof, is effective to reduce the level of serum HCV in the individual to from about 1000 genome copies/mL serum to about 5000 genome copies/mL serum, to from about 500 genome copies/mL serum to about 1000 genome copies/mL serum, or to from about 100 genome copies/mL serum to about 500 genome copies/mL serum. In some embodiments, an effective amount of an adenovirus composition is an amount that, when administered in one or more doses to an individual in need thereof, is effective to reduce HCV viral load to lower than 100 genome copies/mL serum.

[00138] In some embodiments, an effective amount of an adenovirus composition is an amount that, when administered in one or more doses to an individual in need thereof, is effective to achieve a sustained viral response, e.g., non-detectable or substantially non-detectable HCV RNA (e.g., less than about 500, less than about 400, less than about 200, or less than about 100 genome copies per milliliter serum) is found in the patient's serum for a period of at least about one month, at least about two months, at least about three months, at least about four months, at least about five months, or at least about six months following cessation of therapy.

[00139] Viral load can be measured by measuring the titer or level of virus in serum. These methods include, but are not limited to, a quantitative polymerase chain reaction (PCR) and a branched DNA (bDNA) test. Quantitative assays for measuring the viral load (titer) of HCV RNA have been developed. Many such assays are available commercially, including a quantitative reverse transcription PCR (RT-PCR) (Amplicor HCV Monitor™, Roche Molecular Systems, New Jersey); and a branched DNA (deoxyribonucleic acid) signal amplification assay (Quantiplex™ HCV RNA Assay (bDNA), Chiron Corp., Emeryville, California). See, e.g., Gretch et al. (1995) *Ann. Intern. Med.* 123:321-329. Also of interest is a nucleic acid test (NAT), developed by Gen-Probe Inc. (San Diego) and Chiron Corporation, and sold by Chiron Corporation under the trade name Procleix®, which NAT simultaneously tests for the presence of HIV-1 and HCV. See, e.g., Vargo et al. (2002) *Transfusion* 42:876-885.

[00140] As described above, in some cases, an adenovirus composition for use in a subject method does not include nucleotide sequences encoding non-adenovirus polypeptides.

[00141] As described above, in some cases, an adenovirus composition for use in a subject method includes nucleotide sequences encoding non-adenovirus polypeptides. As described above, in some cases, the non-adenovirus polypeptide is an antigen, e.g., an antigen associated with a pathogen, a cancer-associated antigen, etc. In some cases, an adenovirus composition containing antigens other than an adenoviral antigen, or comprising an adenovirus encoding a polypeptide antigen other than an adenovirus polypeptide, can comprise one or more of an adjuvant, a surfactant, a detergent, and a mucoadhesive, where suitable adjuvants, surfactants, detergents, and mucoadhesives are described elsewhere herein.

[00142] In some cases, an adenovirus composition containing antigens other than an adenoviral antigen, or comprising an adenovirus encoding a polypeptide antigen other than an adenovirus polypeptide, can comprise an immunostimulatory or an immunomodulatory agent, where suitable immunostimulatory and immunomodulatory agents are described elsewhere herein. In some cases, an adenovirus encoding a polypeptide antigen other than an adenovirus polypeptide can comprise a nucleotide sequence encoding an immunostimulatory polypeptide or an

immunomodulatory polypeptide, where suitable immunostimulatory and immunomodulatory polypeptides are described elsewhere herein.

[00143] In some cases, a mixture of different recombinant adenovirus vectors containing antigen and/or immunostimulatory sequences and/or immunomodulatory sequences can be mixed together for administration or administered at different sites simultaneously or sequentially.

[00144] In certain embodiments, an adenovirus composition containing antigens other than an adenoviral antigen, or comprising an adenovirus encoding a polypeptide antigen other than an adenovirus polypeptide, can be used in a prime-boost immunization regimen simultaneously or sequentially.

Combination therapy

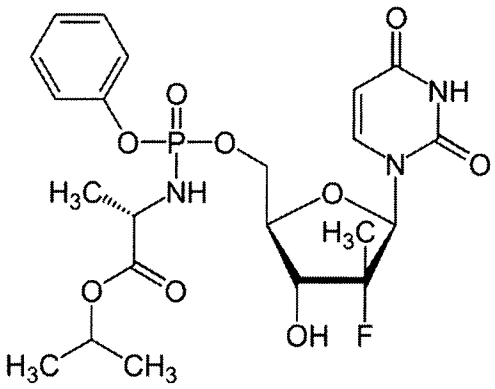
[00145] In some embodiments, a subject method of treating an HCV infection in an individual comprises: a) inducing an immune response in the individual to one or more HCV antigens, as described above; and b) administering at least one additional therapeutic agent that treats an HCV infection. In some embodiments, the at least one additional therapeutic agent is an anti-viral agent, e.g., an agent that has activity in inhibiting HCV or an agent that has activity in inhibiting a coinfecting pathogen (e.g., an agent that has activity in inhibiting HIV, HBV etc.).

[00146] In some embodiments, the at least one additional therapeutic agent is an anticancer agent such as chemotherapeutic cytotoxic agents, oncolytic viruses, anticancer therapeutic antibodies and vaccines, which can treat hepatocellular carcinoma and/or other cancers along with inducing immune responses against HCV.

[00147] In some embodiments, the at least one additional therapeutic agent is a combination of ombitasvir, paritaprevir, and ritonavir. In some embodiments, the at least one additional therapeutic agent is Solvaldi. In some embodiments, the at least one additional therapeutic agent is Harvoni. In some embodiments, the at least one additional therapeutic agent is Olysio. In some embodiments, the at least one additional therapeutic agent is an agent described in U.S. Patent Publication No. 2014/0309189 or 2014/0309164.

[00148] In some embodiments, the at least one additional therapeutic agent is an HCV polymerase inhibitor, ribavirin, viramidine, clemizole, filibuvir (PF-00868554), HCV POL, NM 283 (valopicitabine), MK-0608, 7-Fluoro-MK-0608, MK-3281, IDX-375, ABT-072, ABT-333, ANA598, BI 207127, GS 9190, PSI-6130, R1626, PSI-6206, PSI-938, PSI-7851, sofosbuvir (Sovaldi, PSI-7977, GS-7977), RG1479, RG7128, HCV-796 VCH-759 or VCH-916.

[00149] Sovaldi has the structure:



[00150] In some embodiments, the at least one additional therapeutic agent is a p38 MAPK inhibitor. Suitable p38 MAPK inhibitors include, e.g., SB203580 (4-(4-Fluorophenyl)-2-(4-methylsulfinylphenyl)-5-(4-pyridyl)-1H-imidazole).

[00151] In some embodiments, the at least one additional therapeutic agent includes interferon-alpha (IFN-α). Any known IFN-α can be used in a combination therapy. The term "interferon-alpha" as used herein refers to a family of related polypeptides that inhibit viral replication and cellular proliferation and modulate immune response. The term "IFN-α" includes naturally occurring IFN-α; synthetic IFN-α; derivatized IFN-α (e.g., PEGylated IFN-α, glycosylated IFN-α, and the like); and analogs of naturally occurring or synthetic IFN-α; essentially any IFN-α that has antiviral properties, as described for naturally occurring IFN-α.

[00152] Suitable alpha interferons include, but are not limited to, naturally-occurring IFN-α (including, but not limited to, naturally occurring IFN-α2a, IFN-α2b); recombinant interferon alpha-2b such as Intron-A interferon available from Schering Corporation, Kenilworth, N.J.; recombinant interferon alpha-2a such as Roferon interferon available from Hoffmann-La Roche, Nutley, N. J.; recombinant interferon alpha-2C such as Berofor alpha 2 interferon available from Boehringer Ingelheim Pharmaceutical, Inc., Ridgefield, Conn.; interferon alpha-n1, a purified blend of natural alpha interferons such as Sumiferon available from Sumitomo, Japan or as Wellferon interferon alpha-n1 (INS) available from the Glaxo-Wellcome Ltd., London, Great Britain; and interferon alpha-n3 a mixture of natural alpha interferons made by Interferon Sciences and available from the Purdue Frederick Co., Norwalk, Conn., under the Alferon Tradename.

[00153] The term "IFN-α" also encompasses consensus IFN-α. Consensus IFN-α (also referred to as "CIFN" and "IFN-con" and "consensus interferon") encompasses but is not limited to the amino acid sequences designated IFN-con$_1$, IFN-con$_2$ and IFN-con$_3$ which are disclosed in U.S. Pat. Nos. 4,695,623 and 4,897,471; and consensus interferon as defined by determination of a consensus sequence of naturally occurring interferon alphas (e.g., Infergen®, InterMune, Inc.,

Brisbane, Calif.). IFN-con_1 is the consensus interferon agent in the Infergen® alfacon-1 product. The Infergen® consensus interferon product is referred to herein by its brand name (Infergen®) or by its generic name (interferon alfacon-1). DNA sequences encoding IFN-con may be synthesized as described in the aforementioned patents or other standard methods.

[00154] The term "IFN-α" also encompasses derivatives of IFN-α that are derivatized (e.g., are chemically modified) to alter certain properties such as serum half-life. As such, the term "IFN-α" includes glycosylated IFN-α; IFN-α derivatized with polyethylene glycol ("PEGylated IFN-α"); and the like. PEGylated IFN-α, and methods for making same, is discussed in, e.g., U.S. Patent Nos. 5,382,657; 5,981,709; and 5,951,974. PEGylated IFN-α encompasses conjugates of PEG and any of the above-described IFN-α molecules, including, but not limited to, PEG conjugated to interferon alpha-2a (Roferon, Hoffman La-Roche, Nutley, N.J.), interferon alpha 2b (Intron, Schering-Plough, Madison, N.J.), interferon alpha-2c (Berofor Alpha, Boehringer Ingelheim, Ingelheim, Germany); and consensus interferon as defined by determination of a consensus sequence of naturally occurring interferon alphas (Infergen®, InterMune, Inc., Brisbane, Calif.).

[00155] Effective dosages of Infergen™ consensus IFN-α include about 3 μg, about 6 μg, about 9 μg, about 12 μg, about 15 μg, about 18 μg, about 21 μg, about 24 μg, about 27 μg, or about 30 μg, of drug per dose. Effective dosages of IFN-α2a and IFN-α2b range from 3 million Units (MU) to 10 MU per dose. Effective dosages of PEGASYS™PEGylated IFN-α2a contain an amount of about 90 μg to 270 μg, or about 180 μg, of drug per dose. Effective dosages of PEG-INTRON™ PEGylated IFN-α2b contain an amount of about 0.5 μg to 3.0 μg of drug per kg of body weight per dose. Effective dosages of PEGylated consensus interferon (PEG-CIFN) contain an amount of about 18 μg to about 90 μg, or from about 27 μg to about 60 μg, or about 45 μg, of CIFN amino acid weight per dose of PEG-CIFN. Effective dosages of monoPEG (30 kD, linear)-ylated CIFN contain an amount of about 45 μg to about 270 μg, or about 60 μg to about 180 μg, or about 90 μg to about 120 μg, of drug per dose. IFN-α can be administered daily, every other day, once a week, three times a week, every other week, three times per month, once monthly, substantially continuously or continuously.

[00156] In some embodiments, the at least one additional suitable therapeutic agent includes ribavirin. Ribavirin, 1-β-D-ribofuranosyl-1H-1,2,4-triazole-3-carboxamide, available from ICN Pharmaceuticals, Inc., Costa Mesa, Calif., is described in the Merck Index, compound No. 8199, Eleventh Edition. Its manufacture and formulation is described in U.S. Pat. No. 4,211,771. The invention also contemplates use of derivatives of ribavirin (see, *e.g.*, U.S. Pat. No. 6,277,830). The ribavirin may be administered orally in capsule or tablet form, or in the same or different administration form and in the same or different route as the adenovirus. Of course, other types

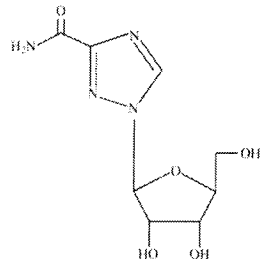
of administration are contemplated, such as by nasal spray, transdermally, by suppository, by sustained release dosage form, etc. Any suitable form of administration can be utilized so long as the proper dosages are delivered without destroying the active ingredient.

[00157] Ribavirin can be administered in an amount ranging from about 400 mg to about 1200 mg, from about 600 mg to about 1000 mg, or from about 700 to about 900 mg per day.

Levovirin

[00158] In some embodiments, the at least one additional suitable therapeutic agent includes levovirin. Levovirin is the L-enantiomer of ribavirin. Levovirin is manufactured by ICN Pharmaceuticals.

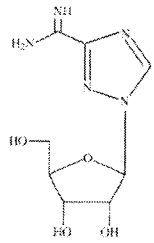
[00159] Levovirin has the following structure:



Viramidine

[00160] In some embodiments, the at least one additional suitable therapeutic agent includes viramidine. Viramidine is a 3-carboxamidine derivative of ribavirin, and acts as a prodrug of ribavirin. It is efficiently converted to ribavirin by adenosine deaminases.

[00161] Viramidine has the following structure:



[00162] Nucleoside analogs that are suitable for use in a subject combination therapy include, but are not limited to, ribavirin, levovirin, viramidine, isatoribine, an L-ribofuranosyl nucleoside as disclosed in U.S. Patent No. 5,559,101 and encompassed by Formula I of U.S. Patent No. 5,559,101 (e.g., 1-β-L-ribofuranosyluracil, 1-β-L-ribofuranosyl-5-fluorouracil, 1-β-L-ribofuranosylcytosine, 9-β-L-ribofuranosyladenine, 9-β-L-ribofuranosylhypoxanthine, 9-β-L-ribofuranosylguanine, 9-β-L-ribofuranosyl-6-thioguanine, 2-amino-α-L-ribofuranl[1',2':4,5]oxazoline, O^2,O^2-anhydro-1-α-L-ribofuranosyluracil, 1-α-L-ribofuranosyluracil, 1-(2,3,5-tri-O-benzoyl-α—ribofuranosyl)-4-thiouracil, 1-α-L-

ribofuranosylcytosine, 1-α-L-ribofuranosyl-4-thiouracil, 1-α-L-ribofuranosyl-5-fluorouracil, 2-amino-β-L-arabinofurano[1',2':4,5]oxazoline, O^2,O^2-anhydro-β-L-arabinofuranosyluracil, 2'-deoxy-β-L-uridine, 3'5'-Di-O-benzoyl-2'deoxy-4-thio β-L-uridine, 2'-deoxy-β-L-cytidine, 2'-deoxy-β-L-4-thiouridine, 2'-deoxy-β-L-thymidine, 2'-deoxy-β-L-5-fluorouridine, 2',3'-dideoxy-β-L-uridine, 2'-deoxy-β-L-5-fluorouridine, and 2'-deoxy-β-L-inosine); a compound as disclosed in U.S. Patent No. 6,423,695 and encompassed by Formula I of U.S. Patent No. 6,423,695; a compound as disclosed in U.S. Patent Publication No. 2002/0058635, and encompassed by Formula 1 of U.S. Patent Publication No. 2002/0058635; a nucleoside analog as disclosed in WO 01/90121 A2 (Idenix); a nucleoside analog as disclosed in WO 02/069903 A2 (Biocryst Pharmaceuticals Inc.); a nucleoside analog as disclosed in WO 02/057287 A2 or WO 02/057425 A2 (both Merck/Isis); and the like.

HCV NS3 inhibitors

[00163] In some embodiments, the at least one additional suitable therapeutic agent includes an HCV NS3 inhibitor. Suitable HCV non-structural protein-3 (NS3) inhibitors include, but are not limited to, a tri-peptide as disclosed in U.S. Patent Nos. 6,642,204, 6,534,523, 6,420,380, 6,410,531, 6,329,417, 6,329,379, and 6,323,180 (Boehringer-Ingelheim); a compound as disclosed in U.S. Patent No. 6,143,715 (Boehringer-Ingelheim); a macrocyclic compound as disclosed in U.S. Patent no. 6,608,027 (Boehringer-Ingelheim); an NS3 inhibitor as disclosed in U.S. Patent Nos. 6,617,309, 6,608,067, and 6,265,380 (Vertex Pharmaceuticals); an azapeptide compound as disclosed in U.S. Patent No. 6,624,290 (Schering); a compound as disclosed in U.S. Patent No. 5,990,276 (Schering); a compound as disclosed in Pause et al. (2003) *J. Biol. Chem.* 278:20374-20380; NS3 inhibitor BILN 2061 (Boehringer-Ingelheim; Lamarre et al. (2002) *Hepatology* 36:301A; and Lamarre et al. (Oct. 26, 2003) *Nature* doi:10.1038/nature02099); NS3 inhibitor VX-950 (Vertex Pharmaceuticals; Kwong et al. (Oct. 24-28, 2003) 54th Ann. Meeting AASLD); NS3 inhibitor SCH6 (Abib et al. (October 24-28, 2003) Abstract 137. Program and Abstracts of the 54th Annual Meeting of the American Association for the Study of Liver Diseases (AASLD). October 24-28, 2003. Boston, MA.); any of the NS3 protease inhibitors disclosed in WO 99/07733, WO 99/07734, WO 00/09558, WO 00/09543, WO 00/59929 or WO 02/060926 (e.g., compounds 2, 3, 5, 6, 8, 10, 11, 18, 19, 29, 30, 31, 32, 33, 37, 38, 55, 59, 71, 91, 103, 104, 105, 112, 113, 114, 115, 116, 120, 122, 123, 124, 125, 126 and 127 disclosed in the table of pages 224-226 in WO 02/060926); an NS3 protease inhibitor as disclosed in any one of U.S. Patent Publication Nos. 2003019067, 20030187018, and 20030186895; and the like.

[00164] In some cases, the at least one additional therapeutic agent includes NS3 inhibitors that are specific NS3 inhibitors, e.g., NS3 inhibitors that inhibit NS3 serine protease activity and that

do not show significant inhibitory activity against other serine proteases such as human leukocyte elastase, porcine pancreatic elastase, or bovine pancreatic chymotrypsin, or cysteine proteases such as human liver cathepsin B.

NS5B inhibitors

[00165] In some embodiments, the at least one additional suitable therapeutic agent includes an NS5B inhibitor. Suitable HCV non-structural protein-5 (NS5; RNA-dependent RNA polymerase) inhibitors include, but are not limited to, a compound as disclosed in U.S. Patent No. 6,479,508 (Boehringer-Ingelheim); a compound as disclosed in any of International Patent Application Nos. PCT/CA02/01127, PCT/CA02/01128, and PCT/CA02/01129, all filed on July 18, 2002 by Boehringer Ingelheim; a compound as disclosed in U.S. Patent No. 6,440,985 (ViroPharma); a compound as disclosed in WO 01/47883, e.g., JTK-003 (Japan Tobacco); a dinucleotide analog as disclosed in Zhong et al. (2003) *Antimicrob. Agents Chemother.* 47:2674-2681; a benzothiadiazine compound as disclosed in Dhanak et al. (2002) *J. Biol Chem.* 277(41):38322-7; an NS5B inhibitor as disclosed in WO 02/100846 A1 or WO 02/100851 A2 (both Shire); an NS5B inhibitor as disclosed in WO 01/85172 A1 or WO 02/098424 A1 (both Glaxo SmithKline); an NS5B inhibitor as disclosed in WO 00/06529 or WO 02/06246 A1 (both Merck); an NS5B inhibitor as disclosed in WO 03/000254 (Japan Tobacco); an NS5B inhibitor as disclosed in EP 1 256,628 A2 (Agouron); JTK-002 (Japan Tobacco); JTK-109 (Japan Tobacco); and the like.

[00166] Of particular interest in many embodiments are NS5 inhibitors that are specific NS5 inhibitors, e.g., NS5 inhibitors that inhibit NS5 RNA-dependent RNA polymerase and that lack significant inhibitory effects toward other RNA dependent RNA polymerases and toward DNA dependent RNA polymerases.

[00167] In some embodiments, the at least one additional therapeutic agent is a peptide, peptide mimetic or modified peptide, which inhibits the interaction of HCV proteins with host receptors.

[00168] In some cases, a method of the present disclosure of inducing immune responses against HCV antigens comprises administering an adenoviral composition to an individual in need thereof, and further comprising administering to the individual an effective amount of at least one additional therapeutic agent, e.g., a monoclonal antibody directed against negative receptors such as PD1 and CTLA-4; antibody directed against co-stimulatory receptors e.g., CD134 and CD137; CDP-860 (anti-CD18), antibody directed against cytokines such as IL-10 and TGF-b, antibody directed against apoptotic cells e.g., anti-phosphatidylserine Mab and the like.

[00169] In some cases, an adenovirus can be engineered to express siRNA, shRNA or antisense DNA against a pathogen to inhibit the replication of pathogens such as HIV, HBV, HCV etc., along with providing immunotherapy to induce HCV specific immune responses.

[00170] In some embodiments, the at least one additional therapeutic agent is an antiviral agent e.g., an agent that has activity in inhibiting HIV, HBV influenza, etc. In some embodiments, the at least one additional therapeutic agent is an anticancer agent.

[00171] In some embodiments, the methods of present disclosure can be used to induce immune responses in humans. In some embodiments, the methods of present disclosure can be used to induce immune responses in non-human mammals, such as mouse, rat, donkey, rabbit, monkey, dogs, or cats. Delivery to non-human mammals can be for therapeutic purposes. Delivery to non-human mammals can be for use in an experimental context, for instance examining the mechanisms of inducing HCV specific immune responses and modulation of immune responses etc.

[00172] In some embodiments, the methods of present disclosure can be used to enhance and/or modify the therapeutic and protective effects and/or reduce frequency and dosing of therapeutic agent and/or vaccine.

FORMULATIONS, DOSAGES, AND ROUTES OF ADMINISTRATION

[00173] An adenovirus composition to be administered according to a method of the present disclosure can include one or more pharmaceutically acceptable excipients; and can be formulated in any of a variety of ways, that may depend, e.g., on the route of administration. Pharmaceutically acceptable excipients are known to those skilled in the art, and have been amply described in a variety of publications, including, for example, A. Gennaro (1995) "Remington: The Science and Practice of Pharmacy", 19th edition, Lippincott, Williams, & Wilkins. Suitable excipient vehicles include, for example, water, saline, dextrose, glycerol, ethanol, or the like, and combinations thereof. In addition, if desired, the vehicle may contain minor amounts of auxiliary substances such as wetting or emulsifying agents or pH buffering agents. Actual methods of preparing such dosage forms are known, or will be apparent, to those skilled in the art. See, e.g., Remington's Pharmaceutical Sciences, Mack Publishing Company, Easton, Pennsylvania, 17th edition, 1985; Remington: The Science and Practice of Pharmacy, A.R. Gennaro, (2000) Lippincott, Williams & Wilkins.

[00174] An adenovirus composition to be administered according to a method of the present disclosure can be incorporated into a variety of formulations for administration. More particularly, an adenovirus composition can be formulated into pharmaceutical compositions by combination with appropriate, pharmaceutically acceptable carriers or diluents, and may be

formulated into preparations in solid, semi-solid, freeze-dried, liquid or gaseous forms, such as tablets, capsules, powders, granules, ointments, solutions, suppositories, injections, skin patches, inhalants and aerosols.

[00175] In pharmaceutical dosage forms, an adenovirus composition may be administered alone or in appropriate association, as well as in combination, with other pharmaceutically active compounds. An adenovirus composition, an antigen, adjuvant and/or therapeutic drug can be administered concurrently, simultaneously, sequentially or at different times and via different routes. The following methods and excipients are merely exemplary and are in no way limiting.

[00176] For oral preparations, an adenovirus composition can be used alone or in combination with appropriate additives to make tablets, powders, granules or capsules, for example, with conventional additives, such as lactose, mannitol, corn starch or potato starch; with binders, such as crystalline cellulose, cellulose derivatives, acacia, corn starch or gelatins; with disintegrators, such as corn starch, potato starch or sodium carboxymethylcellulose; with lubricants, such as talc or magnesium stearate; and if desired, with diluents, buffering agents, moistening agents, preservatives and flavoring agents.

[00177] An adenovirus composition can be formulated into preparations for injection by dissolving, suspending or emulsifying the composition in an aqueous or nonaqueous solvent, such as vegetable or other similar oils, synthetic aliphatic acid glycerides, esters of higher aliphatic acids or propylene glycol; and if desired, with conventional additives such as solubilizers, isotonic agents, suspending agents, emulsifying agents, stabilizers and preservatives.

[00178] An adenovirus composition can be utilized in aerosol formulation to be administered via inhalation. An adenovirus composition to be administered according to a method of the present disclosure can be formulated into pressurized acceptable propellants such as dichlorodifluoromethane, propane, nitrogen and the like.

[00179] Furthermore, an adenovirus composition can be made into suppositories by mixing with a variety of bases such as emulsifying bases or water-soluble bases. An adenovirus composition can be administered rectally via a suppository. The suppository can include vehicles such as cocoa butter, carbowaxes and polyethylene glycols, which melt at body temperature, yet are solidified at room temperature.

[00180] An adenovirus composition to be administered according to a method of the present disclosure can also be administered in the form of liposomes. Liposomes can be given by a variety of routes, oral, nasal, parenteral, trans-dermal, inhalation etc. As is known in the art, liposomes are derived from phospholipids or other lipid substances. Liposomes are formed by

mono- or multilamellar hydrated liquid crystals that are dispersed in an aqueous medium. Any non-toxic, physiologically acceptable and metabolizable lipid capable of forming liposomes can be used. An adenovirus composition in liposome form can contain, in addition to an adenovirus composition, one or more of a stabilizer, a preservative, an excipients, and the like. Exemplary lipids are the phospholipids and the phosphatidylcholines (lecithins), both natural and synthetic. Methods to form liposomes are known in the art. for example, Prescott, Ed., Methods in Cell Biology, Volume XIV, Academic Press, New York, N.Y. (1976), p. 33 et seq.

[00181] An adenovirus composition to be administered according to a method of the present disclosure can also be administered in the form of microspheres, nanoparticles etc.

[00182] Unit dosage forms for oral or rectal administration such as syrups, elixirs, and suspensions may be provided wherein each dosage unit, for example, teaspoonful, tablespoonful, tablet or suppository, contains a predetermined amount of the composition containing one or more active agents. Similarly, unit dosage forms for injection or intravenous administration may comprise an adenovirus composition as a solution in sterile water, normal saline or another pharmaceutically acceptable carrier.

[00183] An adenovirus composition to be administered according to a method of the present disclosure can be formulated for topical administration. Topical administration includes administration to the skin or mucosa, including surfaces of the lung eye, nose, and ear. Suitable topical preparations include, e.g., skin patch preparation, transdermal patch preparation, cream, lotion, gel preparations, powder, ointment, paste, intranasal drops or gels.

[00184] Ointments are semi-solid preparations, which are typically based on petrolatum or other petroleum derivatives. Suitable ointments include oleaginous bases; emulsifiable bases; emulsion bases; and water-soluble bases. Oleaginous ointment bases include, for example, vegetable oils, fats obtained from animals, and semisolid hydrocarbons obtained from petroleum. Emulsifiable ointment bases, also known as absorbent ointment bases, contain little or no water and include, for example, hydroxystearin sulfate, anhydrous lanolin and hydrophilic petrolatum. Emulsion ointment bases are either water-in-oil (WIO) emulsions or oil-in-water (OIW) emulsions, and include, for example, cetyl alcohol, glyceryl monostearate, lanolin and stearic acid. Exemplary water-soluble ointment bases are prepared from polyethylene glycols of varying molecular weight.

[00185] Lotions are preparations to be applied to the skin surface without friction, and are typically liquid or semi liquid preparations in which solid particles, including the active agent, are present in a water or alcohol base. Lotions are usually suspensions of solids, and preferably, for the present purpose, comprise a liquid oily emulsion of the oil-in-water type. Lotions can be

used for treating large body areas, because of the ease of applying a more fluid composition. Lotions may contain suspending agents to produce better dispersions as well as compounds useful for localizing and holding the active agent in contact with the skin, e.g., methyl cellulose, sodium carboxymethyl-cellulose, or the like. An example of a lotion formulation for use in conjunction with the present invention contains propylene glycol mixed with a hydrophilic petrolatum such as that which may be obtained under the trademark Aquaphor® from Beiersdorf, Inc. (Norwalk, Coon.).

[00186] Suitable creams can be viscous liquid or semisolid emulsions, either oil-in-water or water-in-oil. Cream bases are water-washable, and contain an oil phase, an emulsifier and an aqueous phase. The oil phase, also sometimes called the "internal" phase, is generally comprised of petrolatum and a fatty alcohol such as cetyl or stearyl alcohol; the aqueous phase usually, although not necessarily, exceeds the oil so phase in volume, and generally contains a humectant. The emulsifier in a cream formulation, as explained in Remington, supra, is generally a nonionic, anionic, cationic or amphoteric surfactant.

[00187] Gels formulations can be used. Gels are semisolid, suspension-/type systems. Single-phase gels contain organic macromolecules distributed substantially uniformly throughout the carrier liquid, which can be aqueous, but may also contain an alcohol and, optionally, an oil.

[00188] A topical formulation may also be delivered to the skin using conventional "transdermal"-type patches, wherein the agent (adenovirus composition) is contained within a laminated structure that serves as a delivery device to be affixed to the skin. In such a structure, the adenovirus composition is contained in a layer, or "reservoir," underlying an upper backing layer. The laminated structure may contain a single reservoir, or it may contain multiple reservoirs. In one embodiment, the reservoir comprises a polymeric matrix of a pharmaceutically acceptable contact adhesive material that serves to affix the system to the skin during drug delivery. Examples of suitable skin contact adhesive materials include, but are not limited to, polyethylenes, polysioxanes, polyisobutylenes, polyacrylates, polyurethanes, and the like. The particular polymeric adhesive selected will depend on the particular adenovirus composition, vehicle, etc., i.e., the adhesive must be compatible with all components of the drug-containing composition. In an alternative embodiment, the adenovirus composition-containing reservoir and skin contact adhesive are present as separate and distinct layers, with the adhesive underlying the reservoir which, in this case, may be either a polymeric matrix as described above, or it may be a liquid or hydrogel reservoir, or may take some other form.

[00189] The term "unit dosage form," as used herein, refers to physically discrete units suitable as unitary dosages for human and animal subjects, each unit containing a predetermined quantity of an active agent (e.g., adenovirus composition) calculated in an amount sufficient to produce

the desired effect in association with a pharmaceutically acceptable diluent, carrier or vehicle. The specifications for the active agents depend on the particular compound employed and the effect to be achieved, and the pharmacodynamics associated with each compound in the host.

[00190] Other modes of administration will also find use. For instance, an adenovirus composition can be formulated in suppositories and, in some cases, aerosol and intranasal compositions. For suppositories, the vehicle composition will include traditional binders and carriers such as, polyalkylene glycols, or triglycerides. Such suppositories may be formed from mixtures containing the active ingredient in the range of about 0.5% to about 10% (w/w), or about 1% to about 2%.

[00191] Intranasal formulations will usually include vehicles that neither cause irritation to the nasal mucosa nor significantly disturb ciliary function. Diluents such as water, aqueous saline or other known substances can be employed with the subject invention. The nasal formulations may also contain preservatives such as, but not limited to, chlorobutanol and benzalkonium chloride. A surfactant may be present to enhance absorption of the subject proteins by the nasal mucosa.

[00192] An adenovirus composition to be administered according to a method of the present disclosure can be administered as an injectable formulation. For example, injectable compositions are prepared as liquid solutions or suspensions; solid forms suitable for solution in, or suspension in, liquid vehicles prior to injection may also be prepared. The preparation may also be emulsified or the active ingredient encapsulated in liposome vehicles.

[00193] Suitable excipient vehicles are, for example, water, saline, dextrose, glycerol, ethanol, or the like, and combinations thereof. In addition, if desired, the vehicle may contain minor amounts of auxiliary substances such as wetting or emulsifying agents or pH buffering agents. Actual methods of preparing such dosage forms are known, or will be apparent, to those skilled in the art. See, e.g., Remington's Pharmaceutical Sciences, Mack Publishing Company, Easton, Pennsylvania, 17th edition, 1985; Remington: The Science and Practice of Pharmacy, A.R. Gennaro, (2000) Lippincott, Williams & Wilkins. The composition or formulation to be administered will, in any event, contain a quantity of an active agent (e.g., HKCC; antigen; etc.) adequate to achieve the desired state in the subject being treated.

[00194] The pharmaceutically acceptable excipients, such as vehicles, adjuvants, carriers or diluents, are readily available to the public. Moreover, pharmaceutically acceptable auxiliary substances, such as pH adjusting and buffering agents, tonicity adjusting agents, stabilizers, wetting agents and the like, are readily available to the public.

Oral formulations

[00195] In some embodiments, an adenovirus composition is formulated for oral delivery to an individual in need of such a composition.

[00196] For oral delivery, a formulation comprising an adenovirus composition will in some embodiments include an enteric-soluble coating material. Suitable enteric-soluble coating material include hydroxypropyl methylcellulose acetate succinate (HPMCAS), hydroxypropyl methyl cellulose phthalate (HPMCP), cellulose acetate phthalate (CAP), polyvinyl phthalic acetate (PVPA), Eudragit™, and shellac.

[00197] Suitable oral formulations also include an adenovirus composition, formulated with any of the following: microgranules (see, e.g., U.S. Patent No. 6,458,398); biodegradable macromers (see, e.g., U.S. Patent No. 6,703,037); biodegradable hydrogels (see, e.g., Graham and McNeill (1989) *Biomaterials* 5:27-36); biodegradable particulate vectors (see, e.g., U.S. Patent No. 5,736,371); bioabsorbable lactone polymers (see, e.g., U.S. Patent No. 5,631,015); slow release protein polymers (see, e.g., U.S. Patent No. 6,699,504; Pelias Technologies, Inc.); a poly(lactide-co-glycolide/polyethylene glycol block copolymer (see, e.g., U.S. Patent No. 6,630,155; Atrix Laboratories, Inc.); a composition comprising a biocompatible polymer and particles of metal cation-stabilized agent dispersed within the polymer (see, e.g., U.S. Patent No. 6,379,701; Alkermes Controlled Therapeutics, Inc.); and microspheres (see, e.g., U.S. Patent No. 6,303,148; Octoplus, B.V.).

[00198] Suitable oral formulations also include an adenovirus composition formulated with any of the following: a carrier such as Emisphere® (Emisphere Technologies, Inc.); TIMERx, a hydrophilic matrix combining xanthan and locust bean gums which, in the presence of dextrose, form a strong binder gel in water (Penwest); Geminex™ (Penwest); Procise™ (GlaxoSmithKline); SAVIT™ (Mistral Pharma Inc.); RingCap™ (Alza Corp.); Smartrix® (Smartrix Technologies, Inc.); SQZgel™ (MacroMed, Inc.); Geomatrix™ (Skye Pharma, Inc.); Oros® Tri-layer (Alza Corporation); and the like.

[00199] Also suitable for use are formulations such as those described in U.S. Patent No. 6,296,842 (Alkermes Controlled Therapeutics, Inc.); U.S. Patent No. 6,187,330 (Scios, Inc.); and the like.

[00200] Also suitable for use herein are formulations comprising an intestinal absorption enhancing agent. Suitable intestinal absorption enhancers include, but are not limited to, calcium chelators (e.g., citrate, ethylenediamine tetracetic acid); surfactants (e.g., sodium dodecyl sulfate, bile salts, palmitoylcarnitine, and sodium salts of fatty acids); toxins (e.g., zonula occludens toxin); and the like.

[00201] Suitable oral formulations also include an adenovirus composition, formulated as a food supplement (e.g. nutraceuticals, yogurt, bars) etc.

Controlled release formulations

[00202] In some embodiments, an adenovirus composition is formulated in a controlled release formulation.

[00203] Controlled release can be taken to mean any one of a number of extended release dosage forms. The following terms may be considered to be substantially equivalent to controlled release, for the purposes of the present invention: continuous release, controlled release, delayed release, depot, gradual release, long-term release, programmed release, prolonged release, proportionate release, protracted release, repository, retard, slow release, spaced release, sustained release, time coat, timed release, delayed action, extended action, layered-time action, long acting, prolonged action, repeated action, slowing acting, sustained action, sustained-action medications, and extended release. Further discussions of these terms may be found in Lesczek Krowczynski, Extended-Release Dosage Forms, 1987 (CRC Press, Inc.).

[00204] The various controlled release technologies cover a very broad spectrum of drug dosage forms. Controlled release technologies include, but are not limited to physical systems and chemical systems.

[00205] Physical systems include, but are not limited to, reservoir systems with rate-controlling membranes, such as microencapsulation, macroencapsulation, and membrane systems; reservoir systems without rate-controlling membranes, such as hollow fibers, ultra microporous cellulose triacetate, and porous polymeric substrates and foams; monolithic systems, including those systems physically dissolved in non-porous, polymeric, or elastomeric matrices (e.g., nonerodible, erodible, environmental agent ingression, and degradable), and materials physically dispersed in non-porous, polymeric, or elastomeric matrices (e.g., nonerodible, erodible, environmental agent ingression, and degradable); laminated structures, including reservoir layers chemically similar or dissimilar to outer control layers; and other physical methods, such as osmotic pumps, or adsorption onto ion-exchange resins.

[00206] Chemical systems include, but are not limited to, chemical erosion of polymer matrices (e.g., heterogeneous, or homogeneous erosion), or biological erosion of a polymer matrix (e.g., heterogeneous, or homogeneous). Additional discussion of categories of systems for controlled release may be found in Agis F. Kydonieus, Controlled Release Technologies: Methods, Theory and Applications, 1980 (CRC Press, Inc.).

[00207] There are a number of controlled release drug formulations that are developed for oral administration. These include, but are not limited to, osmotic pressure-controlled gastrointestinal

delivery systems; hydrodynamic pressure-controlled gastrointestinal delivery systems; membrane permeation-controlled gastrointestinal delivery systems, which include microporous membrane permeation-controlled gastrointestinal delivery devices; gastric fluid-resistant intestine targeted controlled-release gastrointestinal delivery devices; gel diffusion-controlled gastrointestinal delivery systems; and ion-exchange-controlled gastrointestinal delivery systems, which include cationic and anionic drugs. Additional information regarding controlled release drug delivery systems may be found in Yie W. Chien, Novel Drug Delivery Systems, 1992 (Marcel Dekker, Inc.). Some of these formulations will now be discussed in more detail.

[00208] Enteric coatings are applied to tablets to prevent the release of active agents in the stomach either to reduce the risk of unpleasant side effects or to maintain the stability of the drug which might otherwise be subject to degradation of expose to the gastric environment. Most polymers that are used for this purpose are polyacids that function by virtue or the fact that their solubility in aqueous medium is pH-dependent, and they require conditions with a pH higher than normally encountered in the stomach.

[00209] One exemplary type of oral controlled release structure is enteric coating of a solid or liquid dosage form. The enteric coatings are designed to disintegrate in intestinal fluid for ready absorption. Delay of absorption of the active agent that is incorporated into a formulation with an enteric coating is dependent on the rate of transfer through the gastrointestinal tract, and so the rate of gastric emptying is an important factor. Some investigators have reported that a multiple-unit type dosage form, such as granules, may be superior to a single-unit type.

[00210] Suitable enteric coating agents include, but are not limited to, hydroxypropylmethylcellulose phthalate, methacryclic acid-methacrylic acid ester copolymer, polyvinyl acetate-phthalate and cellulose acetate phthalate.

[00211] Another type of useful oral controlled release structure is a solid dispersion. A solid dispersion may be defined as a dispersion of one or more active ingredients in an inert carrier or matrix in the solid state prepared by the melting (fusion), solvent, or melting-solvent method.

[00212] Examples of carriers useful in solid dispersions include, but are not limited to, water-soluble polymers such as polyethylene glycol, polyvinylpyrrolidone, and hydroxypropylmethyl cellulose. Alternative carriers include phosphatidylcholine. Phosphatidylcholine is an amphoteric but water-insoluble lipid, which may improve the solubility of otherwise insoluble active agents in an amorphous state in phosphatidylcholine solid dispersions.

[00213] Other carriers include polyoxyethylene hydrogenated castor oil. An adenovirus composition can be included in a solid dispersion system with an enteric polymer such as hydroxypropylmethylcellulose phthalate and carboxymethylethylcellulose, and a non-enteric

polymer, hydroxypropylmethylcellulose. Another solid dispersion dosage form includes incorporation of the drug of interest (e.g., an active agent) with ethyl cellulose and stearic acid in different ratios.

[00214] There are various methods commonly known for preparing solid dispersions. These include, but are not limited to, the melting method, the solvent method and the melting-solvent method.

[00215] Injectable microspheres are another controlled release dosage form. Injectable micro spheres may be prepared by non-aqueous phase separation techniques, and spray-drying techniques. Microspheres may be prepared using polylactic acid or copoly(lactic/glycolic acid).

[00216] Other controlled release technologies that may be used include, but are not limited to, SODAS (Spheroidal Oral Drug Absorption System), INDAS (Insoluble Drug Absorption System), IPDAS (Intestinal Protective Drug Absorption System), MODAS (Multiporous Oral Drug Absorption System), EFVAS (Effervescent Drug Absorption System), PRODAS (Programmable Oral Drug Absorption System), and DUREDAS (Dual Release Drug Absorption System) available from Elan Pharmaceutical Technologies. SODAS are multi particulate dosage forms utilizing controlled release beads. INDAS are a family of drug delivery technologies designed to increase the solubility of poorly soluble drugs. IPDAS are multi particulate tablet formation utilizing a combination of high density controlled release beads and an immediate release granulate. MODAS are controlled release single unit dosage forms. Each tablet consists of an inner core surrounded by a semipermeable multiparous membrane that controls the rate of drug release. EFVAS is an effervescent drug absorption system. PRODAS is a family of multi particulate formulations utilizing combinations of immediate release and controlled release mini-tablets. DUREDAS is a bilayer tablet formulation providing dual release rates within the one dosage form. Although these dosage forms are known to one of skill, certain of these dosage forms will now be discussed in more detail.

[00217] An adenovirus composition can be incorporated into any one of the aforementioned controlled released dosage forms, or other conventional dosage forms. The amount of active agent contained in each dose can be adjusted, to meet the needs of the individual patient, and the indication. One of skill in the art and reading this disclosure will readily recognize how to adjust the level of an active agent and the release rates in a controlled release formulation, in order to optimize delivery of an active agent and its bioavailability.

Inhalational formulations

[00218] An adenovirus composition to be administered according to a method of the present disclosure will in some embodiments be administered to a patient by means of a pharmaceutical

delivery system for the inhalation route. The adenovirus composition may be formulated in a form suitable for administration by inhalation. The inhalational route of administration provides the advantage that the inhaled drug can bypass the blood-brain barrier. The pharmaceutical delivery system is one that is suitable for respiratory therapy by delivery of an active agent to mucosal linings of the bronchi. A system that depends on the power of a compressed gas to expel the adenovirus composition from a container can also be used. An aerosol or pressurized package can be employed for this purpose.

[00219] As used herein, the term "aerosol" is used in its conventional sense as referring to very fine liquid or solid particles carries by a propellant gas under pressure to a site of therapeutic application. When a pharmaceutical aerosol is employed, the aerosol contains the therapeutically active compound (e.g., active agent), which can be dissolved, suspended, or emulsified in a mixture of a fluid carrier and a propellant. The aerosol can be in the form of a solution, suspension, emulsion, powder, or semi-solid preparation. Aerosols can be used for administration as fine, solid particles or as liquid mists via the respiratory tract of a patient. Various types of propellants known to one of skill in the art can be utilized. Suitable propellants include, but are not limited to, hydrocarbons or other suitable gas. In the case of the pressurized aerosol, the dosage unit may be determined by providing a value to deliver a metered amount.

[00220] An adenovirus composition can also be formulated for delivery with a nebulizer, which is an instrument that generates very fine liquid particles of substantially uniform size in a gas. For example, a liquid containing the adenovirus composition is dispersed as droplets. The small droplets can be carried by a current of air through an outlet tube of the nebulizer. The resulting mist penetrates into the respiratory tract of the patient.

[00221] There are several different types of inhalation methodologies which can be employed in connection with an adenovirus composition to be administered according to a method of the present disclosure. An adenovirus composition can be formulated with low boiling point propellants. Such formulations are generally administered by conventional meter dose inhalers (MDI's). Alternatively, an adenovirus composition can be formulated in aqueous or ethanolic solutions and delivered by conventional nebulizers. In some embodiments, such solution formulations are aerosolized using devices and systems such as disclosed within U.S. Patent 5,497,763; 5,544,646; 5,718,222; and 5,660,166. An adenovirus composition can be formulated into dry powder formulations. Such formulations can be administered by simply inhaling the dry powder formulation after creating an aerosol mist of the powder. Technology for carrying such out is described within U.S. Patent 5,775,320 issued July 7, 1998 and U.S. Patent 5,740,794 issued April 21, 1998.

[00222] An adenovirus composition to be administered according to a method of the present disclosure will in some embodiments be formulated for vaginal delivery. An adenovirus composition for intravaginal administration can be formulated as an intravaginal bioadhesive tablet, intravaginal bioadhesive microparticle, intravaginal cream, intravaginal lotion, intravaginal foam, intravaginal ointment, intravaginal paste, intravaginal solution, or intravaginal gel.

[00223] An adenovirus composition will in some embodiments be formulated for rectal delivery. A formulation for intrarectal administration comprises an adenovirus composition formulated as an intrarectal bioadhesive tablet, intrarectal bioadhesive microparticle, intrarectal cream, intrarectal lotion, intrarectal foam, intrarectal ointment, intrarectal paste, intrarectal solution, or intrarectal gel.

[00224] An adenovirus composition can include one or more of an excipient (e.g., sucrose, starch, mannitol, sorbitol, lactose, glucose, cellulose, talc, calcium phosphate or calcium carbonate), a binder (e.g., cellulose, methylcellulose, hydroxymethylcellulose, polypropylpyrrolidone, polyvinylpyrrolidone, gelatin, gum arabic, poly(ethylene glycol), sucrose or starch), a disintegrator (e.g., starch, carboxymethylcellulose, hydroxypropyl starch, low substituted hydroxypropylcellulose, sodium bicarbonate, calcium phosphate or calcium citrate), a lubricant (e.g., magnesium stearate, light anhydrous silicic acid, talc or sodium lauryl sulfate), a flavoring agent (e.g., citric acid, menthol, glycine or orange powder), a preservative (e.g., sodium benzoate, sodium bisulfite, methylparaben or propylparaben), a stabilizer (e.g., citric acid, sodium citrate or acetic acid), a suspending agent (e.g., methylcellulose, polyvinylpyrrolidone or aluminum stearate), a dispersing agent (e.g., hydroxypropylmethylcellulose), a diluent (e.g., water), and base wax (e.g., cocoa butter, white petrolatum or polyethylene glycol).

[00225] Tablets comprising an adenovirus composition may be coated with a suitable film-forming agent, e.g., hydroxypropylmethyl cellulose, hydroxypropyl cellulose or ethyl cellulose, to which a suitable excipient may optionally be added, e.g., a softener such as glycerol, propylene glycol, diethylphthalate, or glycerol triacetate; a filler such as sucrose, sorbitol, xylitol, glucose, or lactose; a colorant such as titanium hydroxide; and the like.

Dosages

[00226] The dosage of an adenovirus composition to be administered according to a method of the present disclosure can vary, depending on factors such as the clinical goals to be achieved, the age of the individual being treated, the physical status of the individual being treated, etc.

[00227] An adenovirus composition to be administered according to a method of the present disclosure can comprise adenovirus in an amount of from about 10^3 genome copies per unit dosage form to about 10^{20} genome copies per unit dosage form. For example, an adenovirus composition can comprise adenovirus in an amount of from about 10^3 genome copies per unit dosage form to about 10^4 genome copies per unit dosage form, from about 10^4 genome copies per unit dosage form to about 10^5 genome copies per unit dosage form, from about 10^5 genome copies per unit dosage form to about 10^6 genome copies per unit dosage form, from about 10^6 genome copies per unit dosage form to about 10^7 genome copies per ml, from about 10^8 genome copies per unit dosage form to about 10^9 genome copies per unit dosage form, from about 10^9 genome copies per ml to about 10^{10} genome copies per unit dosage form, from about 10^{15} genome copies per unit dosage form to about 10^{20} genome copies per unit dosage form, or more than 10^{20} genome copies per unit dosage form.

[00228] For example, an adenovirus composition can comprise adenovirus in an amount of from about 10^3 genome copies per ml to about 10^{20} genome copies per ml. For example, an adenovirus composition can comprise genome copies in an amount of from about 10^3 genome copies per ml to about 10^4 genome copies per ml, from about 10^4 genome copies per ml to about 10^5 genome copies per ml, from about 10^5 genome copies per ml to about 10^6 genome copies per ml, from about 10^6 genome copies per ml to about 10^7 genome copies per ml, from about 10^8 genome copies per ml to about 10^9 genome copies per ml, from about 10^9 genome copies per ml to about 10^{10} genome copies per ml, from about 10^{15} genome copies per ml to about 10^{20} genome copies per ml, or more than 10^{20} genome copies per ml.

[00229] In some embodiments, multiple doses of an adenovirus composition are administered. The frequency of administration of an adenovirus composition can vary depending on any of a variety of factors, e.g., severity of the symptoms, etc. For example, in some embodiments, an adenovirus composition is administered once per month, twice per month, three times per month, every other week (qow), once per week (qw), twice per week (biw), three times per week (tiw), four times per week, five times per week, six times per week, every other day (qod), daily (qd), twice a day (qid), or three times a day (tid).

[00230] The duration of administration of an adenovirus composition, e.g., the period of time over which an adenovirus composition is administered, can vary, depending on any of a variety of factors, e.g., patient response, etc. For example, an adenovirus composition can be administered over a period of time ranging from about one hour to one day, from about one day to about one week, from about two weeks to about four weeks, from about one month to about two months, from about two months to about four months, from about four months to about six

months, from about six months to about eight months, from about eight months to about 1 year, from about 1 year to about 2 years, or from about 2 years to about 4 years, or more.

[00231] In some embodiments, an adenovirus composition as described herein is administered with at least one additional agent (e.g., an additional immunogen, a therapeutic agent, an adjuvant etc.) as a single dose or in multiple doses, simultaneously or sequentially. Where an adenovirus composition and at least one additional agent are administered in multiple doses, the adenovirus composition and the at least one additional agent can be administered 1 minute apart, 1 day apart, 1 week apart, 2 weeks apart, 4 weeks apart, 6 weeks apart, 8 weeks apart, 10 weeks apart, 12 weeks apart, or more than 12 weeks apart.

Routes of administration

[00232] An adenovirus composition is administered to an individual using any available method and route suitable for drug delivery, including *in vivo* and *ex vivo* methods, as well as systemic and localized routes of administration.

[00233] Conventional and pharmaceutically acceptable routes of administration include intranasal, intramuscular, intratracheal, subcutaneous, intradermal, intranodal, percutaneous, transdermal, intratumoral, topical application, intravenous, rectal, nasal, oral and other enteral and parenteral routes of administration. Routes of administration may be combined, if desired, or adjusted depending upon the agent and/or the desired effect. The composition can be administered in a single dose or in multiple doses.

[00234] An adenovirus composition can be administered to a host using any available conventional methods and routes suitable for delivery of conventional drugs, including systemic or localized routes. In general, routes of administration contemplated by the invention include, but are not necessarily limited to, enteral, parenteral, or inhalational routes.

[00235] Parenteral routes of administration other than inhalation administration include, but are not necessarily limited to, topical, transdermal, subcutaneous, intramuscular, intraorbital, intracapsular, intraspinal, intrasternal, intracranial, and intravenous routes, *i.e.*, any route of administration other than through the alimentary canal. Parenteral administration can be carried to effect systemic or local delivery of the adenovirus composition. Where systemic delivery is desired, administration typically involves invasive or systemically absorbed topical or mucosal administration of pharmaceutical preparations.

[00236] An adenovirus composition can also be delivered to the subject by enteral administration. Enteral routes of administration include, but are not necessarily limited to, oral and rectal (*e.g.*, using a suppository) delivery.

[00237] An adenovirus composition can also be delivered to the subject via a mucosal route of delivery. Mucosal routes of delivery include nasal, buccal, sublingual, vaginal, ocular, and rectal routes of administration.

[00238] In certain embodiments, an adenovirus composition is administered to a subject via a combination of different routes in the order indicated below:

[00239] i. systemic, mucosal;

[00240] ii. systemic, systemic, mucosal, mucosal;

[00241] iii. systemic, mucosal, systemic;

[00242] iv. mucosal, mucosal, systemic, systemic;

[00243] v. mucosal, systemic, systemic;

[00244] vi. mucosal, systemic, mucosal, for example.

[00245] When an adenovirus composition is administered systemically or mucosally more than once, the two or more systemic or mucosal administrations may be by the same systemic (for example, two intramuscular injections) or mucosal route (two IN/SL administrations) or different (for example, one intramuscular injection and one intravenous injection; one IN administration and one SL administration).

[00246] An adenovirus composition is administered to an individual using any available method, delivery or device such as vaccine patches, needles, microneedles, drop, syrup, tablets, capsules, pipette, dose-spray pumps, nasal dropper, inhalation devices, liquid or dry powder, freeze-dried powder, suspensions or solutions, spray devices, Accuspray™, thermoresponsive gels, jet injectors, Biojector™, Nasovak™, Bespak™, ointment, lotions, suppositories, gels etc.

[00247] In some embodiments, an adenovirus composition of the present disclosure may, if desired, be presented in a kit, pack or dispenser which may contain one or more unit dose forms containing the active ingredient. The kit may contain an adjuvant, a device for delivering the vaccine to a host.

SUBJECTS SUITABLE FOR TREATMENT

Methods of inducing an immune response to one or more HCV antigens

[00248] Individuals who are suitable for treatment with method of inducing an immune response to one or more HCV antigens include individuals who are not infected with HCV. For example, a method of the present disclosure of inducing an immune response can be utilized as a prophylactic vaccine to induce immunity in an individual against HCV, such that upon subsequent exposure to HCV, induced preexisting immunity would reduce the likelihood that an HCV infection would be established in the individual. Individuals that are suitable for treatment with method of inducing an immune response to one or more HCV antigens include individuals

who are at greater risk than the general population of becoming infected with HCV, where such individuals include intravenous drug users, medical personnel who come into contact with HCV-infected individuals, and the like. Individuals who are suitable for treatment with method of inducing an immune response to one or more HCV antigens include prospective liver transplant recipients.

Methods of treating an HCV infection

[00249] Individuals that are suitable for treatment with method of treating an HCV infection of the present disclosure include individuals who have been diagnosed with an HCV infection. Any of the above treatment regimens can be administered to individuals who have been diagnosed with an HCV infection. Any of the above treatment regimens can be administered to individuals who have failed previous treatment for HCV infection ("treatment failure patients," including non-responders and relapsers).

[00250] In some cases, individuals have an HCV titer of at least about 10^5, at least about 5×10^5, or at least about 10^6, or at least about 2×10^6, genome copies of HCV per milliliter of serum. The patient may be infected with any HCV genotype (genotype 1, including 1a and 1b, 2, 3, 4, 6, etc. and subtypes (e.g., 2a, 2b, 3a, etc.)), particularly a difficult to treat genotype such as HCV genotype 1 and particular HCV subtypes and quasispecies.

[00251] Individuals that are suitable for treatment with a subject method for treating an HCV infection include individuals who have an HCV infection and, as a result of the HCV infection, suffer from liver fibrosis or hepatocellular carcinoma. Such individuals include HCV-infected individuals as described above.

[00252] Individuals that are suitable for treatment with a subject method for treating an HCV infection include individuals who have an HCV infection and are also co-infected with other pathogens e.g., HIV, HBV etc., or have a cancer. Such individuals include individuals infected with any of a variety of HCV genotypes, subtypes, or quasispecies, as described above.

EXAMPLES

[00253] The following examples are put forth so as to provide those of ordinary skill in the art with a complete disclosure and description of how to make and use the present invention, and are not intended to limit the scope of what the inventors regard as their invention nor are they intended to represent that the experiments below are all or the only experiments performed. Efforts have been made to ensure accuracy with respect to numbers used (e.g. amounts, temperature, etc.) but some experimental errors and deviations should be accounted for. Unless indicated otherwise, parts are parts by weight, molecular weight is weight average molecular

weight, temperature is in degrees Celsius, and pressure is at or near atmospheric. Standard abbreviations may be used, e.g., bp, base pair(s); kb, kilobase(s); pl, picoliter(s); s or sec, second(s); min, minute(s); h or hr, hour(s); aa, amino acid(s); kb, kilobase(s); bp, base pair(s); nt, nucleotide(s); i.m., intramuscular(ly); i.p., intraperitoneal(ly); s.c., subcutaneous(ly); and the like.

MATERIALS AND METHODS

[00254] The following materials and methods were used in the Examples provided below.

[00255] **Sequence Alignment**. Human adenovirus 5 (adenoviral vector, Ad) protein sequences (Table 6; Figure 24) were compared with 15-20 amino acid long peptide sequences from various HCV antigens (Core, F, NS3, NS4 and NS5) by sequence alignment using ClustalW software. Fifteen amino acid long peptides from F, core and NS3 and 20 amino acid long peptides from NS4 and NS5 were used . Sequence homology was documented as pairwise similarity or homology score and a heat map was prepared by Microsoft excel to present the distribution of each HCV antigen peptide homology across the different adenovirus proteins. Pairwise scores are the number of identities between the two sequences, divided by the length of the alignment, and calculated as a percentage, so a score of 25 means 25% homology, 30 means 30% homology and so on, in the aligned region. Number of adenovirus proteins showing various levels of homology (>25, >30, >35-40 and >50) is depicted in the tables 1-5.

[00256] **Adenovirus (Ad) vector**. Replication incompetent human adenovirus 5 with no transgene insert was amplified and titrated in human embryonic cell line 293A (HEK-293A) transformed with adenovirus E1 gene (QBiogene Inc., CA, USA) to provide complementarity for virus production. Recombinant adenoviruses (rAd), which express HCV antigens Core (rAd-core), F (rAd-F), NS3 (rAd-NS3), NS4 (rAd-NS4) or NS5 (rAd-NS5) have been prepared and reported earlier by us previously.

[00257] **DNA Purification and PCR Amplification**. DNA was purified from Ad, rAd-NS5A, rAd-NS5B vector stocks. Briefly, $1x10^9$ pfu of each vector was taken in individual tubes and DNA was prepared by using High-Pure Viral Nucleic Acid Kit^R (Roche Applied Bio). PCR reaction was set up with 10 µl template DNA obtained from the above preparation using 50 ul total reaction volume consisting of 1x PCR buffer, 10 µM dNTP, 25 µM of each primer (forward and reverse) and 1.25 unit of Taq polymerase. PCR tubes containing reaction mixtures was incubated in thermo-cycler with initial denaturation at 95°C and 35 amplification cycles (95°C: 30 Sec, 52°C: 30 Sec, 68°C: 60 Sec). PCR amplification products were run on 1% agarose gel at 80 volt to resolve amplification product along with 1 KB size Quick Load DNA Ladder (NEB Biolab, Germany).

[00258] **Adjuvants**. Toll-like receptor agonists poly I:C (TLR 3 agonist) and resiquimod (TLR 7/8 agonist) were used as adjuvants with Ad vector for immunization.

[00259] **Mice immunizations.** Six to seven weeks old female C57Bl/6 mice were purchased from Charles River Laboratory (Charles River, Canada) and immunized once or twice using various doses ($0.5x10^6$-$2x10^7$ PFU/mouse) of Ad, rAd-HCVNS3, rAd-MtbAg85B, rAd-HIVnef, or a pool of HCV derived NS3 peptides intramuscularly, intranasally, or orally in presence or absence of different adjuvants (Poly I:C, Resiquimod, 20 μg/mouse, or heat-killed *Caulobacter crescentus* HKCC). Details of dose and route of administration are indicated in figures or figure legends. Mice were euthanized 8 days after the first or second immunization(s) and various tissue samples (e.g. spleens, inguinal lymph nodes, ovaries, serum etc.) were collected. All animal experiments were approved by University of Alberta Animal Care and Use Committee in accordance with the Canadian Council of Animal Care guidelines.

[00260] **Immunohistochemistry.** Twelve, 24 and 48 hours after immunization, mice were euthanized and thigh muscle cells were collected. Ten-micrometer sections of quadriceps muscles of hind limbs of immunized mice were fixed on the slides and stained as follows. Briefly, slides were washed two times with 0.05% Tween phosphate-buffered saline (PBS) buffer for 2 min, followed by cleaning with Triton X-100 containing PBS. Nonspecific binding of biotinylated secondary antibody, used later in the procedure, was blocked by incubation with 5% diluted normal goat serum at room temperature for 30 minutes. Sections were then incubated with anti CD16/32 for one hour followed by two washes with 0.05% Tween PBS. Slides were then incubated with anti-core and anti-NS-3 primary antibody in a 1:100 dilution for 30 minutes followed by two washes with 0.05% Tween PBS. Endogenous peroxidase activity was depleted by incubating the sections in 3% H_2O_2 with 0.1% sodium azide in 0.05% Tween PBS buffer for 10 min. After two washes in 0.05% Tween PBS buffer, sections were incubated with 10 pg/ml of biotinylated goat anti-mouse in 1% normal mouse serum (Sigma) for 20 minutes, and washed twice in 0.05% Tween PBS buffer. The sections were then incubated with DAB for 20 minutes and subsequently washed twice in 0.05% Tween PBS buffer. Chromogen was added to each section for 5 minutes, and washed twice in 0.05% Tween PBS buffer. Sections were dried and dehydrated with 95% and 100% ethanol, cleared with xylene and mounted with water-based plastic mount (Polysciences, Inc.).

[00261] **T cell proliferation assay**. Eight days after last immunization, mice were euthanized, spleens and/or inguinal lymph nodes were collected. The spleens were pooled from replicates and ground to a single cell suspension and filtered through a Falcon 100 μm nylon cell strainer. The cells were resuspended in 2 ml of media and passed through an equilibrated nylon wool column. The column was washed after 45 min of incubation at 37°C and the flow through

contained the splenic T cells. These T cells were used in the experiments (~90% $CD3^+$ T cells). Lymph nodes were ground into single cell suspension and used in the assays. Proliferative responses were measured in triplicate cultures in 96-well flat-bottomed microtiter plates. A total of 4×10^5 T cells from immunized mice and 4×10^5 APCs (spleen cells from control mice irradiated with 18 Gy) were mixed with different HCV derived proteins (Core = c22-3, NS3 = c33c; NS4 = c100-3; NS5 = NS5 SOD, polyprotein c25 = Core+NS3+NS4; polyprotein c200 = NS3+NS4 or control protein = rhSOD) or synthetic HCV derived peptides (listed in tables 1-5) at different concentrations as described in figure legends. In experiments using rAd containing non-HCV antigens, respective antigens were used. T cell proliferation was assessed by radioactive ^{3}H-thymidine incorporation assay. Detailed methodologies for T cell proliferation assays have been reported previously.

[00262] **Cytokine ELISA**. Various cytokines (such as IFN-□, IL-10) were assessed in culture supernatants collected from T cell proliferation assays using mouse cytokine ELISA kits supplied by eBiosciences (eBiosciences Inc. San Diego, USA). ELISA was performed according to the manufacturer's instructions manual. Plates were read and data was analyzed in FluoStar ELISA reader (BMG Labtech GmbH, Ortenberg, Germany). Calculated concentration was multiplied with the dilution factor to quantitate the cytokine concentration in per ml of culture supernatant and averages of these concentrations (pg/ml) from duplicate wells were plotted in graphs.

[00263] **Antibody ELISA**. Serum was prepared from the blood of immunized mice and stored at -20°C until use. For the detection of HCV antigen specific cross-reactive IgG and IgG1 antibodies in Ad vector immunized mice, 96-well plates were coated with HCV antigens (Core, NS3, NS4 or NS5) at 1 μg/ml in 1xPBS overnight at 4°C. Next day, after blocking with 1% BSA at room temperature for 1 hour, serial dilutions of serum samples were added to 96-well plate in 2-3 replicates and incubated again at room temperature for 2 hours. After application of serum, anti-mouse IgG or IgG1 labeled with alkaline phosphatase (AP) (Southern Biotech, Alabama, USA) was added and plates were incubated for 1 hour, and finally color was developed by adding PNPP substrate (Southern Biotech, Alabama, USA). Plates were washed with 1xPBST (1xPBS with 0.1% Tween-20) after each incubation step. Absorbance was read using FluoStar Optima ELISA Reader (BMG Labtech GmbH, Ortenberg, Germany).

[00264] **Immunization of mice with bone marrow derived DCs infected with Ad vector**. Female C57 BL/6 mice were euthanized and bone marrow cells were harvested from tibiae and femur bones, and cultured in RPMI-1640 medium supplemented with 10% heat-inactivated fetal bovine serum, 2-mercaptoethanol (50 mM), 400 U/ml murine GM-CSF and 1% Penicillin/Streptomycin at $10x10^6$ cells/100 mm petri dish. Half of the medium was changed on

days 3, 6, 8 and 10. On day 12, differentiated DCs were harvested and counted. DCs obtained were further cultured overnight in presence of Ad vector (100 MOI) or media. Twenty-four hours after, cells were washed, counted and a sample of $0.5x10^6$ cells was taken for flow cytometry analysis, and the remaining DCs were used to inject mice. Naïve (media) or Ad vector infected DCs ($1.0x10^6$/mouse) were injected subcutaneously. Prior to injection, about 74-83% of the cell population was $CD11c^+$. Upon infection with Ad vector, they showed a small up-regulation of maturation markers (MHC class-II and CD86, data not shown). Two immunizations were given on days 0 and 14. Mice were euthanized 8 days after second immunization to evaluate cellular immune responses against HCV antigens.

[00265] **Flow cytometry**. Mouse splenocytes were cultured with 5 μg/ml of HCV protein antigens or peptides for 5 days in RPMI-1640 media supplemented with 10% fetal bovine serum. On the fifth day, cells were harvested and counted, and $1x10^6$ cells per group were stained for intracellular Granzyme B (Alexa fluor 647) and extracellular CD8 (APC efluor 780) markers. To perform intracellular cytokine staining, splenocytes cultured for 5 days with HCV antigens were treated with ionomycin (1 μg/ml), phorbol 12-myristate 13-acetate or PMA (50 ng/ml) and brefeldin A (1.5 μg/ml) for 5 hours and subsequently stained for extracellular lineage markers: CD3 (PE Cy7), CD4 (APC) and CD8 (APC efluor 780); and intracellular cytokines: IFN-γ (PE) and IL-10 (FITC). For T cell proliferation using CFSE dilution assay, splenocytes were enriched for T cells using nylon wool column and stained with 1 uM CFSE in 1 ml of cell suspension for 7-10 minutes at room temperature. These CFSE stained cells were washed in 1x PBS containing 10 % fetal bovine serum, counted and plated in 24-well plate with 1 μg/ml of HCV NS5 antigen and equal number of γ-irradiated syngeneic splenocytes as APCs. After four days, cells were stained for $CD4^+$ and $CD8^+$ T cell markers. The cells were run in BD FACS Canto, and data were analyzed using FACS Diva and FCS Express 4.0 softwares. Fluorescently labeled antibodies against various cell markers were purchased from eBiosciences.

[00266] **T cell cytotoxicity assay:** Spleen T cells harvested from Ad immunized mice were stimulated *in vitro* with the HCV protein antigens Core, NS3, NS4 or NS5 at 5 μg/ml concentration for 4 days. The target EL4 cells were incubated with corresponding HCV peptides (Core peptides: 2, 14, 17, 25, 27, 28, 32; NS3 peptides: 8, 10; NS4 peptides: 3, 4, 8; and NS5 peptides: 1a, 2a, 16a, 20a, 5b, 19b, 23b, 39b; or All: a mixture of the above peptides from core, NS3, NS4 and NS5) overnight at 37°C and peptide loaded EL4 cells were cultured with effectors at 10:1 (effectors: target) ratio for 4-5 hours. CFSE labeled live targets were quantified by flow cytometry and subtracted from background CFSE labeled targets to get numbers of killed targets. Empty (no peptide loaded) EL4 targets were used as a negative control.

[00267] **Chimeric Vac-HCV Challenge**. Eight days after the last immunization with Ad vector or PBS, mice were challenged with $1x10^7$ PFU of Vac-HCV chimeric virus (Vac-Core-NS3 including Core, NS2 and NS3 antigens of HCV or Vac-NS3-NS5 including NS3, NS4 and NS5 antigens of HCV), or wild-type Vaccinia (WT-Vac, not containing HCV antigens) virus intraperitoneally. Five days after virus challenge, mice were euthanized and ovaries were removed, homogenized and freeze-thawed three times in 1x PBS. Homogenized samples were stored at -80° C until used for viral titer.

[00268] **Vac-HCV or WT-Vac titration by plaque assay.** Serially diluted samples of ovary homogenates were added in duplicate wells in 6-well plates containing 80% confluent monolayers of TK-1 cells (ATCC # CRL8303) and incubated for 90 minutes. Subsequently, unbound virus was removed and fresh 1x DMEM media (Gibco by Invitrogen, NY, USA) supplemented with 3% FBS, was added and plates were incubated for 48 hours. At this time, media was removed, and plaques were fixed by using 10% formaldehyde (Fisher Scientific, NJ, USA) at room temperature for 30 minutes. Plates were washed with PBS and the monolayers were stained with 0.5% crystal violet (Sigma-Aldrich Company, MO, USA) for 30 minutes, followed by further washing. Plaques were counted, averaged and multiplied with the dilution factor to determine the viral load/mouse.

[00269] **Statistical Analysis**. Data were analyzed by Graph-pad Prism software (Graph-pad Software Inc., CA, USA). Student t-test was used to determine the significant difference between two groups. p-value less than 0.05 (<0.05) was considered to be statistically significant.

Example 1

[00270] The following example describes the amino acid sequence alignment of adenoviral vector (Ad) proteins from human Ad5 and Chimp Ad25 with the 15 or 20-amino acid long sequences of different HCV proteins to identify the regions of homology in both Ad proteins and HCV proteins by using bioinformatics tool Clustal W.

[00271] The sequence analyses demonstrate that various Ad proteins show extensive regions of homology with HCV peptides ranging from scores 1-50. Figures 18-24 (**Tables 1-6**) summarize the homology of different HCV peptides with the Ad proteins from human Ad5. We found high homology between HCV peptide sequences from core, F, NS3, NS5 proteins and a large number of Ad proteins. We also found that an individual peptide from various HCV antigens showed homology with multiple Ad proteins. These homologies also resulted in high cross-reactive cellular immune responses against HCV antigens as described in following examples. HCV NS4 derived peptides showed the least homology with the lowest number of Ad proteins (Table 4; Figure 21). We compared peptide sequences from HCV core with amino acid sequences of

various proteins from a simian adenovirus (Chimp Ad25) (**Figure 29, Table 8**). Interestingly, there was high level of homology (25-40%) between HCV core peptides and ChAd25 proteins, suggesting that Chimp adenoviruses will also induce HCV cross-reactive immune responses.

Example 2

[00272] PCR Amplification of DNA purified from adenoviral vector (Ad vector) stocks and recombinant Ad vector (rAd) harboring HCV transgenes used in immunization studies.

[00273] The PCR analysis experiment demonstrates that Ad vector used, which was found to induce both cellular and humoral immune responses against HCV antigens, was devoid of any cross contamination with HCV genes (Figure 1A). In the PCR reaction products run on 1% agarose gel, none of the primer sets specific for NS3, core, F, NS4, NS5a and NS5b antigens of HCV were able to detect HCV antigen products from Ad vector DNA template and relevant genes in recombinant Ad virus DNAs containing HCV genes were detected. Therefore, Ad vector stock was devoid of any HCV gene contaminations and the cross-reactive immune responses induced in mice against various HCV antigens described in following examples are solely due to heterologous cross-reactive immunity generated by Ad vector expressing adenoviral antigens.

Example 3

[00274] Cross-reactive binding of anti-core and anti-NS3 MAb to quadriceps muscles of mice immunized with Ad or recombinant Ad containing HCV antigens (rAd-core or rAd-NS3).

[00275] Mice were immunized with replication deficient adenovector (Ad) or Ad containing HCV derived core or NS3 proteins ($2x10^7$PFU/mouse) intramuscularly. Twelve, 24, and 48 hours after immunization, mice were euthanized and the quadriceps muscles of the hind limbs were collected for immunohistochemistry. These time points were chosen because we are using replication-incompetent recombinant adenovirus vectors. At all time points, significant cross-reactive binding of Anti-NS3 and anti-core MAbs was observed in Ad group, and positive controls rAd-NS3 and rAd-core immunized groups (**Fig 1B**). The intensity of cross-reactive binding was qualitatively lower compared to the relevant transgene-expressing Ads. As negative controls, quadriceps muscles from PBS immunized mice were stained with anti-core and anti-NS3 antibody (**Fig 1B**, top panel). Isotype control antibodies did not show any cross-reactive binding.

Example 4

[00276] Induction of cross-reactive T cells, cytokines and antibody responses against HCV antigens (Core, NS3, NS4 and NS5), after two intramuscular (i.m.) immunizations with adenoviral vector (Ad) in the absence or presence of toll like receptor (TLR) agonists.

[00277] Female C57b/6 mice (n = 5/group) were immunized twice (at 14 day interval) intramuscularly with $2x10^7$PFU/mouse adenoviral vector (Ad), Ad+poly I:C, Ad+resiquimod, rAd-NS3 or PBS. The proliferation of spleen and lymph node T cells obtained from mice was determined against HCV Core, NS3 or NS5 protein antigens or pools of selected peptides from HCV proteins (Core Pool: 5, 14, 16, 17 & 27; NS3 Pool: 5, 6, 8, 15 & 17; NS5 Pool: 5a- 6, 24/ b-5, 19 & 27) (**Fig. 2-4**). Both spleen and lymph node T cells from Ad immunized group demonstrated high HCV antigen specific proliferation, which was further increased by co-administration of poly I:C and resiquimod adjuvants. Furthermore, presence of NS3 transgene in Ad vector showed robust cross-reactive proliferation against all of the HCV antigens tested (core, NS3, NS4 and NS5), similar or higher than Ad+poly I:C or Ad+resiquimod groups (**Fig 2A, 3A, 4A**). We also analyzed IFN-γ and IL-10 secretion in culture supernatants collected from T cell proliferation assays. In the Ad immunized group, both spleen and lymph node T cells produced IFN-γ in response to various HCV protein antigens and peptide pools, which was significantly increased in the T cells obtained from mice immunized with Ad vector plus poly I:C adjuvant (**FIG. 2B, 3B, 4B**). IFN-γ levels in culture supernatants upon *in vitro* stimulation with various HCV proteins or peptides was also significantly higher in rAd-NS3 immunized mice in comparison to PBS immunized mice.

[00278] Further, we assessed cross-reactive antibodies against HCV protein antigens (Core, NS3, NS4 and NS5) in the serum samples Ad vector immunized mice (**FIG. 5 A-H**). Ad vector induced significant amounts of cross-reactive antibodies against various HCV antigens (Core, NS3, NS4 and NS5), which correlated with the T cell proliferation responses. Interestingly, co-administration of TLR agonists poly I:C or presence of HCV NS3 transgene in Ad vector enhanced the levels of anti-HCV Core, NS3, NS4 and NS5 cross-reactive antibodies. PBS immunized mice did not show IgG or IgG1 binding to HCV core, NS3, NS4 and NS5 antigens (**FIG. 5**).

[00279] Unexpectedly, broadly directed and robust T cell and antibody responses against several HCV conserved antigens were observed upon i.m. immunization with replication defective adenoviral vector (Ad). Thus, immunization with Ad virus alone can induce broad, robust multifunctional immune responses against HCV.

[00280] Further, recombinant Ad-NS3 unexpectedly demonstrated similar or higher cross-reactive cellular and humoral immune responses against HCV antigens (core, NS3, NS4 and NS5) compared to immunization with transgene free Ad.

[00281] We also observed that the cellular immune responses generated against HCV antigens upon immunization with rAd-NS3 were similar or higher then immunization with Ad vector co-administered with poly I:C or resiquimod adjuvants.

Example 5

[00282] Induction of cross-reactive cellular immune responses against HCV antigens (Core, NS3, NS4 and NS5) in mice immunized with adenoviral vector (Ad) through various routes of immunizations.

[00283] Female C57bl/6 mice were immunized with two doses of Ad vector ($2x10^7$ pfu/mouse) or PBS at two weeks' time interval through intramuscular (i.m.), intranasal (i.n.) or oral routes. The proliferation of splenocytes harvested from immunized mice was determined in response to HCV Core, NS3, NS4 or NS5 protein antigens or pools of selected peptides from HCV proteins (Core Pool: 5, 14, 16, 17 & 27; NS3 Pool: 5, 6, 8, 15 & 17; NS4 Pool: 4, 8, 9, 13 & 14; NS5 Pool: 5a- 6, 24/ b-5, 19 & 27). With all of the routes tested, spleen T cells from Ad immunized mice showed significantly high proliferation in in vitro stimulation with HCV protein antigens **(Fig.6A)**. We also analyzed IFN-γ secretion in culture supernatant collected from T cell proliferation assay. Spleen T cells produce high amount of IFN-γ cytokine in response to HCV protein antigens and peptide pools in Ad immunized mice via both i.m. and i.n. routes but not by oral route **(Fig.6B)**. Overall, broadly directed T cell and antibody responses (data not shown) against several HCV conserved antigens were observed upon i.m., i.n. and oral immunization with replication defective adenoviral vector (Ad).

[00284] To determine whether single immunization with Ad and rAd-NS3 also induces cellular immune responses against various recombinant HCV proteins, mice were immunized once intramuscularly or intranasally with different doses ($0.5x10^6$, $1x10^6$ and $2x10^7$ pfu/mouse) of Ad or rAd-NS3 vector (**Fig 6C**). Splenocytes and/or inguinal lymph node T cells were isolated 8 days after immunization and HCV antigens-specific T cell proliferation was determined. At all of the doses tested by i.m. or i.n. route, both Ad and rAd-NS3 vector induced robust HCV specific T cell proliferative responses in spleens and/or lymph nodes (**Fig. 6C**).

Example 6

[00285] Cross-reactive T cell responses against a large number of synthetic peptide epitopes of various HCV proteins in mice after intramuscular immunization with adenoviral vector (Ad).

[00286] To characterize and identify the domains of cross-reactivity in various HCV antigens with respect to amino acid sequences, we immunized mice with Ad vector and compared spleen T cell proliferation in response to HCV Core, F, NS3, NS5a and NS5b derived synthetic individual peptides with un-immunized (PBS) mice. Several of the HCV Core, F, NS3, NS5a and

NS5b peptides are able to induce T cell proliferation ex vivo (**Fig 7-12**), which also translated in to production of IFN-γ and IL-10 (**Fig. 7-12**). Several peptides, which possess a very high amino acid sequence homology with the different Ad vector proteins and with multiple high scoring regions in adenovirus proteins showed T cell proliferation and IFN-γ production. However, some HCV peptides which showed high homology with respect to high score (>35) and number of regions in Ad proteins (**Table 1-5; provided in Figures 18-23**), did not show cross-reactive responses in mice immunized with Ad vector. This could be explained on the basis of homology in TCR contact vs. the non-contact amino acids of the peptides or the overall immunogenicity of those epitopes. Further, to confirm that the high proliferation responses observed in ^{3}H-Tdr assay are due to actual proliferation of HCV specific CD4 and CD8 T cells, we performed CFSE proliferation assay along with staining for CD3, CD4 and CD8, which allows one to demonstrate actual proliferating cells in response to a given protein (**FIG 13A, B**) or peptide (**FIG 14 A, B**) antigen. Spleen T cells obtained from Ad vector immunized mice and stimulated ex vivo with various recombinant HCV protein antigens (Core, NS3, NS4 and NS5, **Fig 13A, B**); and selected representative peptides from these proteins (**FIG. 14A,B**), showed antigen dependent proliferation of cross-reactive $CD4^+$ and $CD8^+$ T cells.

[00287] Further, to demonstrate that the cytokines produced in culture supernatants observed in experiments described in figures 2-6 are from $CD4^+$ and $CD8^+$ T cells, we performed intracellular cytokine expression analyses of spleen T cells obtained from Ad vector immunized mice and stimulated ex vivo with various recombinant HCV protein antigens (Core, NS3, NS4 and NS5); or selected representative peptides from HCV proteins. Both $CD4^+$ and $CD8^+$ T cells from Ad vector immunized mice showed increased expression of IFN-γ upon stimulation with HCV protein antigens (**FIG. 13 C, D**) or peptide antigens (**FIG. 14 C, D**) in comparison to PBS immunized mice. T Cells expressing both IFN-γ and IL-10 simultaneously were also in higher frequency in Ad vector immunized mice when stimulated with HCV core, NS3 or NS4 antigens, except NS5 antigen. HCV core stimulated $CD4^+$ and $CD8^+$ T cells show high frequency of IL-10 expressing cells compared to other HCV antigens. HCV NS5 antigen did not increase IFN-γ production in $CD4^+$ T cells; however in $CD8^+$ T cells it was significantly increased in Ad vector immunized mice compared to PBS control (**FIG. 13 C, D**).

[00288] Intracellular cytokine analyses were also performed in splenic T cells obtained from Ad vector or PBS immunized mice and cultured with selected representative peptides derived from various HCV antigens core, NS3, NS4 and NS5 (**FIG 14 C, D**). Cross-reactive $CD4^+$ T cells from Ad immunized mice showed enhanced IFN-γ and IL-10 expression upon stimulation with all of the peptides tested. Interestingly, frequency of $CD4^+$ T cells which express both IFN-γ and

IL-10 was also higher in comparison to PBS group. Further, IFN-γ producing $CD8^+$ T cells were significantly high with all the peptides used for *in vitro* stimulation (**FIG. 14C, D**).

[00289] The CFSE proliferation data and intracellular cytokine analyses described in figure 13 and 14 provided conclusive evidence that $CD4^+$ and $CD8^+$ T cells obtained from Ad vector immunized mice are highly cross-reactive against various HCV antigens and peptides derived from them.

[00290] Multi-antigen specific $CD4^+$, $CD8^+$ T cells, effector cells producing GrB and IFN-γ against highly conserved HCV core, NS3, NS4 and NS5 antigens were also observed.

[00291] T cell immunity against conserved epitopes of adenoviruses has been shown to be conserved across various human serotypes and also across species. It was observed that the homology between HCV epitopes and adenoviruses spans across a number of adenoviral proteins including conserved antigens, and thus it can be extended to a number of human (e.g., Human Ad5, Ad6, Ad24 and Ad35) and non-human adenoviruses (such as Chimpanzees Ad3, Bovine etc.). Therefore, cross-reactive heterologous immunity against HCV antigens epitopes may be induced by a number of different rare human and non-human adenoviruses. In this regard, the adenovirus used to induce immunity against HCV could be a bovine adenovirus, a canine adenovirus, non-human primate adenovirus, a chicken adenovirus, a porcine adenovirus, a swine adenovirus, an adeno associated virus or a helper dependent adeno virus, and their various serotypes (e.g., 57 different serotypes from 7 subtypes of human adenoviruses).

Example 7

[00292] Cytotoxic activity of the T cells derived from mice immunized with adenoviral vector (Ad) against EL4 target cells loaded with HCV peptide antigens.

[00293] The cytotoxic activity of the cross-reactive effector T cells, obtained from spleens of Ad vector immunized mice and stimulated *in vitro* (4 days) with 5 μg/ml concentration of HCV protein antigens (Core, NS3, NS4, NS5 or polyprotein), was examined against EL4 targets cells loaded with pools of respective HCV antigen peptides. Briefly, different sets of CFSE stained EL4 targets were prepared by loading them with pools of peptides obtained from different HCV antigens as follows: Core (peptide # 2, 14, 17, 25, 27, 28, 32), NS3 (peptides # 8, 10), NS4 (peptides # 3, 4, 8), NS5 (peptides # NS5a: 1, 2, 16, 20 and NS5b: 5, 19, 23, 39), all of the above peptides (ALL) or no peptide loaded (No). These different EL4 targets were cultured for 4-5 hours with corresponding effector T cells stimulated with HCV protein antigens. The results obtained demonstrated that T cells induced after Ad vector immunization act as potent effector T cells, which can kill HCV peptide antigens loaded EL4 target cells in an antigen specific manner

(**FIG. 15**). Therefore, Ad vector immunization can lead to the induction of strong cytotoxic effectors T cells, which demonstrate HCV antigen specific killing of target cells (**FIG. 15**).

Example 8

[00294] This example illustrates the use of dendritic cells infected with adenoviral vector (Ad) to induce cross-reactive immune responses against HCV proteins as cellular vaccines.

[00295] The cross-reactive immune responses against HCV antigen NS5 were examined after immunizations with bone marrow derived dendritic cells (DCs) infected with Ad vector. Mice immunized with uninfected DCs were used as control. Briefly, after 8 days of two subcutaneous immunizations with Ad vector expressing DCs (Ad DCs) or uninfected DCs (DCs only), spleen T cells were harvested, labeled with CFSE, and cultured for five days in the presence of NS5 antigen (5 μg/ml) and APCs. Proliferation of $CD4^+$ and $CD8^+$ T cells and granzyme B expression by $CD8^+$ T cells was evaluated by flow cytometry (**FIG. 16**). The results demonstrated that immunization with Ad vector infected DCs generated HCV NS5 cross-reactive $CD4^+$ and $CD8^+$ T cells (**FIG. 16A**). Further, $CD8^+$ T cells also showed high granzyme B expression when restimulated with HCV NS5 protein (**FIG. 16B**). These results indicate that cross-reactive immune response against HCV antigen is also induced by cellular immunization with DCs infected with Ad vector.

[00296] Unexpectedly, Ad induces cross reactive HCV antigen specific $CD4^+$ and $CD8^+$ T cell responses upon immunization with DC infected with Ad virus ex vivo.

Example 9

[00297] This example describes the role of HCV specific cross-reactive immune responses induced by adenoviral vector (Ad) in the reduction of viral loads in Vaccinia-HCV challenged mice.

[00298] To demonstrate the protective potential of Ad induced cross-reactive immune responses against HCV infection, a surrogate Vac-HCV infection model was used. The recombinant HCV-vaccinia infected mouse model has been reported as a surrogate small animal model for HCV infection. Although this model does not demonstrate exact features of HCV infection, it can be used to assess the ability of the induced immunity to kill targets expressing HCV antigens, a critical parameter in the antiviral response against HCV. Briefly, C57bl/6 mice after two intramuscular immunizations with Ad vector with or without poly I:C as adjuvant, were challenged intraperitoneally with Vaccinia-HCV (Vac-NS3-NS5) chimeric virus. After 5 days of challenge, mice were euthanized and viral loads in ovaries of individual mice were evaluated (**FIG 17 A**). The results obtained clearly demonstrate that Ad vector immunized mice had significantly reduced viral loads in comparison to PBS immunized mice, and mice immunized

with Ad vector in presence of poly I:C had further reduced viral loads. Therefore, Ad vector immunization can induce antiviral cross-reactive immune response against HCV infection (**FIG. 17 A**).

[00299] To further prove the role of antigen specific heterologous immunity and exclude the possibility of non-specific immunity in viral reduction in HCV-vaccinia challenge model, we used recombinant Vac-HCV (Vac-Core-NS3) or WT-Vac (wild-type vaccinia not including sequences from HCV genome) infection models. Briefly, two groups of mice (n = 5/group) were immunized twice with Ad vector ($2x10^7$ pfu/mouse) and other two groups (n = 5/group) with HEK lysate (cell line used to cultivate Ad, as control) intramuscularly. Each immunized group of mice was challenged with WT-Vac or Vac-HCV ($1x10^7$ pfu/mouse, intraperitoneally). Five days after the virus challenge, ovaries were harvested from individual mouse and viral titers were determined using plaque assay in TK-1 cells (**Fig 17B**). Immunization with Ad vector led to significant reduction in viral titers in mice infected with Vac-HCV ($p < 0.05$, Student's t test) in contrast to mice infected with WT-Vac ($p > 0.05$), providing conclusive evidence that immunization with Ad leads to cross protective immunity against HCV antigens allowing the reduction in viral titer of Vac-HCV and not WT-Vac. Immunizations with HEK lysate did not lead to significant changes in viral titers in WT-Vac or Vac-HCV infected mice.

Example 10

[00300] This example illustrates the induction of cross-reactive T cell responses against HCV antigens (core, NS3, NS4 and NS5), after two intranasal (i.n) immunizations with recombinant adenoviral vector expressing HIV nef protein (rAd-nef) in the absence or presence of adjuvant Poly I: C.

[00301] Male C57bl/6 mice (n = 5/group) were immunized twice (at 14 day interval) intranasally with $2x10^7$PFU/mouse recombinant adenoviral vector expressing HIV-nef (rAd-nef), rAd-nef+poly I:C or PBS. After 8 days of second immunization the proliferation of spleen T cells obtained from mice was determined against HCV Core, NS3, NS4 or NS5 protein antigens and HIV-nef protein (**Fig. 26**). Splenic T cells from rAd-nef immunized group demonstrated robust cross-reactive proliferation against all of the HCV antigens tested (core, NS3, NS4 and NS5) and HIV antigen specific proliferation, which was further increased by co-administration of poly I:C adjuvant comparison to PBS immunized mice. Therefore, recombinant Ad vector expressing multiple antigens from other viral or cancer-associated antigens can induce cross-reactive HCV-specific immunity and can be used as a single multi-pathogen vaccine to protect and/or prevent two or more disease.

Example 11

[00302] This example demonstrates the induction of cross-reactive T cell responses against HCV antigens (core, NS3 and NS4), after two intramuscular (i.m) immunizations with recombinant adenoviral vector expressing mycobacteria antigen 85B (rAd-Ag85B) or transgene free Ad .

[00303] Male C57bl/6 mice (n = 5/group) were immunized twice (at 14 day interval) intramuscularly with $2x10^7$PFU/mouse recombinant adenoviral vector expressing Mycobacterial antigen 85B (rAd-Ag85B), or Ad vector. After 8 days of second immunization the proliferation of spleen T cells obtained from mice was determined against HCV Core, NS3 or NS4 protein antigens and sonicated-mycobacteria at different concentrations (**Fig. 27**). Splenic T cells from rAd-Ag85B immunized group demonstrated robust cross-reactive proliferation against all of the HCV antigens tested (core, NS3 ad NS4) and mycobacterial antigen specific proliferation, while T cells obtained from Ad vector immunized group showed only HCV-specific T cell responses and Mtb-specific T cell responses were absent in Ad vector group. Therefore, recombinant Ad vector expressing antigens from other bacterial/parasites antigens can induce cross-reactive HCV-specific immunity and can be used as a single multi-pathogen vaccine to protect and/or prevent bacterial and viral disease.

Example 12

[00304] This example demonstrates the protective potential of heterologous priming with Ad vector (i.m) and boosting with pool of HCV-NS3 peptide antigens (i.n) with immunomodulator heat-killed *Caulobacter crescentus* (HKCC) in the reduction in viral loads in Vaccinia-HCV challenged mice.

[00305] Female C57bl/6 mice were first immunized intramuscularly with Ad vector. After 14 days interval, mice were boosted with a pool of HCV-NS3 peptides with HKCC intranasally or PBS. Each immunized group of mice was challenged intraperitoneally with Vaccinia-HCV (Vac-Core-NS3) chimeric virus. Five days after the virus challenge, ovaries were harvested from individual mouse and viral titers were determined using plaque assay in TK-1 cells (**Fig 28**). Priming with Ad vector and boosting with NS3 peptides and HKCC [Ad (i.m)-Peptides+HKCC (i.n)] led to significant reduction in viral titers in mice infected with Vac-HCV ($p < 0.05$, Student's t test) in contrast to PBS immunized mice.

[00306] The results obtained demonstrate that the potential of cross HCV specific immunity with adenoviral vector can be further enhanced with the use of structural or non-structural HCV peptide/protein antigens with or without an immunomodulator or viral/bacterial vector expressing HCV antigens.

[00307] While the present invention has been described with reference to the specific embodiments thereof, it should be understood by those skilled in the art that various changes may be made and equivalents may be substituted without departing from the true spirit and scope of the invention. In addition, many modifications may be made to adapt a particular situation, material, composition of matter, process, process step or steps, to the objective, spirit and scope of the present invention. All such modifications are intended to be within the scope of the claims appended hereto.

CLAIMS

What is claimed is:

1. A method of inducing an immune response in an individual to a hepatitis C virus (HCV) protein, the method comprising administering to the individual an effective amount of an immunogenic composition comprising an adenoviral nucleic acid or an adenovirus polypeptide.

2. The method of claim 1, wherein the adenoviral nucleic acid or adenovirus polypeptide is administered via an oral, intranasal, subcutaneous, transdermal, intratracheal, rectal, intramuscular or parenteral route of administration.

3. The method of any one of claims 1-2, wherein the adenoviral nucleic acid or adenovirus polypeptide is administered multiple times.

4. The method of claim 3, wherein the multiple administrations comprise a first administration wherein the adenoviral nucleic acid or adenovirus polypeptide is a first adenovirus serotype or subtype, and at least a second administration wherein the adenoviral nucleic acid or adenovirus polypeptide is a second adenovirus serotype or subtype.

5. The method of any one of claims 1-4, wherein said immune response comprises a humoral and/or a cellular immune response.

6. The method of any one of claims 1-4, wherein the adenoviral nucleic acid is a full-length adenovirus nucleic acid or an adenovirus nucleic acid comprising a deletion.

7. The method of any one of claims 1-6, wherein the adenoviral nucleic acid does not encode a non-adenovirus polypeptide.

8. The method of any one of claims 1-5, wherein the adenoviral nucleic acid comprises a nucleotide sequence encoding one or more HCV polypeptides.

9. The method of any one of claims 1-5, wherein the adenoviral nucleic acid comprises a nucleotide sequence encoding an antigen associated with a pathogen other than HCV or comprises a nucleotide sequence encoding a cancer-associated antigen.

10. The method of any one of claims 1-6, 8, and 9, wherein the adenoviral nucleic acid comprises a nucleotide sequence associated with an immunostimulatory or immunomodulatory sequence.

11. The method of claim 9, further comprising simultaneously administering a non-recombinant adenovirus.

12. The method of claims 1-9, further comprising administering a structural or a non-structural HCV polypeptide or a nucleic acid comprising a nucleotide sequence encoding the structural or non-structural HCV polypeptide.

13. The method of claim 12, wherein the HCV polypeptide is one or more of E1, E2, F, core, P7, NS2, NS3, NS4 and NS5.

14. The method of claim 12, wherein the structural or non-structural HCV antigen is administered before the adenovirus nucleic acid or the adenovirus polypeptide.

15. The method of claim 12, wherein the structural or non-structural HCV antigen is administered after the adenovirus nucleic acid or the adenovirus polypeptide.

16. The method of any one of claims 1-15, wherein the immunogenic composition comprises an adjuvant.

17. The method of any one of claims 1-15, wherein the composition comprises a cytokine and/or an antibody.

18. The method of any one of claims 1-15, wherein the composition comprises adenoviral nucleic acid or adenovirus polypeptide from two or more different serotypes or subtypes of adenovirus.

19. A method of inducing an immune response in an individual to a hepatitis C virus antigen, the method comprising:

a) obtaining dendritic cells (DCs) from the individual;

b) genetically modifying the DCs to express one or more adenoviral proteins; and

c) administering the genetically modified DCs to the individual.

20. A method of inducing an immune response in an individual to a hepatitis C virus antigen, the method comprising:

a) obtaining DCs from the individual;

b) infecting the DCs with replication competent adenovirus or replication-defective adenovirus; and

c) administering the infected DCs to the individual.

21. A method of inducing an immune response in an individual to a hepatitis C virus antigen, the method comprising:

a) obtaining DCs from the individual;

b) introducing one or more adenoviral proteins, or nucleic acids encoding one or more adenoviral proteins, into the DCs, thereby generating adenoviral protein-expressing DCs; and

c) administering the adenoviral protein-expressing DCs to the individual.

22. A method of treating a hepatitis C virus (HCV) infection in an individual, the method comprising inducing an immune response to one or more HCV antigens in the individual, wherein said inducing comprises a method of any one of claims 1-21.

23. The method of claim 22, comprising administering to the individual an effective amount of at least a second therapeutic agent that treats an HCV infection.

24. The method of claim 22 or 23, wherein the HCV-infected individual is a treatment-naïve individual.

25. The method of claim 22 or 23, wherein the HCV-infected individual failed a prior treatment for HCV infection.

26. The method of any one of claims 22-25, wherein the HCV is HCV of any genotype or subtype.

Figure 1A: PCR Amplification of DNA purified from adenovirus vector stock using HCV specific primers

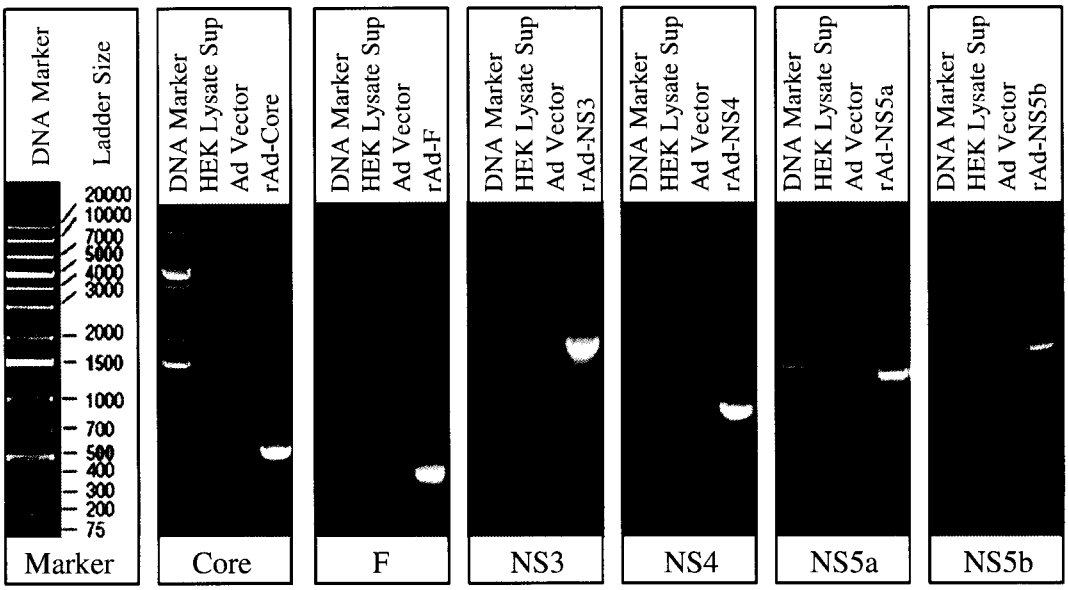


Figure 1B: Cross-reactive binding of anti-core and anti-NS3 antibodies to mouse quadricep muscles upon intramuscular immunization with adenoviral vector (Ad) or recombinant adenoviral vectors (rAd-NS3 and rAd-Core)

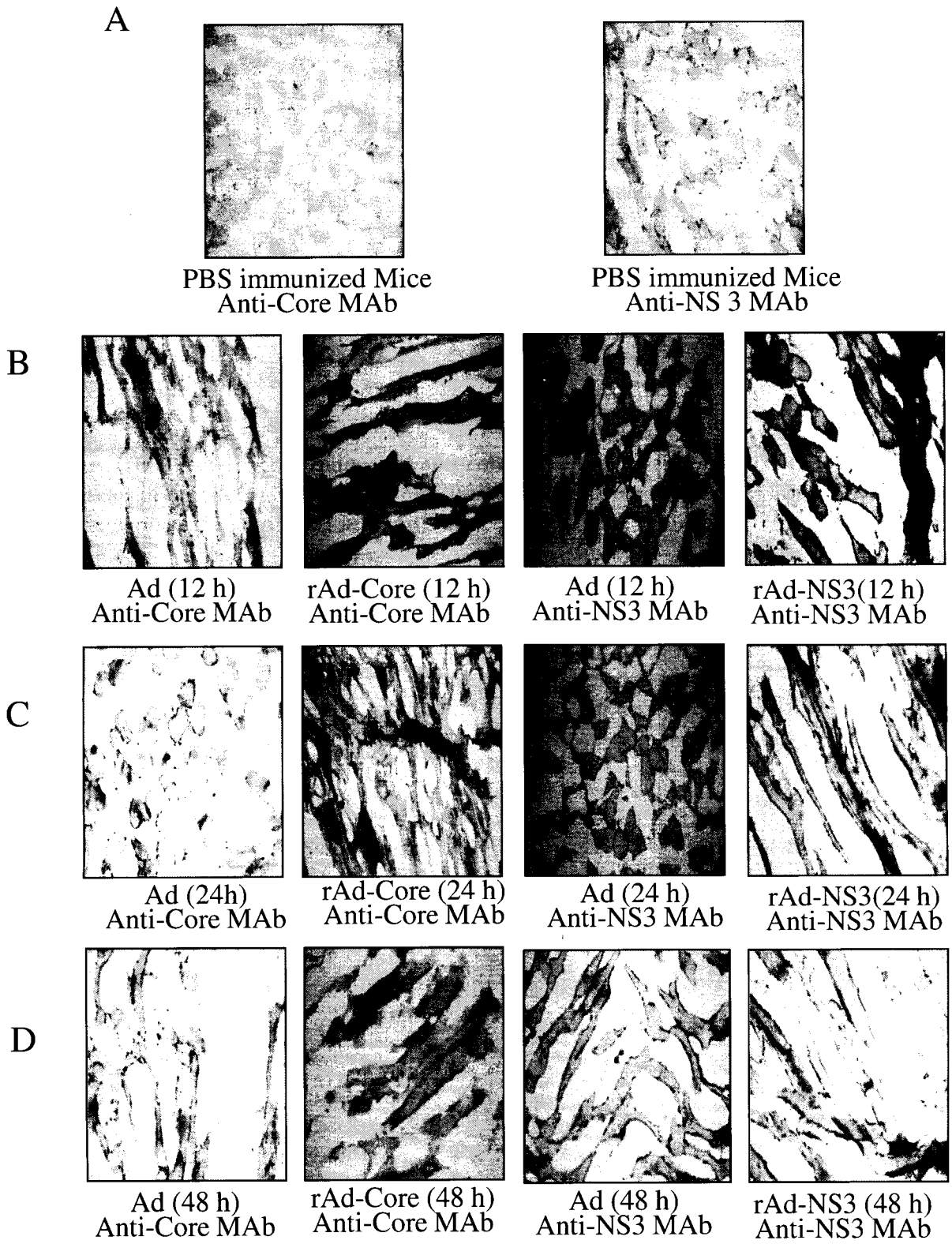


Figure 2: Immunization of mice with adenoviral vector (Ad and rAd-NS3) twice intramuscularly in the absence or presence of adjuvants induces cross reactive cellular immune responses against HCV core

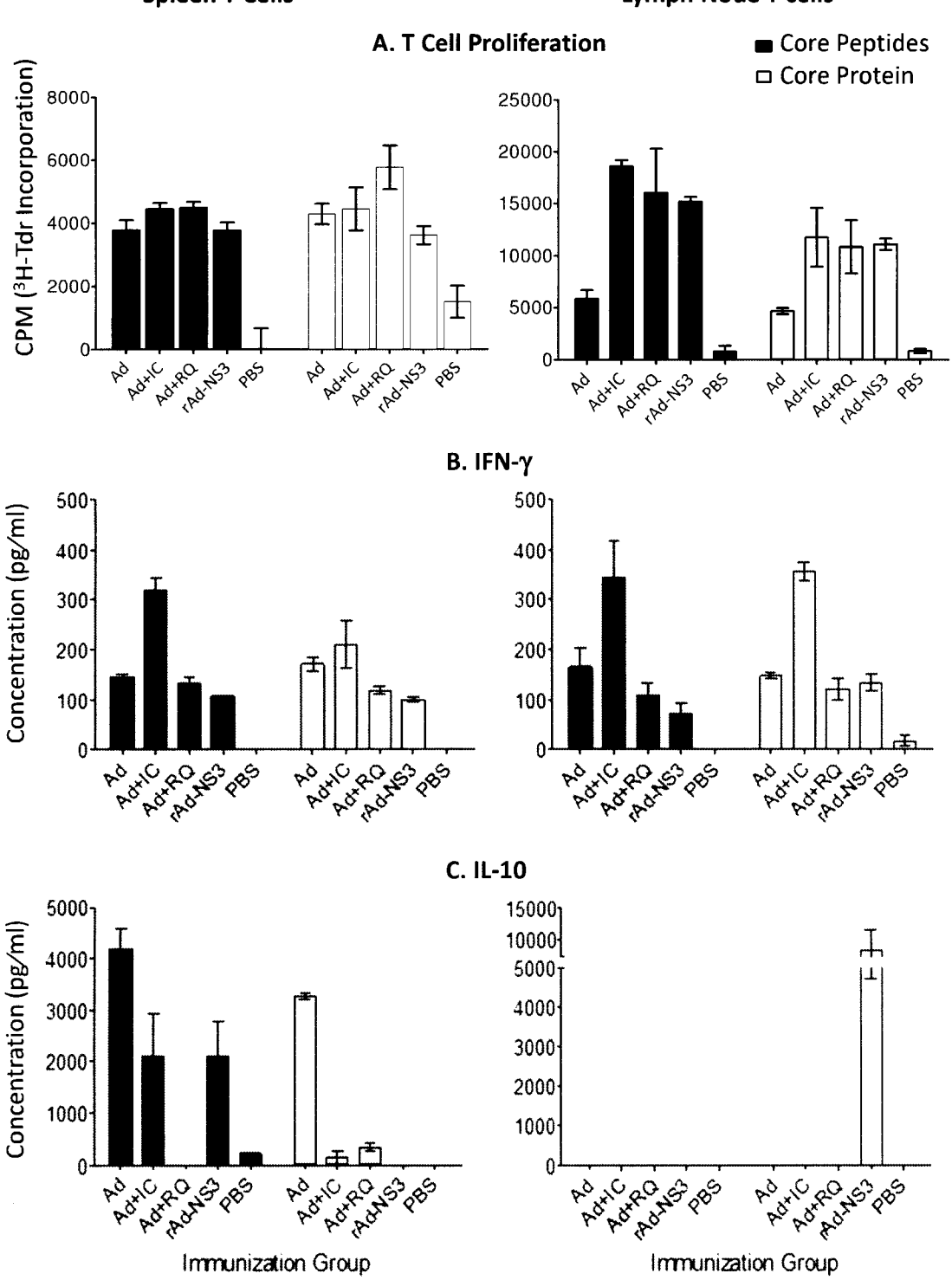


Figure 3: Immunization of mice with adenoviral vector (Ad and rAd-NS3) twice intramuscularly in the absence or presence of adjuvants induces cross reactive cellular Immune responses against HCV NS3

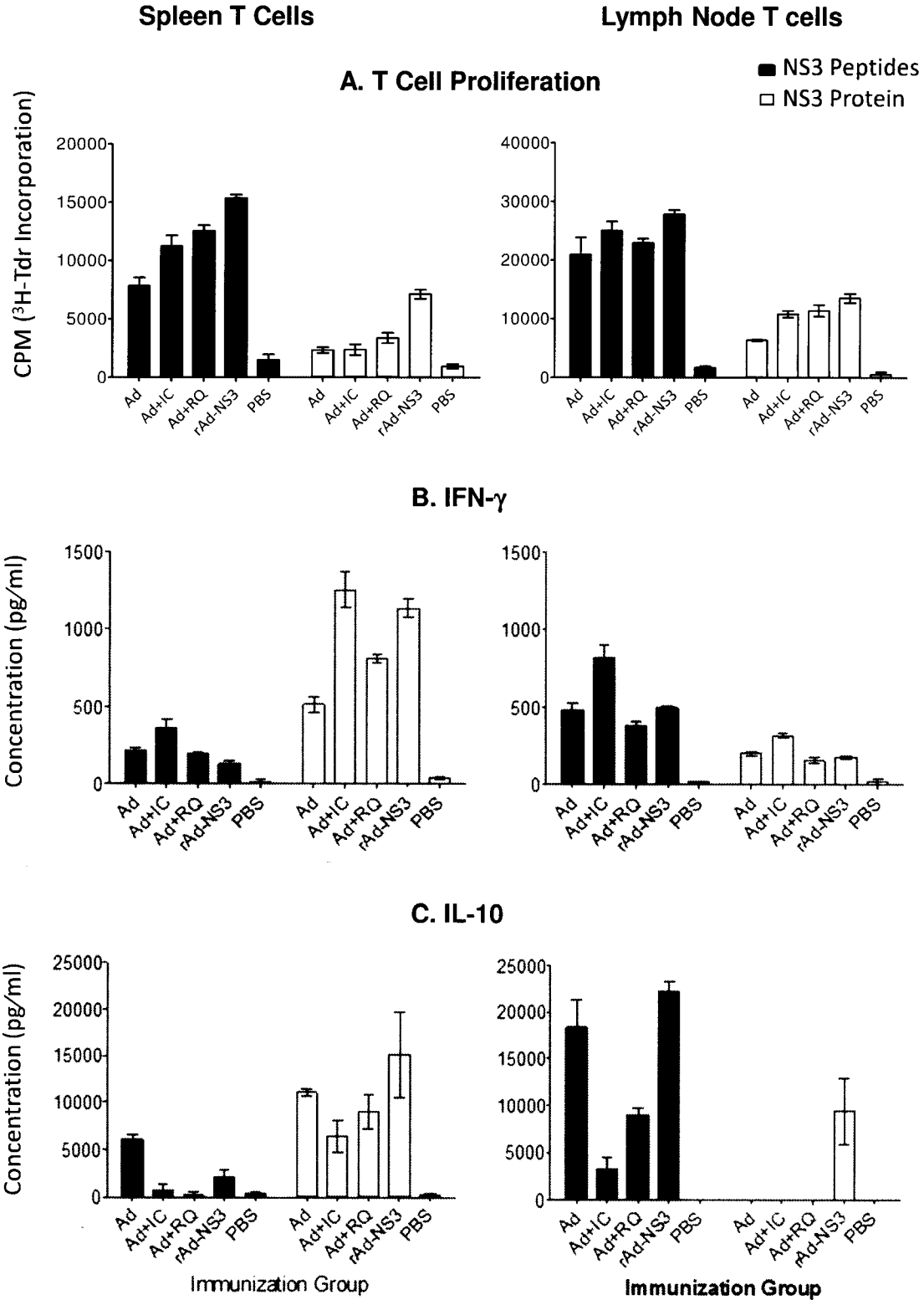


Figure 4: Immunization of mice with adenoviral vector (Ad and rAd-NS3) twice intramuscularly in the absence or presence of adjuvants induces cross reactive cellular immune responses against HCV NS5

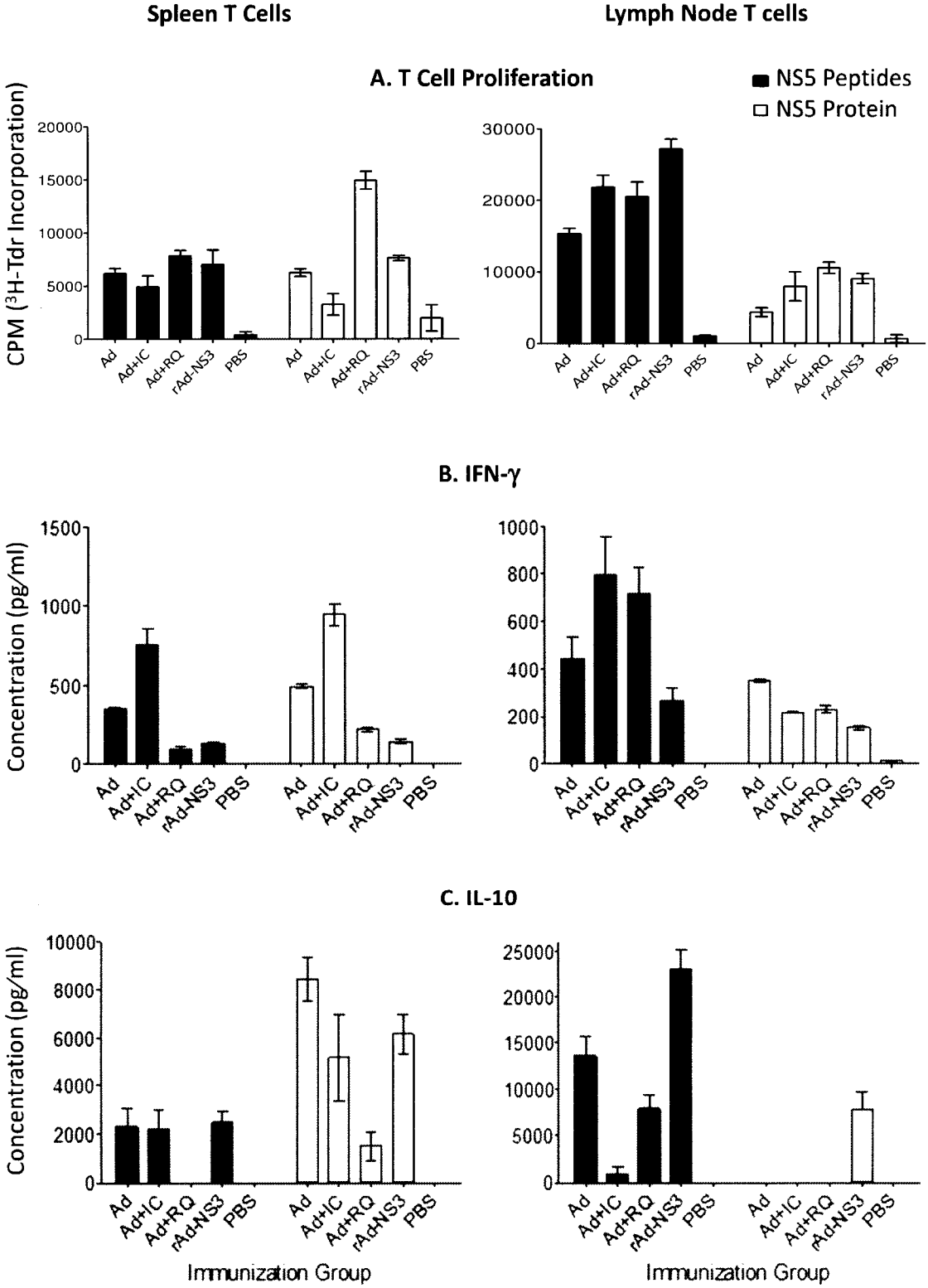


Figure 5: Immunization of mice with adenoviral vector (Ad and rAd-NS3) twice intramuscularly in the absence or presence of adjuvants induces cross reactive antibody responses against HCV proteins

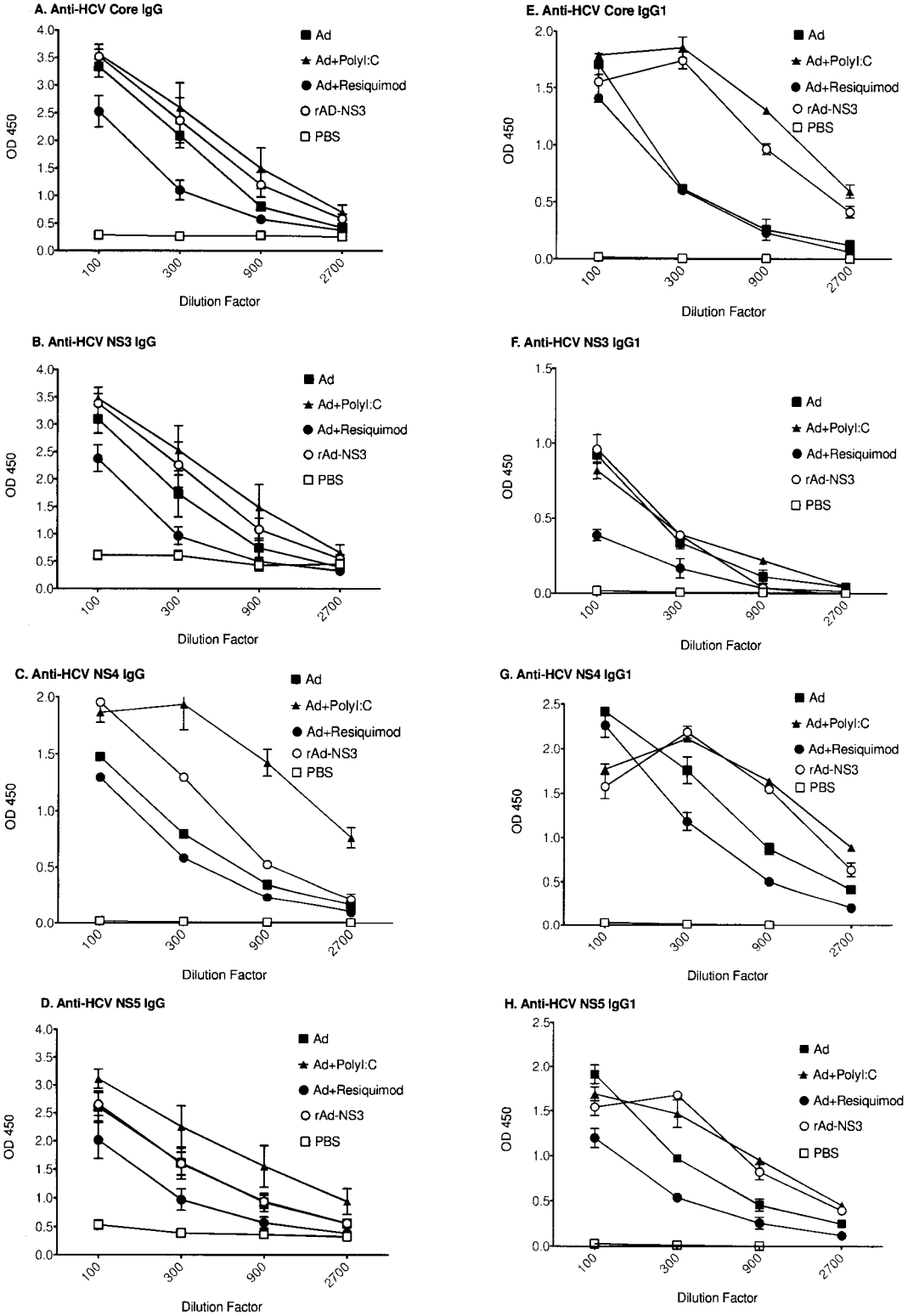


Figure 6 A: Cross-reactive cellular immune responses against HCV antigens in mice immunized twice with adenoviral vector (Ad) through different routes of immunization.

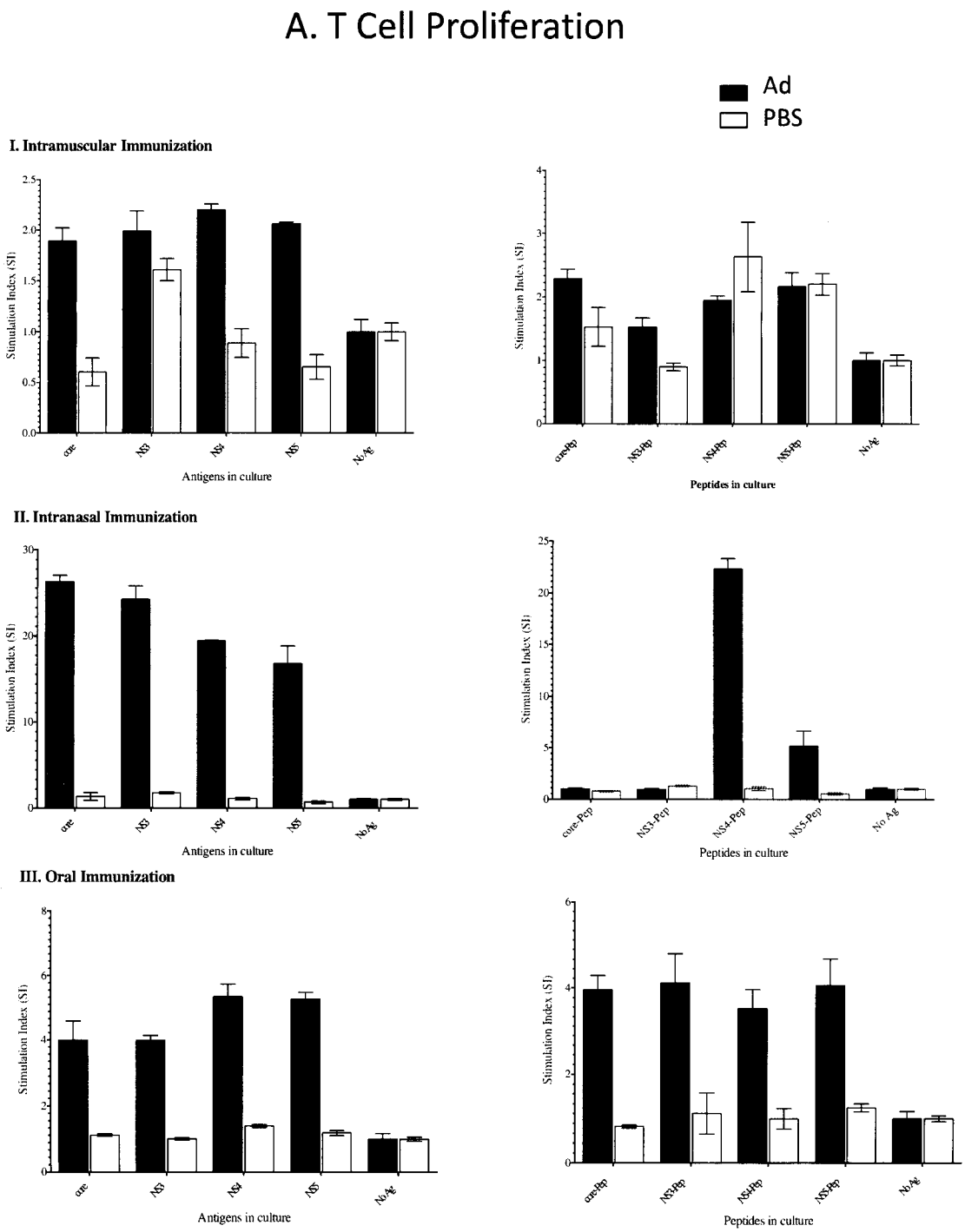


Figure 6B: Cross-reactive cytokine responses against HCV proteins in mice immunized twice with adenoviral vector through different routes of immunization.

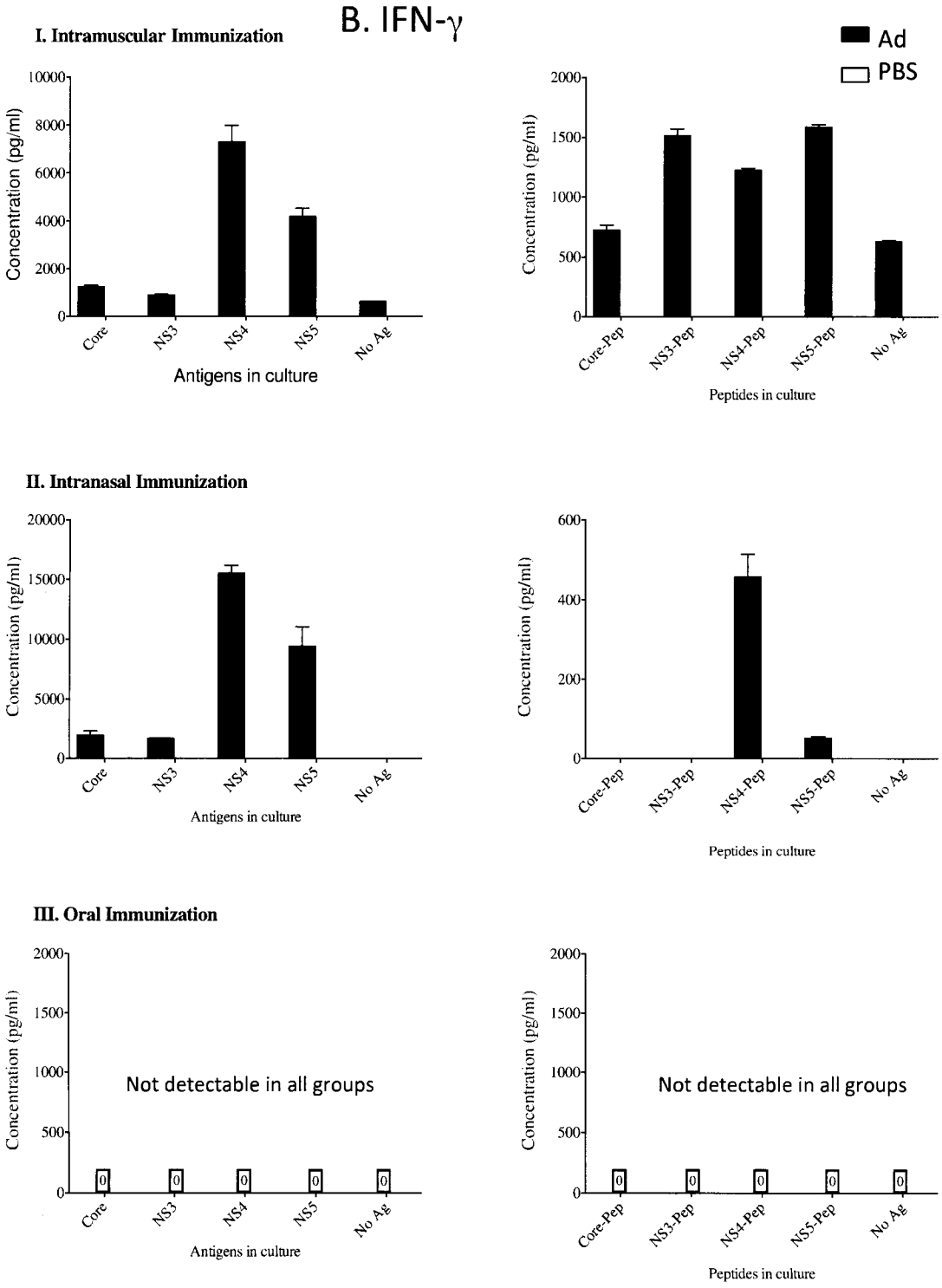


Figure 6C: Cellular immune responses against HCV proteins in mice upon single intramuscular (i.m.) or intranasal (i.n.) immunization with different doses of Ad or rAd-NS3: Antigen specific T cell proliferation of splenocytes and lymph nodes

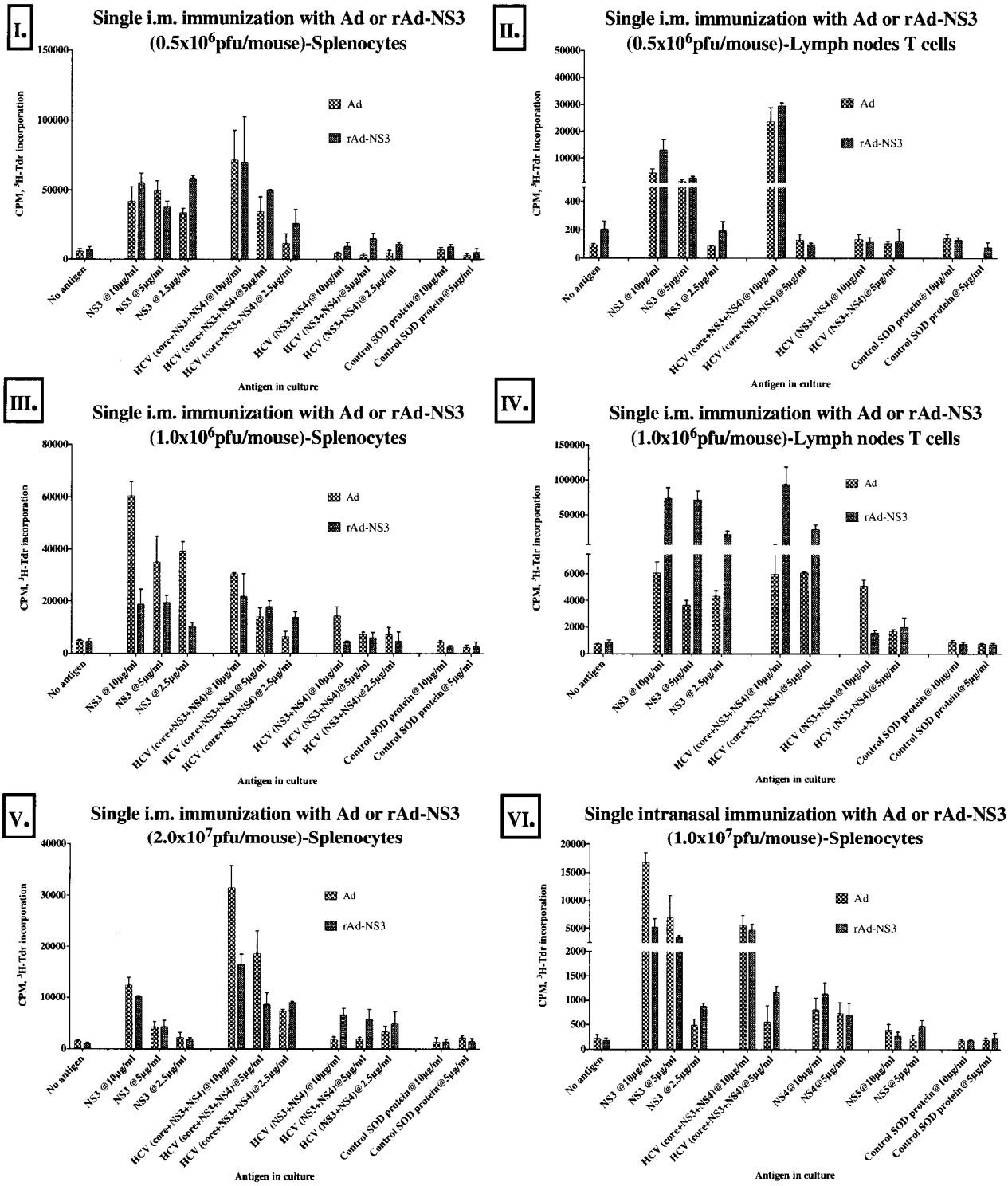


Figure 7: Cross-reactive T cell proliferation and cytokine responses against HCV Core peptides in mice after two intramuscular (i.m.) immunizations with adenoviral vector (Ad)

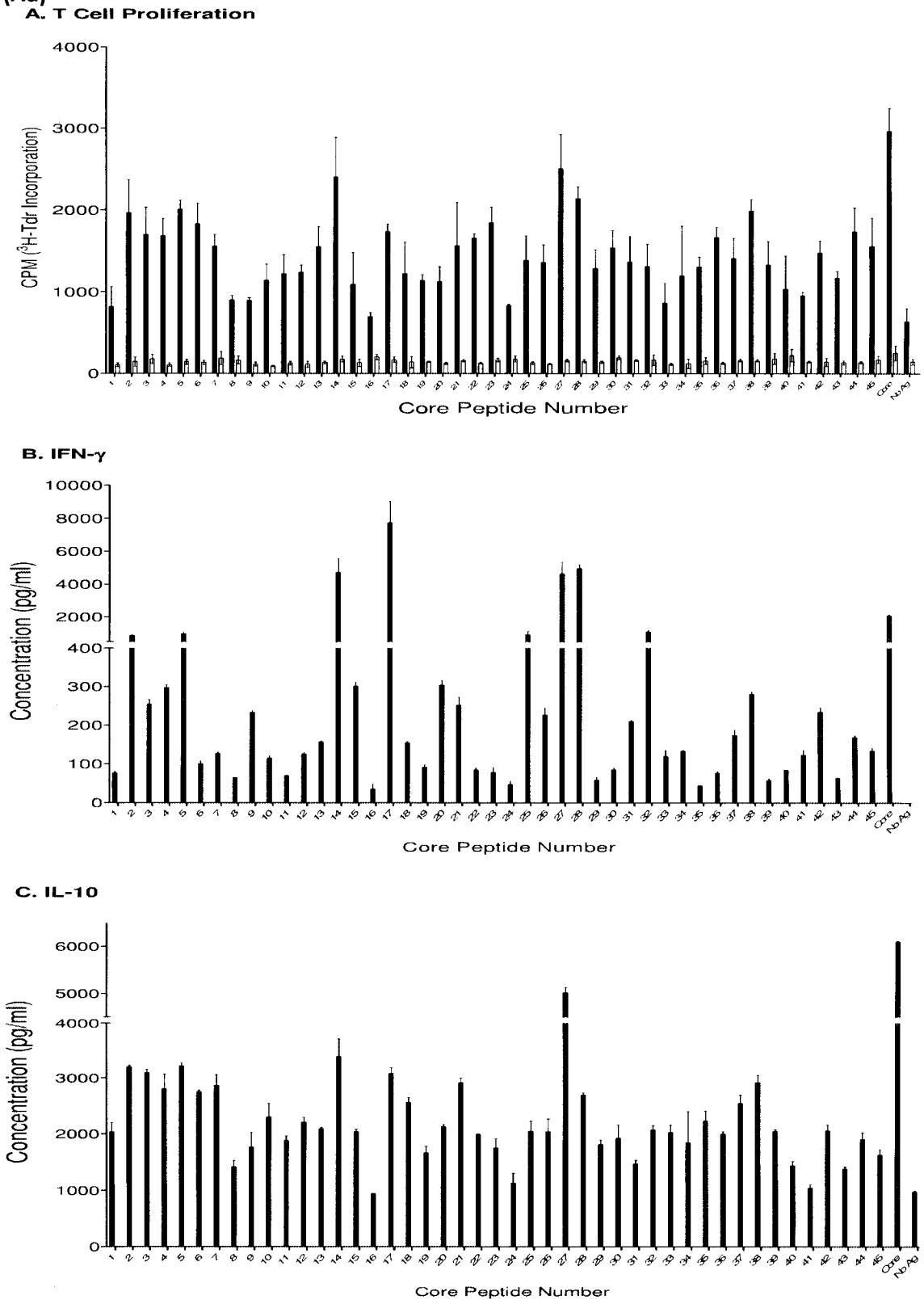


Figure 8: Cross-reactive T cell proliferation and cytokine responses against HCV F peptides in mice after two I.M. immunizations with adenoviral vector (Ad)

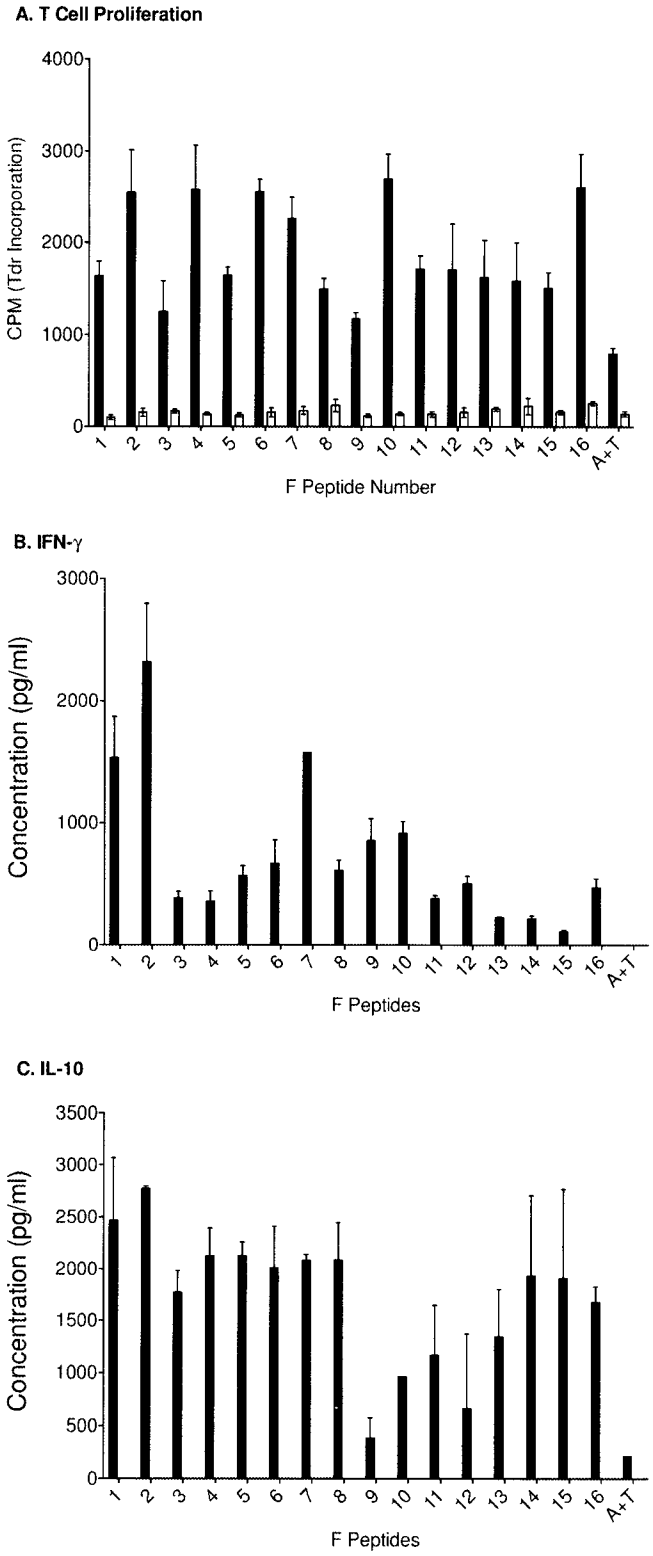


Figure 9: Cross-reactive T cell proliferation and cytokine responses against HCV NS3 peptides in mice after two I.M. immunizations with adenoviral vector (Ad)

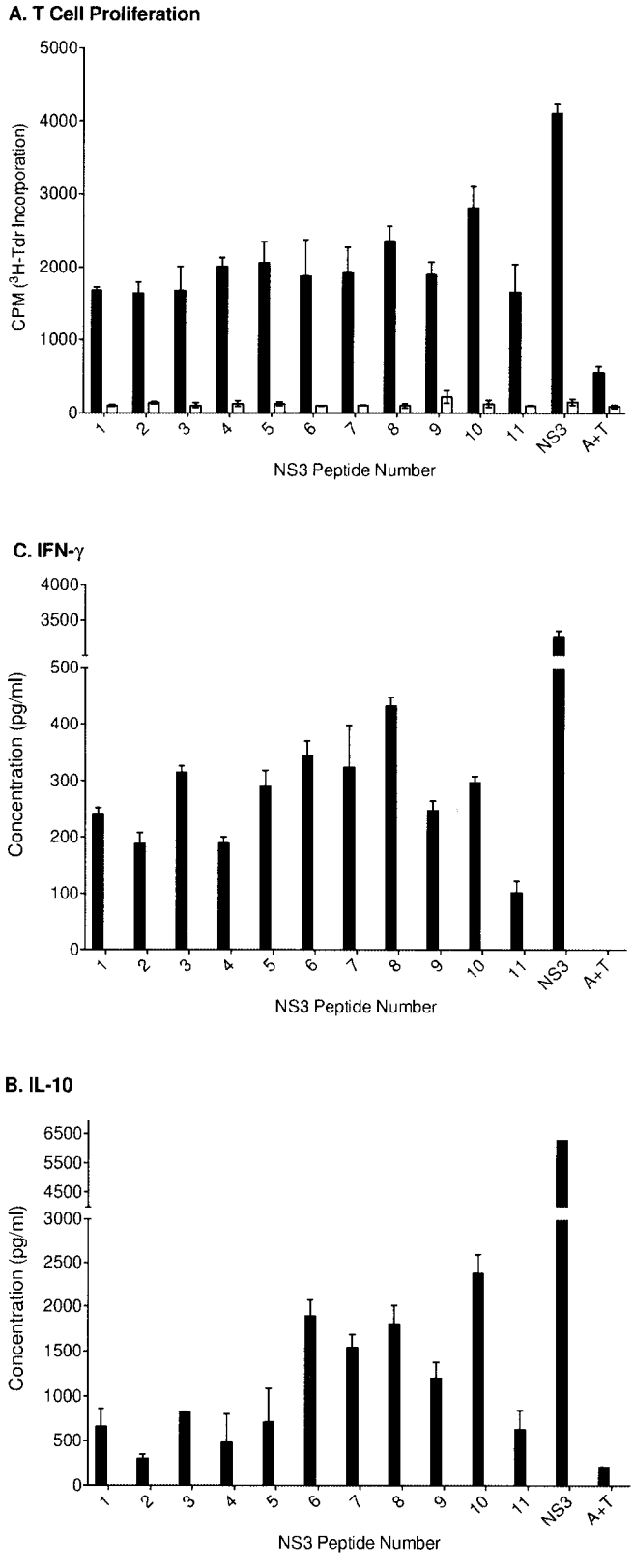


Figure 10: Cross-reactive T cell proliferation and cytokine responses against HCV NS4 peptides in mice after two I.M. immunizations with adenoviral vector (Ad)

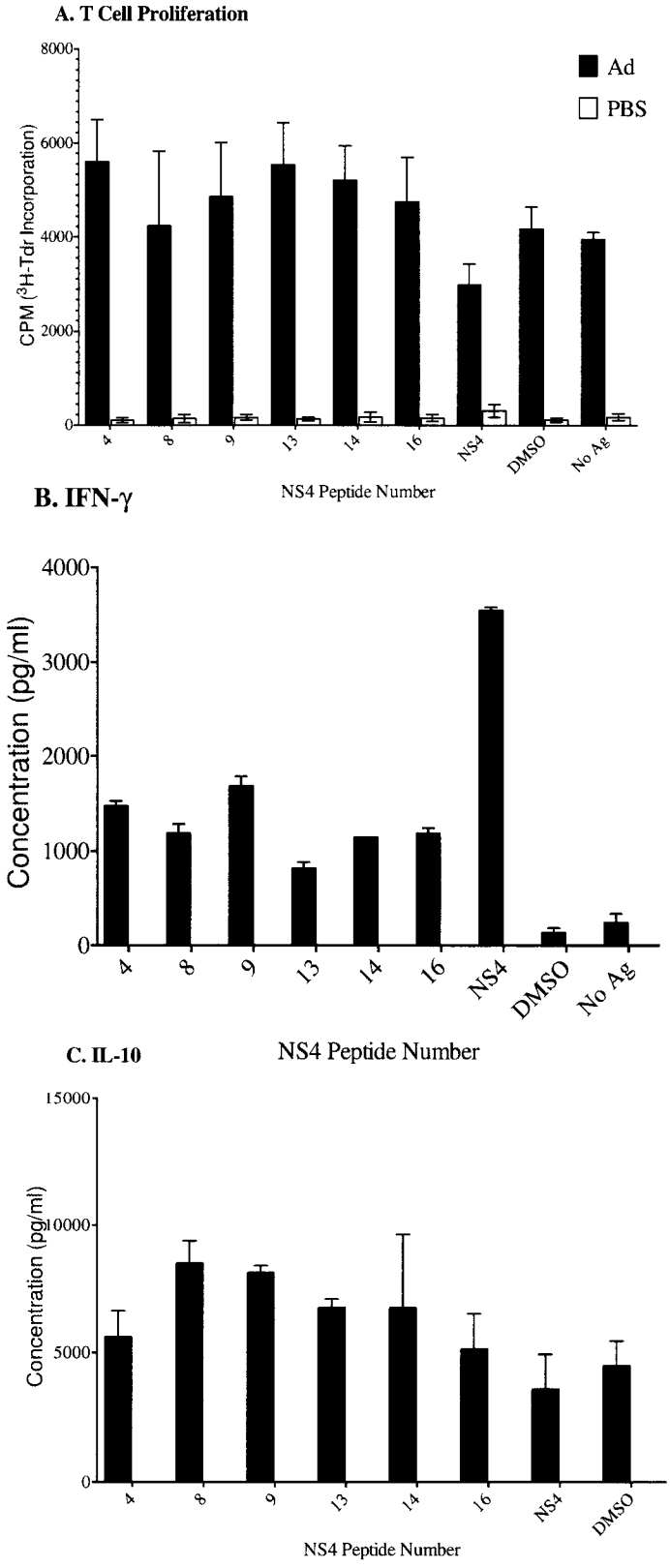


Figure 11: Cross-reactive T cell proliferation and cytokine responses against HCV NS5A peptides in mice after two I.M. immunizations with adenoviral vector (Ad)

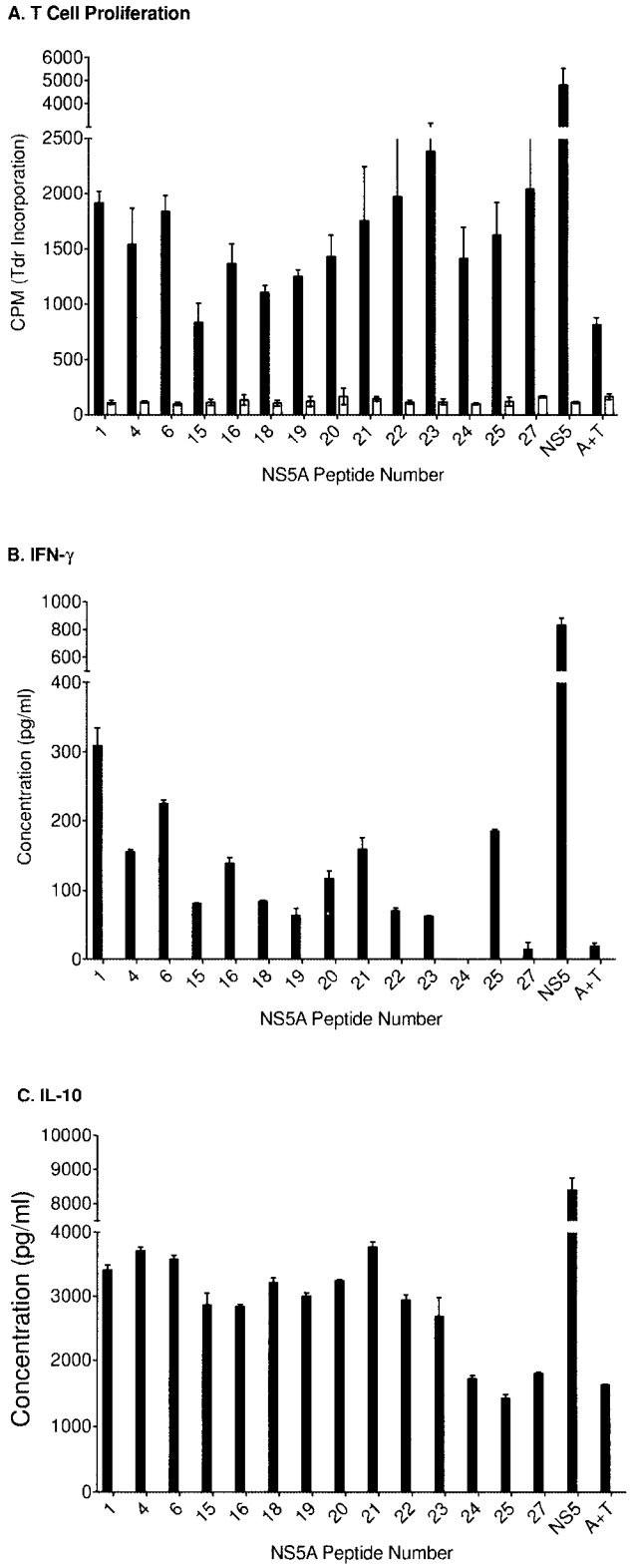


Figure 12: Cross-reactive T cell proliferation and cytokine responses against HCV NS5B peptides in icme after two I.M. immunizations with adenoviral vector (Ad)

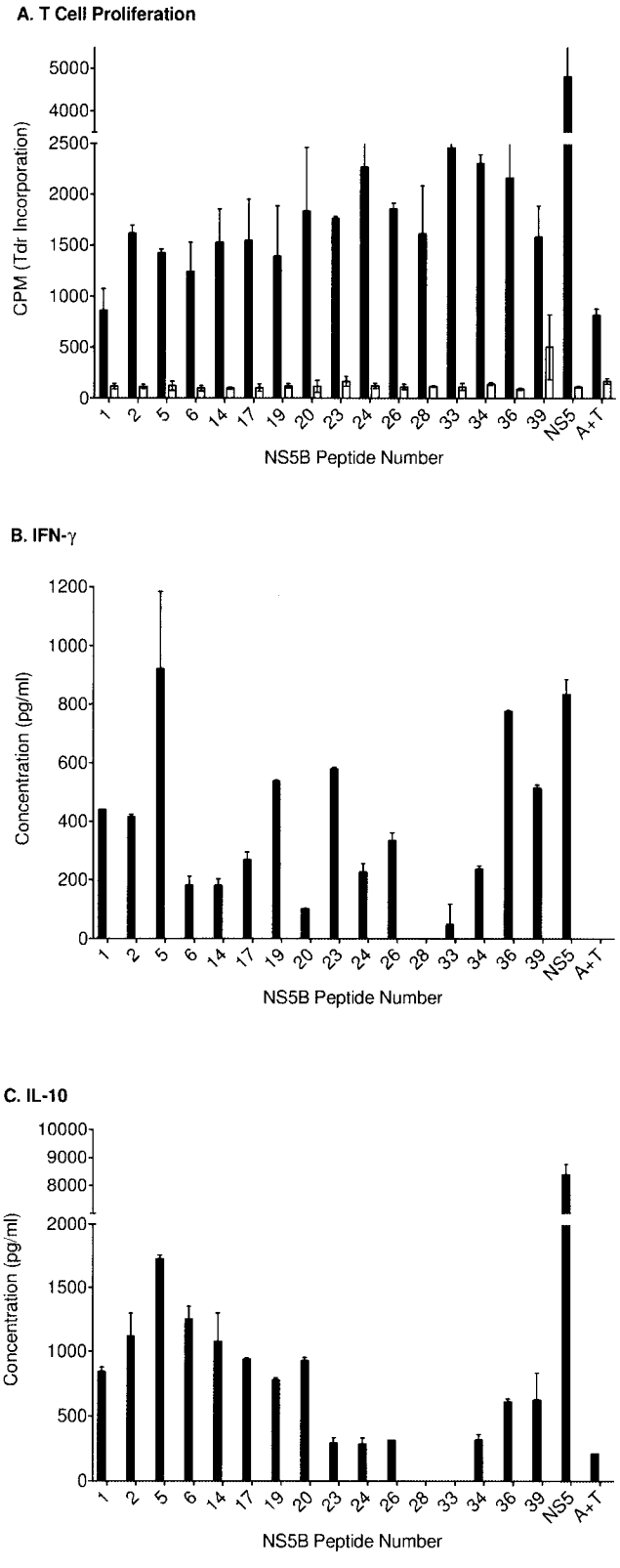
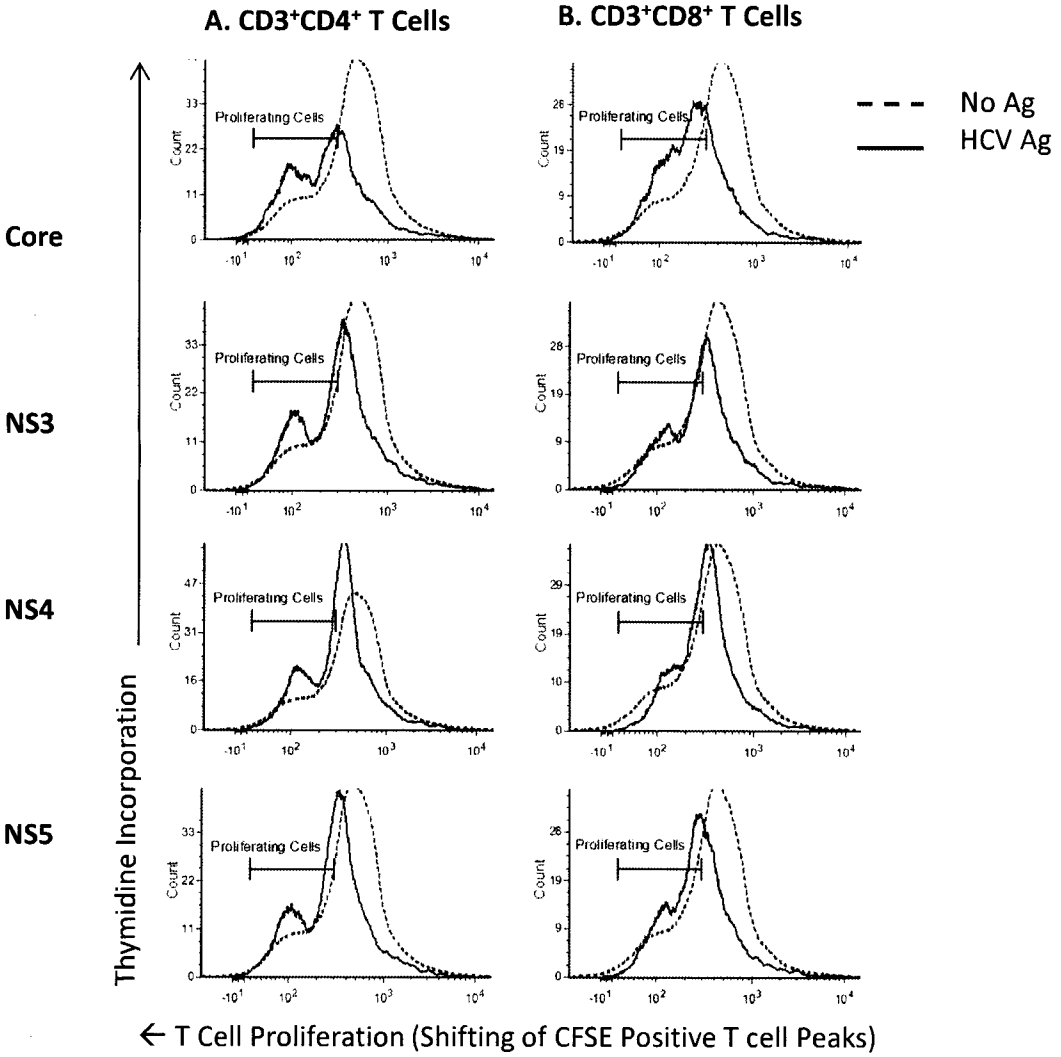
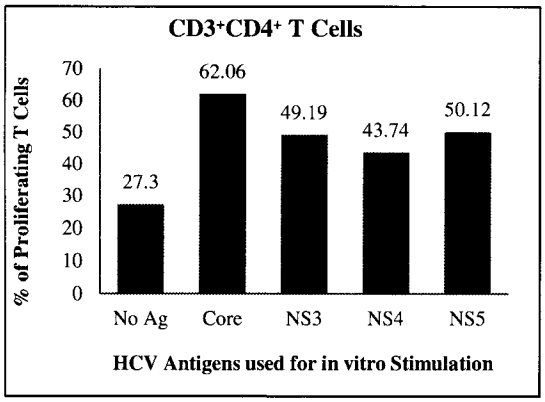


Figure 13A,B: Proliferation of spleen derived CD4+ (panel A) and CD8+ (panel B) T cells from adenoviral vector immunized mice in response to HCV proteins (by CFSE dilution assay)





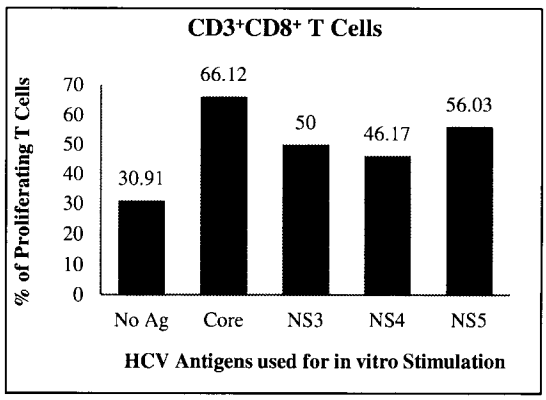


Figure 13C,D: Intracellular IFN-γ and IL-10 expression profile of CD4+ (panel C) and CD8+ (panel D) T cells from adenoviral vector (Ad) immunized mice stimulated with HCV proteins.

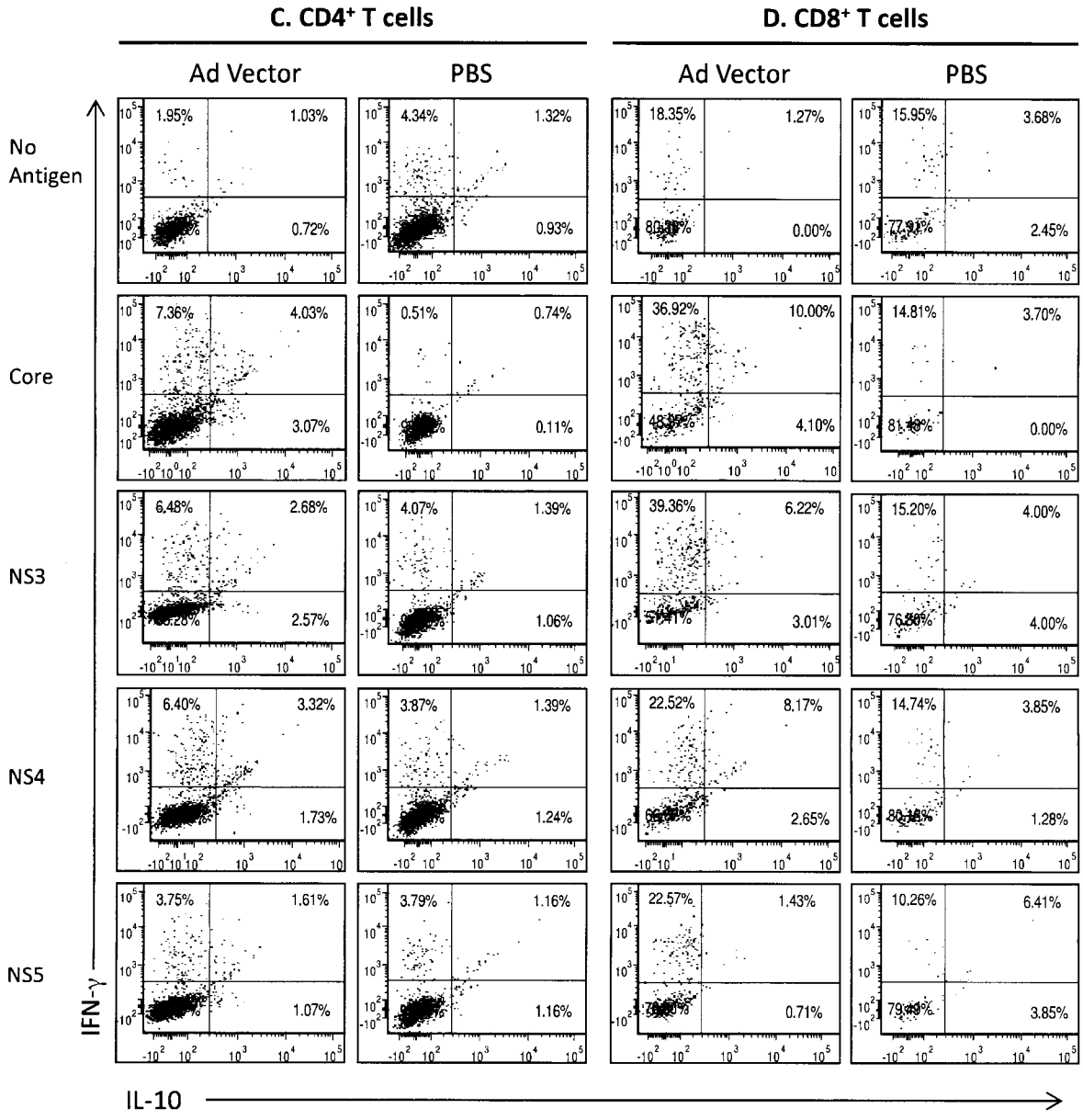


Figure 14A, B: Proliferation of spleen derived CD4+ (Panel A) and CD8+ (B) T cells against specific HCV peptides from Adenoviral vector immunized mice (CFSE dilution assay)

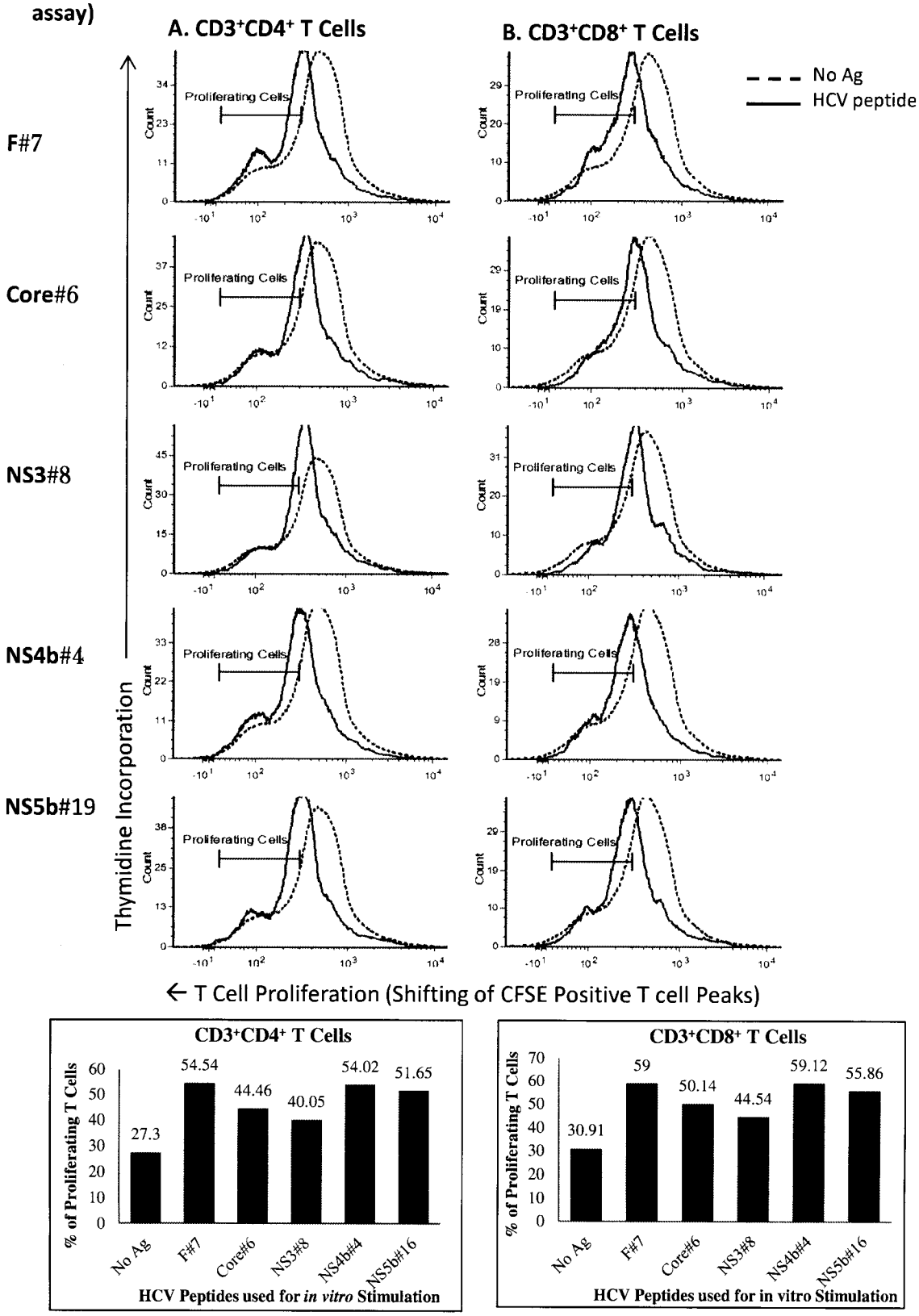


Figure 14C, D: Intracellular IFN-γ and IL-10 expression profile of CD4+ (panel C) and CD8+ (panel D) T cells from adenoviral vector (Ad) immunized mice stimulated with specific synthetic peptides derived from HCV antigens.

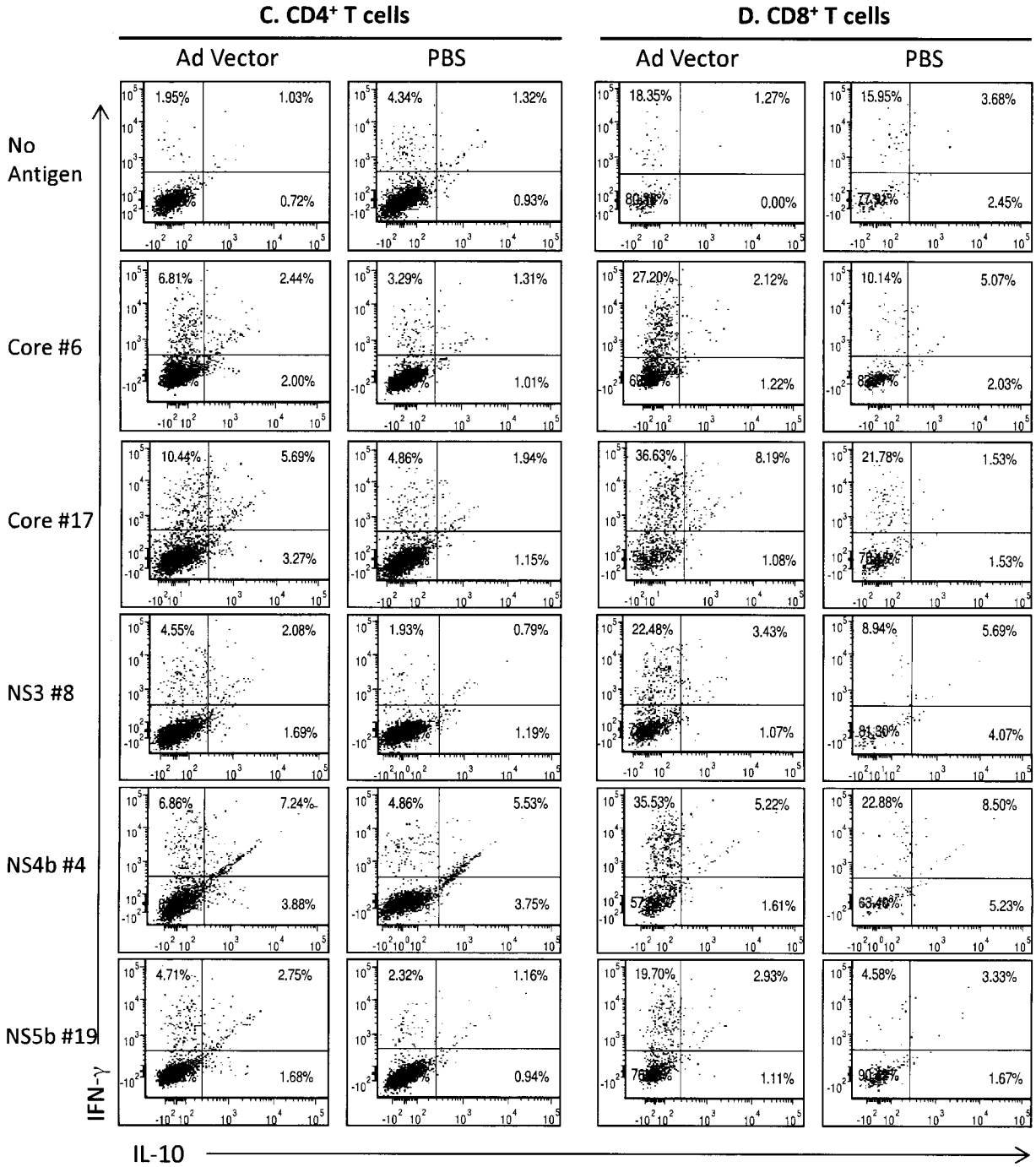


Figure 15: Cytotoxic activity of T cells derived from mice immunized with adenoviral vector against HCV peptides loaded EL-4 target cells

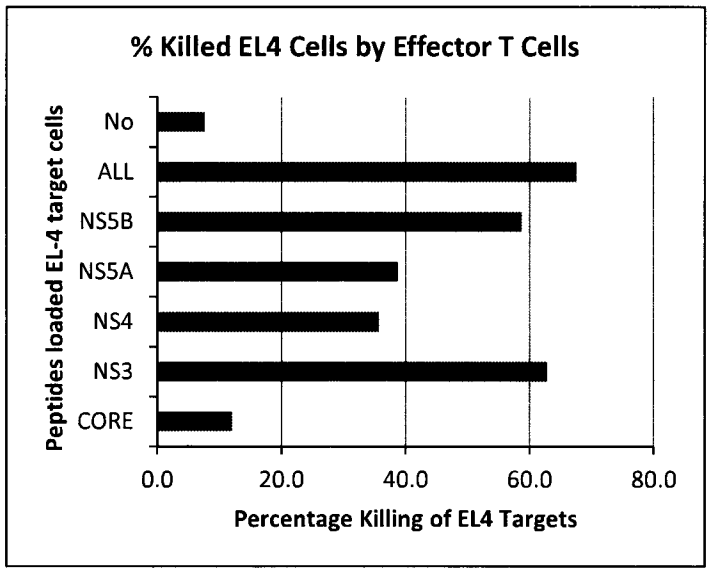
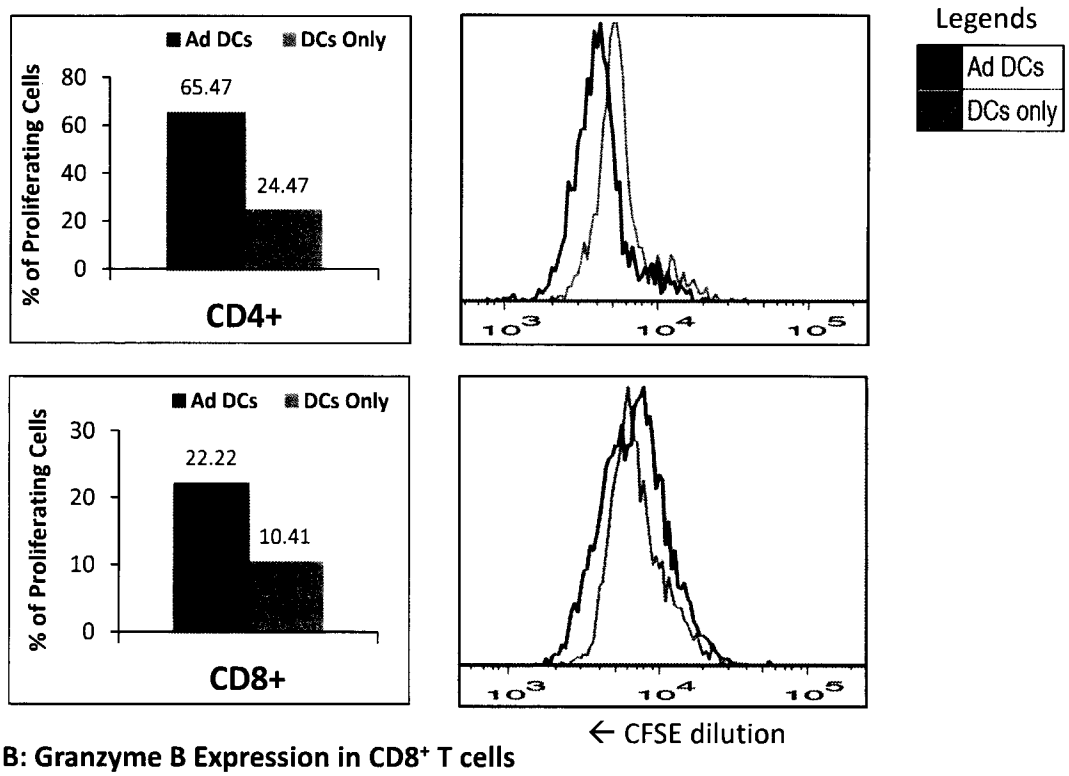
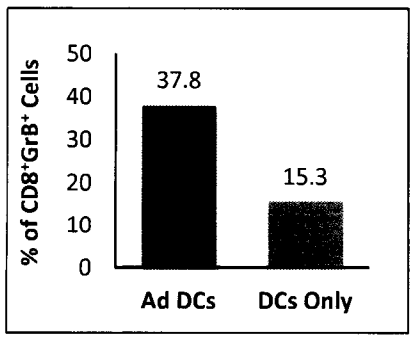


Figure 16: Immunization with bone marrow derived dendritic cells infected with adenovirus vector (Ad) also induces cross reactive HCV antigen (NS5) specific T cell proliferation and Granzyme B responses

A: CD4⁺ and CD8⁺ T cell Proliferation



B: Granzyme B Expression in CD8⁺ T cells



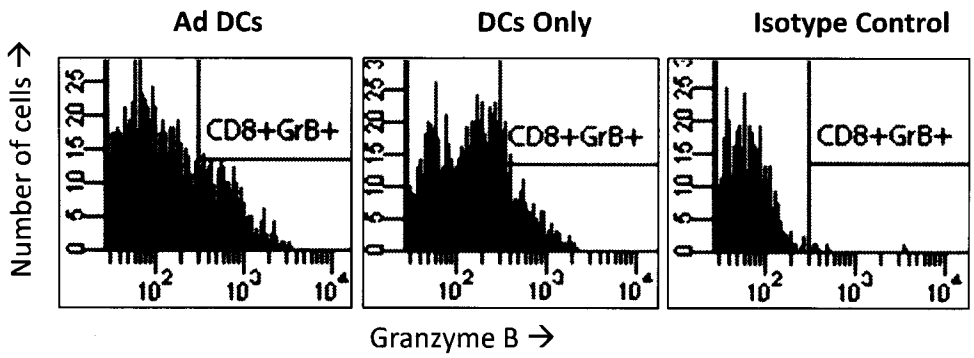


Figure 17: A. Mice immunized intramuscularly twice with adenoviral vector (Ad) in the absence or presence of poly I:C and challenged with chimeric Vac-HCV NS3-NS5 have reduced viral loads

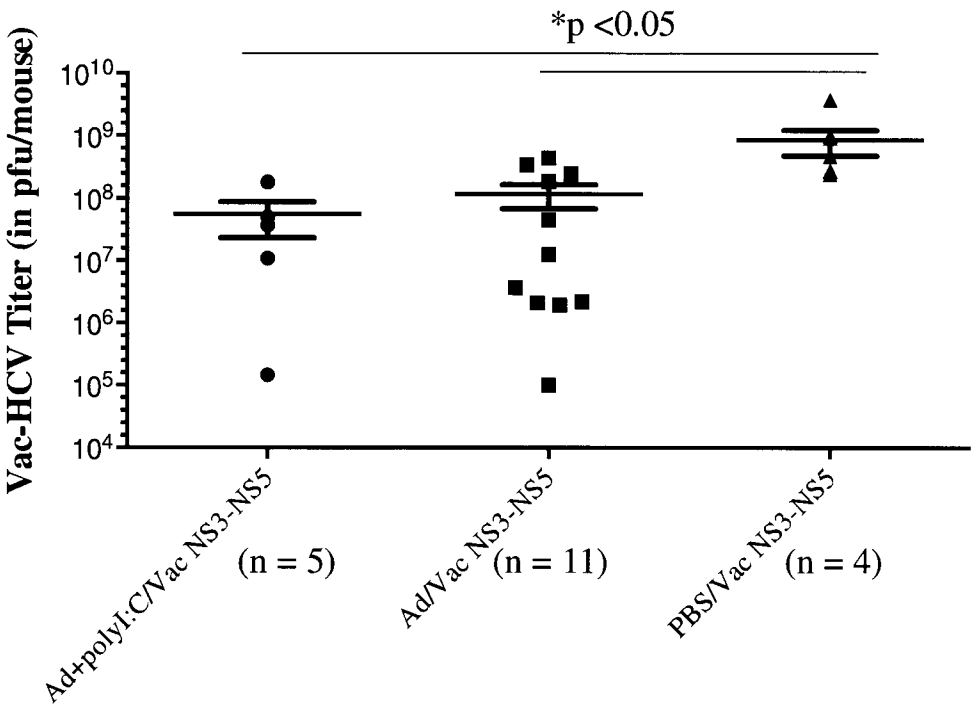


Figure 17: B. Mice immunized with adenoviral vector (Ad) or HEK cell lysate supernatant and challenged with wild-type Vaccinia (WT-Vac) or chimeric Vaccinia-HCV Core-NS3 (Vac-Core-NS3) after 8 days of two i.m. immunizations.

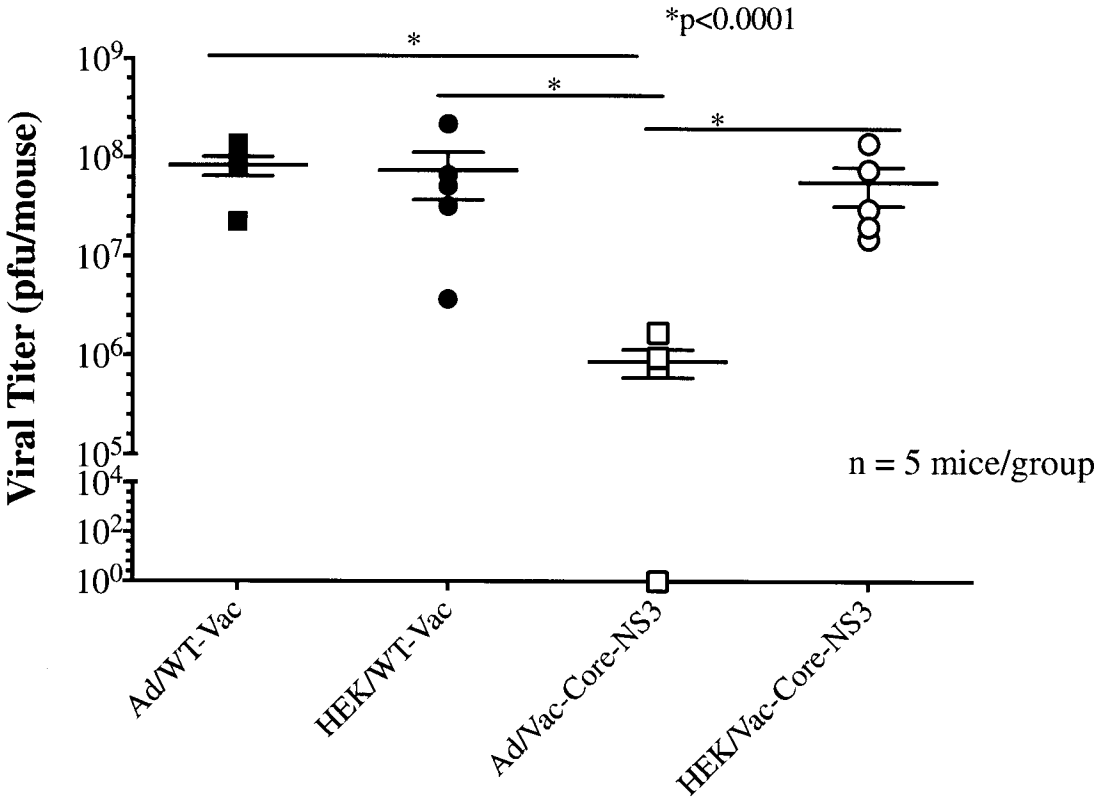


Figure 18. Table 1: Frequency of HCV Core peptides and number of Ad proteins which show high homology score(>25)

1	Core-1	1-15	MSTNPKPQRKTKRNT	5	2	0	0	0	0
2	Core-2	5-19	PKPQRKTKRNTNRRP	10	2	1	0	0	0
3	Core-3	9-23	RKTKRNTNRRPQDVK	8	3	0	0	0	0
4	Core-4	13-27	RNTNRRPQDVKFPGG		1	1	0	0	0
5	Core-5	17-31	RRPQDVKFPGGGQIV	10	2	0	0	0	0
6	Core-6	21-35	DVKFPGGGQIVGGVY	5	3	0	0	0	
7	Core-7	25-39	PGGGQIVGGVYLLPR	10	3	0	0	0	0
8	Core-8	29-43	QIVGGVYLLPRRGPR		6	3	0	0	0
9	Core-9	33-47	GVYLLPRRGPRLGVR	10	7	1	0		0
10	Core-10	37-51	LPRRGPRLGVRATRK	8		0	0		0
11	Core-11	41-55	GPRLGVRATRKTSER		3	2	0	0	0
12	Core-12	45-59	GVRATRKTSERSQPR	10	5	1	0	0	0
13	Core-13	49-63	TRKTSERSQPRGRRQ	8	6	1	0	0	0
14	Core-14	53-67	SERSQPRGRRQPIPK	7	6	1	0	0	0
15	Core-15	57-71	QPRGRRQPIPKARRP	7	7	2	0	0	0
16	Core-16	61-75	RRQPIPKARRPEGRT	8		0	0	0	0
17	Core-17	65-79	IPKARRPEGRTWAQP	6	1	1	0	0	0
18	Core-18	69-83	RRPEGRTWAQPGYPW	6	2	1	0	0	0
19	Core-19	73-87	GRTWAQPGYPWPLYG	3	3	0	0	0	0
20	Core-20	77-91	AQPGYPWPLYGNEGC	4	1	1	0	0	0
21	Core-21	81-95	YPWPLYGNEGCGWAG	6	3	1	0	0	0
22	Core-22	85-99	LYGNEGCGWAGWLLS	5	4	0	0	0	0
23	Core-23	89-103	EGCGWAGWLLSPRGS	5	3	1	0	0	0
24	Core-24	93-107	WAGWLLSPRGSRPSW	5	2	1	0	0	0
25	Core-25	97-111	LLSPRGSRPSWGPTD	9	3	1	0	0	0
26	Core-26	101-115	RGSRPSWGPTDPRRR	1	2	1	0	0	0
27	Core-27	105-119	PSWGPTDPRRRSRNL	8	7	2	0	0	0
28	Core-28	109-123	PTDPRRRSRNLGKVI		5	1	0	0	0
29	Core-29	113-127	RRRSRNLGKVIDTLT		3	0	0	1	0
30	Core-30	117-131	RNLGKVIDTLTCGFA	3	2	0	0	0	0
31	Core-31	121-135	KVIDTLTCGFADLMG	3	2	0	0	0	0
32	Core-32	125-139	TLTCGFADLMGYIPL	4	1	0	0	0	0
33	Core-33	129-143	GFADLMGYIPLVGAP	4	4	0	0	1	0
34	Core-34	133-147	LMGYIPLVGAPLGGA	4	3	0	0	1	0
35	Core-35	137-151	IPLVGAPLGGAARAL	9	4	3	0	0	0
36	Core-36	141-155	GAPLGGAARALAHGV	7	6		0	1	0
37	Core-37	145-159	GGAARALAHGVRVLE	1	8	2	0	0	0
38	Core-38	149-163	RALAHGVRVLEDGVN	10	3	0	0	0	0
39	Core-39	153-167	HGVRVLEDGVNYATG	8	2	0	0	0	0
40	Core-40	157-171	VLEDGVNYATGNLPG	9	1	0	0	0	0
41	Core-41	161-175	GVNYATGNLPGCSFS	4	2	1	0	0	0
42	Core-42	165-179	ATGNLPGCSFSIFLL	5	1	1	0	0	0
43	Core-43	169-183	LPGCSFSIFLLALLS	3	0	1	0	1	0
44	Core-44	173-187	SFSIFLLALLSCLTV	4	1	1	0	0	0
45	Core-45	177-191	FLLALLSCLTVPASA	7	0	2	0	0	0
Cummulative Number of Ad Proteins showing homology with each HCV Core Peptide				331	154	39	0	9	1
Total Number of Core Peptides Showing Homology				45	43	27	0	7	1

Figure 19. Table 2: Frequency of HCV F peptides and number of Ad proteins which show high homology score(>25)

1	F-1	1-15	MSTNPKPQRKPNVTP	2			0	0	0
2	F-2	11-25	PNVTPTVAHRTSSSR		2	0	0	0	0
3	F-3	21-35	TSSSRVAVRSLVEFT	7	1	0	0	0	0
4	F-4	31-45	LVEFTCCRAGALDWV	5	1	0	0	0	0
5	F-5	41-55	ALDWVCARRGRLPSG	6	3	0	0	0	0
6	F-6	51-65	RLPSGRNLEVDVSLS	7	3	0	0	0	0
7	F-7	61-75	DVSLSPRHVGPRAGP	9	3		0	0	0
8	F-8	71-85	PRAGPGLSPGTLGPS	9	6		0	0	0
9	F-9	81-95	TLGPSMAMRVAGGRD	5	4	0	0		0
10	F-10	91-105	AGGRDGSCLPVALGL		3	0	0		0
11	F-11	101-115	VALGLAGAPQTPGVG	6	6		0	0	0
12	F-12	111-125	TPGVGRAIWVRSSIP	7	2	0	0	0	0
13	F-13	121-135	RSSIPLRAASPTSWG	8	3	0	0	0	0
14	F-14	131-145	PTSWGTYRSSAPLLE	4	2	0	0	0	0
15	F-15	141-155	APLLEALPGPWRMAS	7	5	0	0		0
16	F-16	148-162	LPGPWRMASGFWKTA	4	0	0	0	0	
Cummulative Number of Ad Proteins showing homology with each HCV Core Peptide				107	52	4	0	3	1
Total Number of Core Peptides Showing Homology				16	15	4	0	3	1

Figure 20. Table 3: Frequency of HCV NS3 peptides and number of Ad proteins which show high homology score(>25)

1	NS3-1	1367-1381	LSTTGEIPFYGKAIP	7	1		0	0	0
2	NS3-2	1411-1425	GINAVAYYRGLDVS	5	0	1		0	0
3	NS3-3	1415-1429	VAYYRGLDVSVIPTS	6		1	0		0
4	NS3-4	1372-1386	EIPFYGKAIPLEVIK	5	2	0	0	0	0
5	NS3-5	1450-1464	SVIDCNTCVTQTVDF	3	0	1	0	0	0
6	NS3-6	1127-1142	SSDLYLVTRHADVIP	5	1	1	0	0	0
7	NS3-7	1621-1635	PTPLLYRLGAVQNEV	6	3	1	0	0	0
8	NS3-8	1467-1482	RRGRTGRGKPGIYRF		3		0	0	0
9	NS3-9	1187-1201	RGVAKAVDFIPVENL	4	1	1	0	0	0
10	NS3-10	1607-1622	MWKCLIRLKPTLHGP	3	2	0	0	0	0
11	NS3-11	1067-1081	QTFLATCINGVCWTV	0	0	0	0	0	0
Cummulative Number of Ad Proteins showing homology with each HCV Core Peptide				54	17	10	3	1	0
Total Number of Core Peptides Showing Homology				10	8	8	1	1	0

Figure 21. Table 4: Frequency of HCV NS4 peptides and number of Ad proteins which show high homology score(>25)

1	NS4a-1	1658-1677	STWVLVGGVLAALAAYCLST	7	0	0	0	0	0
2	NS4a-2	1673-1692	YCLSTGCVVIVGRIVLSGKP	0	1	0	0	0	0
3	NS4a-3	1688-1707	LSGKPAIIPDREVLYQEFDE	3	1	0	0	0	0
4	NS4a-4	1703-1721	QEFDEMEECSQHLPYIEQG	0	1	0	0	0	0
1	NS4b-1	1712-1731	SQHLPYIEQGMMLAEQFKQK	3	0	0	0	0	0
2	NS4b-2	1727-1746	QFKQKALGLLQTASRHAEVI	2	0	0	0	0	0
3	NS4b-3	1742-1761	HAEVITPAVQTNWQKLEVFW	2	0	0	0	0	0
4	NS4b-4	1757-1776	LEVFWAKHMWNFISGIQYLA	2		0	0	0	0
5	NS4b-5	1772-1791	IQYLAGLSTLPGNPAIASLM	6	0	0	0	0	0
6	NS4b-6	1787-1806	IASLMAFTAAVTSPLTTGQT	6	0	0	0	0	0
7	NS4b-7	1802-1821	TTGQTLLFNILGGWVAAQLA	5	0	0	0	0	0
8	NS4b-8	1817-1836	AAQLAAPGAATAFVGAGLAG	5	0		0	0	0
9	NS4b-9	1832-1851	AGLAGAAIGSVGLGKVLVDI		1	0	0	0	0
10	NS4b-10	1847-1866	VLVDILAGYGAGVAGALVAF	3	0	0	0	0	0
11	NS4b-11	1862-1881	ALVAFKIMSGEVPSTEDLVN	0	1	0	0	0	0
12	NS4b-12	1877-1896	EDLVNLLPAILSPGALVVGV	6	0	0	0	0	0
13	NS4b-13	1892-1911	LVVGVVCAAILRRHVGPGEG	0	0		0	0	0
14	NS4b-14	1907-1926	GPGEGAVQWMNRLIAFASRG	5		0	0	0	0
15	NS4b-15	1922-1941	FASRGNHVSPTHYVPESDAA	0	0	0	0	0	0
16	NS4b-16	1937-1956	ESDAAARVTAILSSLTVTQL		0	0	0	0	0
Cummulative Number of Ad Proteins showing homology with each HCV Core Peptide				75	9	2	0	0	0
Total Number of Core Peptides Showing Homology				15	7	2	0	0	0

Figure 22. Table 5a: Frequency of HCV NS5A peptides and number of Ad proteins which show high homology score(>25)

1	NS5a-1	1972-1991	CSGSWLRDIWDWICEVLSDF	3	0	1	0	0	0
2	NS5a-2	1987-2006	VLSDFKTWLKAKLMPQLPGI	3	0	0	0	0	0
3	NS5a-3	2002-2021	QLPGIPFVSCQRGYRGVWRG	5	2	1	0	0	0
4	NS5a-4	2017-2036	GVWRGDGIMHTRCHCGAEIT	3	1		0	0	0
5	NS5a-5	2032-2051	GAEITGHVKNGTMRIVGPRT	5	0	0	0	0	0
6	NS5a-6	2047-2066	VGPRTCRNMWSGTFPINAYT	3	0	0	0	0	0
7	NS5a-7	2062-2081	INAYTTGPCTPLPAPNYKFA	5	0	0	0	0	0
8	NS5a-8	2077-2096	NYKFALWRVSAEEYVEIRRV	3	0	0	0	0	0
9	NS5a-9	2092-2111	EIRRVGDFHYVSGMTTDNLK	3	0	0	0	0	0
10	NS5a-10	2107-2126	TDNLKCPCQIPSPEFFTELD	1	0	0	0	0	0
11	NS5a-11	2122-2141	FTELDGVRLHRFAPPCKPLL	4	0	0	0	0	0
12	NS5a-12	2137-2156	CKPLLREEVSFRVGLHEYPV	6	0	0	0	0	0
13	NS5a-13	2152-2171	HEYPVGSQLPCEPEPDVAVL	5	0	0	0	0	0
14	NS5a-14	2167-2186	DVAVLTSMLTDPSHITAEAA	5	1	0	0	0	0
15	NS5a-15	2182-2201	TAEAAGRRLARGSPPSMASS		0	0	0	0	0
16	NS5a-16	2197-2216	SMASS SASQLSAPSLKATCT	4	0	0		0	0
17	NS5a-17	2212-2231	KATCTANHDSPDAELIEANL	7	0	0	0	0	0
18	NS5a-18	2227-2246	IEANLLWRQEMGGNITRVES	4	0	0	0	0	0
19	NS5a-19	2242-2261	TRVESENKVVILDSFDPLVA	4	1	0	0	0	0
20	NS5a-20	2257-2276	DPLVAEEDEREVSVPAEILR	4	1	1	0	0	0
21	NS5a-21	2272-2291	AEILRKSRRFARALPVWARP	8	1	1	0	0	0
22	NS5a-22	2287-2306	VWARPDYNPPLVETWKKPDY	4	0	0	0	0	0
23	NS5a-23	2302-2321	KKPDYEPPVVHGCPLPPPRS	9	1		0	0	0
24	NS5a-24	2317-2336	PPPRSPPVPPPRKKRTVVLT			0		0	0
25	NS5a-25	2332-2351	TVVLTESTLSTALAELATKS	8	0	0	0	0	0
26	NS5a-26	2347-2366	LATKSFGSSSTSGITGDNTT	6	0	0	0	0	0
27	NS5a-27	2362-2381	GDNTTTSSEPAPSGCPPDSD	7	2	0	0	0	0
28	NS5a-28	2377-2396	PPDSDVESYSSMPPLEGEPG	9	1	1	0	0	0
29	NS5a-29	2392-2420	EGEPGDPDLSDGSWSTVSSG ADTEDVVCC	1	0	0	0	0	0
Cummulative Number of Ad Proteins showing homology with each HCV Core Peptide				158	15	9	2	0	0
Total Number of Core Peptides Showing Homology				29	10	7	2	0	0

Figure 23. Table 5b: Frequency of HCV NS5B peptides and number of Ad proteins which show high homology score(>25)

1	NS5b-1	2421-2440	SMSYSWTGALVTPCAAEEQK	2	0	0	0	0	0
2	NS5b-2	2436-2455	AEEQKLPINALSNSLLRHHN	5	0	0	0	0	0
3	NS5b-3	2451-2470	LRHHNLVYSTTSRSACQRQK	3	0	0	0	0	0
4	NS5b-4	2466-2485	CQRQKKVTFDRLQVLDSHYQ	2	0	0	0	0	0
5	NS5b-5	2481-2500	DSHYQDVLKEVKAAASKVKA	4	9	0	0	0	0
6	NS5b-6	2496-2515	SKVKA NLLSVEEACSLTPPH	6	0	0	0	0	0
7	NS5b-7	2511-2530	LTPPHSAKSKFGYGAKDVRC	1	0	0	0	0	0
8	NS5b-8	2526-2545	KDVRCHARKAVAHINSVWKD	3	0	0	0	0	0
9	NS5b-9	2541-2560	SVWKDLLEDSVTPIDTTIMA	2	0	0	0	0	0
10	NS5b-10	2556-2575	TTIMAKNEVFCVQPEKGGRK	7	0	0	0	0	0
11	NS5b-11	2571-2590	KGGRKPARLIVFPDLGVRVC	4	0	0	0	0	0
12	NS5b-12	2586-2605	GVRVCEKMALYDVVSKLPLA	3	0	0	0	0	0
13	NS5b-13	2601-2620	KLPLAVMGSSYGFQYSPGQR	9	0	0	0	0	0
14	NS5b-14	2616-2635	SPGQRVEFLVQAWKSKKTPM	3	0	0	0	0	0
15	NS5b-15	2631-2650	KKTPMGFSYDTRCFDSTVTE	3	0	0	0	0	0
16	NS5b-16	2646-2665	STVTESDIRTEEAIYQCCDL	2	0	0	0	0	0
17	NS5b-17	2661-2680	QCCDLDPQARVAIKSLTERL	6	9	0	0	0	0
18	NS5b-18	2676-2695	LTERLYVGGPLTNSRGENCG	9	0	0	0	0	0
19	NS5b-19	2691-2710	GENCGYRRCRASGVLTTSCG	7	0	9	0	0	0
20	NS5b-20	2706-2725	TTSCGNTLTCYIKARAACRA	3	0	0	0	0	0
21	NS5b-21	2721-2740	AACRAAGLQDCTMLVCGDDL	3	0	9	0	0	0
22	NS5b-22	2736-2755	CGDDLVVICESAGVQEDAAN	3	0	0	0	0	0
23	NS5b-23	2751-2770	EDAANLRAFTEAMTRYSAPP	6	9	0	0	0	0
24	NS5b-24	2766-2785	YSAPPGDPPQPEYDLELITS	9	0	0	0	0	0
25	NS5b-25	2781-2800	ELITSCSSNVSVAHDGAGKR	4	0	0	0	0	0
26	NS5b-26	2796-2815	GAGKRVYYLTRDPTTPLARA	8	0	0	0	0	0
27	NS5b-27	2811-2830	PLARAAWETARHTPVNSWLG	3	0	0	0	0	0
28	NS5b-28	2826-2845	NSWLGNIIMFAPTLWARMIL	2	0	0	0	0	0
29	NS5b-29	2841-2860	ARMILMTHFFSVLIARDQLE	1	0	0	0	0	0
30	NS5b-30	2856-2875	RDQLEQALNCEIYGACYSIE	1	9	0	0	0	0
31	NS5b-31	2871-2890	CYSIEPLDLPPIIQRLHGLS	4	0	0	9	0	0
32	NS5b-32	2886-2905	LHGLSAFSLHSYSPGEINRV	6	0	0	0	0	0
33	NS5b-33	2901-2920	EINRVAACLRKLGVPPLRAW	7	9	9	0	0	0
34	NS5b-34	2916-2935	PLRAWRHRARSVRARLLSRG	9	0	0	0	0	0
35	NS5b-35	2931-2950	LLSRGGRAAICGKYLFNWAV	2	0	0	0	0	0
36	NS5b-36	2946-2965	FNWAVRTKLKLTPITAAGRL	3	0	0	0	0	0
37	NS5b-37	2961-2980	AAGRLDLSGWFTAGYSGGDI	7	9	0	0	0	0
38	NS5b-38	2976-2995	SGGDIYHSVSHARPRWFWFC	5	0	0	0	0	0
39	NS5b-39	2991-3015	WFWFCLLLLAAGVGIYLLPN	5	9	9	0	0	0
Cummulative Number of Ad Proteins showing homology with each HCV Core Peptide				174	7	4	1	0	0
Total Number of Core Peptides Showing Homology				39	7	4	1	0	0

Figure 24. Table 6: List of Ad5 vector proteins whose amino acid sequences were aligned with HCV synthetic peptide sequences

S. No.	Human Ad5 Protein Name	Short Name	GI Number	Accession No.	Transcription Unit
1	E2A, DNA Binding Protein	DBP	58177697	AAW65516	E2
2	E2B, DNA Polymerase	Pol	58177697	AP_000202	E2
3	E2B, Terminal Protein Precursor	pTP	209846	AAW65504	E2
4	34k, Control Protein	34K	58177724	AAW65531.1	E4
5	ORF1, Control Protein	ORF1	56160565	AP_000232.1	E4
6	ORF2, Control protein	ORF2	56160564	AP_000231.1	E4
7	ORF3, Control protein	ORF3	56160563	AP_000230.1	E4
8	ORF4, Control protein	ORF4	56160562	AP_000229.1	E4
9	ORF6/7, Control Protein	ORF6/7	56160560	AP_000227.1	E4
10	13.6k protein	13.6K	58177696	AAW65503.1	L1
11	52k, Encapsidation Protein	52K	58177700	AAW65507.1	L1
12	pIIIa, Capsid Protein precursor	pIIIa	58177701	AAW65508.1	L1
13	III, Penton Base	Penton	5817770	AAW65509	L2
14	pVII, Core Protein Precursor	pVII	209845	AAA96408.1	L2
15	V, Core Protein	V	209846	AP_000208	L2
16	pX, Core Protein Precursor	pX	58177705	AP_000209	L2
17	pVI, Core Protein Precursor	pVI	209848	AP_000210	L3
18	II, Hexon (Capsid) protein	Hexon	157879600	1P30_A	L3
19	Protease	Protease	58177708	AP_000212	L3
20	100k, Hexon Assembly Protein	100k	58177710	AAW65517.1	L4
21	22k Protein	22k	58177711	AAW65518.1	L4
22	33k protein	33K	58177712	AAW65519.1	L4
23	pVIII, Capsid Protein precursor	pVIII	454806	AP_000217	L4
24	IV, Fiber (Capsid) Protein	Fiber	209931	AP_000226	L5
25	Encapsidation Protein IVa2	IVa2	56160534	AP_000201.1	IVa2
26	Capsid Protein IX	IX	56160533	AP_000200.1	IX
27	Protein U	U	158536737	ABW72885.1	U

FIG. 25A

Table 7: List of adenoviral proteins and their amino acid sequences compared with HCV 15-20 amino acid epitopes

1. **E2A DNA-binding protein [Human adenovirus 5], ACCESSION: AAW65516**

```
   1 masreeeqre ttpergrgaa rrpptmedvs spspsppppr appkkrmrrr iesedeedss
  61 qdalvprtps prpstsaadl aiapkkkkkr pspkperpps pevivdseee redvalqmvg
 121 fsnppvlikh gkggkrtvrr lneddpvarg mrtqeeeeep seaeseitvm nplsvpivsa
 181 wekgmeaara lmdkyhvdnd lkanfkllpd qvealaavck twlneehrgl qltftsnktf
 241 vtmmgrflqa ylqsfaevty khheptgcal wlhrcaeieg elkclhgsim inkehviemd
 301 vtsengqral keqsskakiv knrwgrnvvq isntdarccv hdaacpanqf sgkscgmffs
 361 egakaqvafk qikafmqaly pnaqtghghl lmplrcecns kpghapflgr qlpkltpfal
 421 snaedldadl isdksvlasv hhpalivfqc cnpvyrnsra qgggpncdfk isapdllnal
 481 vmvrslwsen ftelprmvvp efkwstkhqy rnvslpvahs darqnpfdf
```

2. **Pol [Human adenovirus 5], ACCESSION: AP_000202**

```
   1 malaqahrar rlhaeapdsg dqpprrrvrq qptraapapa rarrrrapap spggsgappt
  61 sggspaspll dasskdtpaa hrpprgtvva prgcgllqai daatnqplei ryhldlaral
 121 trlcevnlqe lppdltprel qtmdsshlrd vviklrppra diwtlgsrgv vvrstvtple
 181 qpdgqgqaae vedhqpnppg eglkfplcfl vrgrqvnlvq dvqpvhrcqy carfyksqhe
 241 csarrrdfyf hhinshssnw wreiqffpig shprterlfv tydvetytwm gafgkqlvpf
 301 mlvmkfggde plvtaardla anlgwdrweq dpltfycitp ekmaigrqfr tfrdhlqmlm
 361 ardlwssfva snphladwal sehglsspee ltyeelkklp sikgiprfle lyivghning
 421 fdeivlaaqv innrsevpgp fritrnfmpr agkilfndvt falpnprskk rtdfllweqg
 481 gcddtdfkyq ylkvmvrdtf althtslrka aqayalpvek gccayqavnq fymlgsyrse
 541 adgfpiqeyw kdreefvlnr elwkkkgqdk ydiiketldy caldvqvtae lvnklrdsya
 601 sfvrdavglt dasfnvfqrp tissnshaif rqivfraeqp arsnlgpdll apshelydyv
 661 rasirggrcy ptylgilrep lyvydicgmy asalthpmpw gpplnpyera laarawqqal
 721 dlqgckidyf darllpgvft vdadppdetq ldplppfcsr kggrlcwtne rlrgevatsv
 781 dlvtlhnrgw rvhlvpdert tvfpewrcva reyvqlniaa keradrdknq tlrsiaklls
 841 nalygsfatk ldnkkivfsd qmdaatlkgi tagqvnikss sfletdnlsa evmpafqrey
 901 spqqlalads daeesedera ptpfysppsg tpghvaytyk pitfldaeeg dmclhtlerv
 961 dplvdndryp shlasfvlaw trafvsewse flyeedrgtp ledrplksvy gdtdslfvte
1021 rghrlmetrg kkrikkhggn lvfdperpel twlvecetvc gacgadaysp esvflapkly
1081 alkslhcpsc gasskgklra kghaaegldy dtmvkcylad aqgedrqrfs tsrtslkrtl
1141 asaqpgahpf tvtqttltrt lrpwkdmtla rldehrllpy sesrpnprne eicwiemp
```

FIG. 25B

3. Terminal protein precursor [Human adenovirus 5], ACCESSION: AAW65504

```
  1 malsvndcar ltgqsvptme hflplrniwn rvrdfprast taagitwmsr yiygyhrlml
 61 edlapgapat lrwplyrqpp phflvgyqyl vrtcndyvfd sraysrlryt elsqpghqtv
121 nwsvmancty tintgayhrf vdmddfqstl tqvqqailae rvvadlallq pmrgfgvtrm
181 ggrgrhlrpn saaaaaidar dagqeegeee vpverlmqdy ykdlrrcqne awgmadrlri
241 qqagpkdmvl lstirrlkta yfnyiissts arnnpdrrpl ppatvlslpc dcdwldafle
301 rfsdpvdads lrslgggvpt qqllrcivsa vslphgsppp thnrdmtggv fqlrprengr
361 avtetmrrrr gemierfvdr lpvrrrrrrv pppppppeee egealmeeei eeeeeapvaf
421 erevrdtvae lirlleeelt vsarnsqffn favdfyeame rlealgdine stlrrwvmyf
481 fvaehtattl nylfqrlrny avfarhveln laqvvmrard aeggvvysrv wnegglnafs
541 qlmarisndl aatveragrg dlqeeeieqf maeiayqdns gdvqeilrqa avndteidsv
601 elsfrfkltg pvvftqrrqi qeinrrvvaf asnlraqhql lpargadvpl pplpagpepp
661 lppgarprhr f
```

4. 34 kDa protein [Human adenovirus 5], ACCESSION: AAW65531

```
  1 mttsgvpfgm tlrptrsrls rrtpysrdrl ppfetetrat iledhpllpe cntltmhnvs
 61 yvrglpcsvg ftliqewvvp wdmvltreel vilrkcmhvc lccanidimt smmihgyesw
121 alhchcsspg slqciaggqv laswfrmvvd gamfnqrfiw yrevvnynmp kevmfmssvf
181 mrgrhliylr lwydghvgsv vpamsfgysa lhcgilnniv vlccsycadl seirvrccar
241 rtrrlmlrav riiaeettam lyscrterrr qqfirallqh hrpilmhdyd stpm
```

5. E4 ORF1 [Human adenovirus 5], ACCESSION: AP_000232

```
  1 maaavealyv vleregailp rqegfsgvyv ffspinfvip pmgavmlslr lrvcippgyf
 61 grflaltdvn qpdvftesyi mtpdmteels vvlfnhgdqf fyghagmavv rlmlirvvfp
121 vvrqasnv
```

6. E4 ORFB [Human adenovirus 5], ACCESSION: AP_000231

```
  1 mferkmvsfs vvvpeltcly lhehdydvls flrealpdfl sstlhfispp mqqayigatl
 61 vsiapsmrvi isvgsfvmvp ggevaalvra dlhdyvqlal rrdlrdrgif vnvpllnliq
121 vceepeflqs
```

FIG. 25C

7. E4 ORF3 [Human adenovirus 5], ACCESSION: AP_000230

1 mirclrlkve galeqiftma glnirdllrd ilrrwrdeny lgmvegagmf ieeihpegfs
61 lyvhldvrav clleaivqhl tnaiicslav efdhatgger vhlidlhfev ldnlle

8. E4 ORF4 [Human adenovirus 5], ACCESSION: AP_000229

1 mvlpalpapp vcdsqnecvg wlgvaysavv dviraaaheg vyiepeargr ldalrewiyy
61 nyyterskrr drrrrsvcha rtwfcfrkyd yvrrsiwhdt ttntisvvsa hsvq

9. E4 ORF6/7 [Human adenovirus 5], ACCESSION AP_000227

1 mttsgvpfgm tlrptrsrls rrtpysrdrl ppfetetrat iledhpllpe cntltmhnaw
61 tspsppvkqp qvgqqpvaqq ldsdmnlsel pgefinitde rlarqetvwn itpknmsvth
121 dmmlfkasrg ertvysvcwe gggrlntrvl

10. DNA-binding protein [Human adenovirus 5], ACCESSION: AAW65503

1 mradreeldl pppiggvaid vvkvevpatg rtlvlafvkt cavlaavhgl yilhevdltt
61 ahkeaewefe plawrvwlvv fyfgclsltv wllegsyggs dhhaaraqsp dvrarrseld
121 dniaqmgavh glelprrqvr rellqvyla

11. 52 kDa protein [Human adenovirus 5], ACCESSION: AAW65507

1 mhpvlrqmrp ppqqrqeqeq rqtcrapspp ptasggatsa vdaaadgdye pprrrarhyl
61 dleegeglar lgapsperhp rvqlkrdtre ayvprqnlfr dregeepeem rdrkfhagre
121 lrhglnrerl lreedfepda rtgisparah vaaadlvtay eqtvnqeinf qksfnnhvrt
181 lvareevaig lmhlwdfvsa leqnpnskpl maqlflivqh srdneafrda llnivepegr
241 wlldlinilq sivvqersls ladkvaainy smlslgkfya rkiyhtpyvp idkevkiegf
301 ymrmalkvlt lsddlgvyrn erihkavsvs rrrelsdrel mhslqralag tgsgdreaes
361 yfdagadlrw apsrraleaa gagpglavap aragnvggve eydeddeyep edgey

FIG. 25D

12. Protein pIIIa precursor [Human adenovirus 5], ACCESSION: AAW65508

```
  1 mmqdatdpav raalqsqpsg lnstddwrqv mdrimsltar npdafrqqpq anrlsailea
 61 vvparanpth ekvlaivnal aenrairpde aglvydallq rvarynsgnv qtnldrlvgd
121 vreavaqrer aqqqgnlgsm valnaflstq panvprgqed ytnfvsalrl mvtetpqsev
181 yqsgpdyffq tsrqglqtvn lsqafknlqg lwgvraptgd ratvsslltp nsrlllllia
241 pftdsgsvsr dtylghlltl yreaigqahv dehtfqeits vsralgqedt gsleatlnyl
301 ltnrrqkips lhslnseeer ilryvqqsvs lnlmrdgvtp svaldmtarn mepgmyasnr
361 pfinrlmdyl hraaavnpey ftnailnphw lpppgfytgg fevpegndgf lwddiddsvf
421 spqpqtllel qqreqaeaal rkesfrrpss lsdlgaaapr sdasspfpsl igsltstrtt
481 rprllgeeey lnnsllqpqr eknlppafpn ngieslvdkm srwktyaqeh rdvpgprppt
541 rrqrhdrqrg lvwedddsad dssvldlggs gnpfahlrpr lgrmf
```

13. Penton protein [Human adenovirus 5], ACCESSION: AAW65509

```
  1 mrraamyeeg pppsyesvvs aapvaaalgs pfdapldppf vpprylrptg grnsirysel
 61 aplfdttrvy lvdnkstdva slnyqndhsn flttviqnnd yspgeastqt inlddrshwg
121 gdlktilhtn mpnvnefmft nkfkarvmvs rlptkdnqve lkyewveftl pegnysetmt
181 idlmnnaive hylkvgrqng vlesdigvkf dtrnfrlgfd pvtglvmpgv ytneafhpdi
241 illpgcgvdf thsrlsnllg irkrqpfqeg frityddleg gnipalldvd ayqaslkddt
301 eqggggaggs nssgsgaeen snaaaaamqp vedmndhair gdtfatraee kraeaeaaae
361 aaapaaqpev ekpqkkpvik pltedskkrs ynlisndstf tqyrswylay nygdpqtgir
421 swtllctpdv tcgseqvyws lpdmmqdpvt frstrqisnf pvvgaellpv hsksfyndqa
481 vysqlirqft slthvfnrfp enqilarppa ptittvsenv paltdhgtlp lrnsiggvqr
541 vtitdarrrt cpyvykalgi vsprvlssrt f
```

14. Protein pVII [Human adenovirus 5], ACCESSION: AAA96408

```
  1 mraarrlaag ivtvpprsrr raaaaaaaai samtqgrrgn vywvrdsvsg lrvpvrtrpp
 61 rn
```

FIG. 25E

15. Protein pV [Human adenovirus 5], ACCESSION: AP_000208

```
  1 mskrkikeem lqviapeiyg ppkkeeqdyk prklkrvkkk kkddddeldd evellhatap
 61 rrrvqwkgrr vkrvlrpgtt vvftpgerst rtykrvydev ygdedlleqa nerlgefayg
121 krhkdmlalp ldegnptpsl kpvtlqqvlp alapseekrg lkresgdlap tvqlmvpkrq
181 rledvlekmt vepglepevr vrpikqvapg lgvqtvdvqi pttsstsiat ategmetqts
241 pvasavadaa vqavaaaask tstevqtdpw mfrvsaprrp rgsrkygaas allpeyalhp
301 siaptpgyrg ytyrprrrat trrrtttgtr rrrrrrqpvl apisvrrvar eggrtlvlpt
361 aryhpsiv
```

16. Protein pX [Human adenovirus 5], ACCESSION: AP_000209

```
  1 maltcrlrfp vpgfrgrmhr rrgmaghglt ggmrrahhrr rrashrrmrg gilpllipli
 61 aaaigavpgi asvalqaqrh
```

17. Protein pVI [Human adenovirus 5], ACCESSION: AP_000210

```
  1 medinfasla prhgsrpfmg nwqdigtsnm sggafswgsl wsgiknfgst vknygskawn
 61 sstgqmlrdk lkeqnfqqkv vdglasgisg vvdlanqavq nkinskldpr ppveepppav
121 etvspegrge krprpdreet lvtqidepps yeealkqglp ttrpiapmat gvlgqhtpvt
181 ldlpppadtq qkpvlpgpta vvvtrpsras lrraasgprs lrpvasgnwq stlnsivglg
241 vqslkrrrcf
```

FIG. 25F

18. Chain A Hexon [Adenovirus Type 5], ACCESSION: 1P30_A

```
  1 atpsmmpqws ymhisgqdas eylspglvqf aratetyfsl nnkfrnptva pthdvttdrs
 61 qrltlrfipv dredtaysyk arftlavgdn rvldmastyf dirgvldrgp tfkpysgtay
121 nalapkgapn pcewdeaata leinleeedd dnedevdeqa eqqkthvfgq apysginitk
181 egiqigvegq tpkyadktfq pepqigesqw yeteinhaag rvlkkttpmk pcygsyakpt
241 nenggqgilv kqqngklesq vemqffstte atagngdnlt pkvvlysedv dietpdthis
301 ymptikegns relmgqqsmp nrpnyiafrd nfiglmyyns tgnmgvlagq asqlnavvdl
361 qdrntelsyq llldsigdrt ryfsmwnqav dsydpdvrii enhgtedelp nycfplggvi
421 ntetltkvkp ktgqengwek datefsdkne irvgnnfame inlnanlwrn flysnialyl
481 pdklkyspsn vkisdnpnty dymnkrvvap glvdcyinlg arwsldymdn vnpfnhhrna
541 glryrsmllg ngryvpfhiq vpqkffaikn llllpgsyty ewnfrkdvnm vlqsslgndl
601 rvdgasikfd siclyatffp mahntastle amlrndtndq sfndylsaan mlypipanat
661 nvpisipsrn waafrgwaft rlktketpsl gsgydpyyty sgsipyldgt fylnhtfkkv
721 aitfdssvsw pgndrlltpn efeikrsvdg egynvaqcnm tkdwflvqml anynigyqgf
781 yipesykdrm ysffrnfqpm srqvvddtky kdyqqvgilh qhnnsgfvgy laptmregqa
841 ypanfpypli gktavdsitq kkflcdrtlw ripfssnfms mgaltdlgqn llyansahal
901 dmtfevdpmd eptllyvlfe vfdvvrvhrp hrgvietvyl rtpfsagnat t
```

19. Protease [Human adenovirus 5], ACCESSION: AP_000212

```
  1 mgsseqelka ivkdlgcgpy flgtydkrfp gfvsphklac aivntagret ggvhwmafaw
 61 nphsktcylf epfgfsdqrl kqvyqfeyes llrrsaiass pdrcitleks tqsvqgpnsa
121 acglfccmfl hafanwpqtp mdhnptmnli tgvpnsmlns pqvqptlrrn qeqlysfler
181 hspyfrshsa qirsatsfch lknm
```

FIG. 25G

20. 100 kDa hexon-assembly associated protein [Human adenovirus 5], ACCESSION: AAW65517

```
  1 mesvekkdsl tapsefatta stdaanaptt fpveapplee eeviieqdpg fvseddedrs
 61 vptedkkqdq dnaeaneeqv grgderhgdy ldvgddvllk hlqrqcaiic dalqersdvp
121 laiadvslay erhlfsprvp pkrqengtce pnprlnfypv favpevlaty hiffqnckip
181 lscranrsra dkqlalrqga vipdiaslne vpkifeglgr dekraanalq qenseneshs
241 gvlvelegdn arlavlkrsi evthfaypal nlppkvmstv mselivrraq plerdanlqe
301 qteeglpavg deqlarwlqt repadleerr klmmaavlvt velecmqrff adpemqrkle
361 etlhytfrqg yvrqackisn velcnlvsyl gilhenrlgq nvlhstlkge arrdyvrdcv
421 ylflcytwqt amgvwqqcle ecnlkelqkl lkqnlkdlwt afnersvaah ladiifperl
481 lktlqqglpd ftsqsmlqnf rnfilersgi lpatccalps dfvpikyrec ppplwghcyl
541 lqlanylayh sdimedvsgd gllechcrcn lctphrslvc nsqllnesqi igtfelqgps
601 pdeksaapgl kltpglwtsa ylrkfvpedy haheirfyed qsrppnaelt acvitqghil
661 gqlqainkar qefllrkgrg vyldpqsgee lnpipppppqp yqqqpralas qdgtqkeaaa
721 aaathgrggi lgqsgrggfg rgggghdgrl geprrgsfrg rrgvrrntvt lgriplagap
781 eignrfqhgy nlrssgaagt arsptqp
```

21. 22 kDa protein [Human adenovirus 5], ACCESSION: AAW65518

```
  1 mapkkklqlp ppptdeeeyw dsqaeevlde eeedmmedwe sldeeaseve evsdetpsps
 61 vafpspapqk satgssmatt sapqappalp vrrpnrrwdt tgtragkskq ppplaqeqqq
121 rqgyrswrgh knaivaclqd cggnisfarr fllyhhgvaf prnilhyyrh lyspyctggs
181 gsnssghtea katg
```

22. 33 kDa protein [Human adenovirus 5], ACCESSION: AAW65519

```
  1 mapkkklqlp ppptdeeeyw dsqaeevlde eeedmmedwe sldeeaseve evsdetpsps
 61 vafpspapqk satgssmatt sapqappalp vrrpnrrwdt tgtraahtap aaaaataaat
121 qkqrrpdskt ltkpkkstaa aaagggalrl apnepvstre lrnrifptly aifqqsrgqe
181 qelkiknrsl rsltrsclyh ksedqlrrtl edaealfsky caltlkd
```

FIG. 25H

23. Protein pVIII [Human adenovirus 5]., ACCESSION: AP_000217

```
  1 mskeiptpym wsyqpqmgla agaaqdystr inymsagphm isrvngirah rnrilleqaa
 61 itttprnnln prswpaalvy qespapttvv lprdaqaevq mtnsgaqlag gfrhrvrspg
121 qgithltirg rgiqlndesv ssslglrpdg tfqiggagrp sftprqailt lqtssseprs
181 ggigtlqfie efvpsvyfnp fsgppghypd qfipnfdavk dsadgyd
```

24. Fiber [Human adenovirus 5], ACCESSION: AP_000226

```
  1 mkrarpsedt fnpvypydte tgpptvpflt ppfvspngfq esppgvlslr lseplvtsng
 61 mlalkmgngl sldeagnlts qnvttvsppl kktksninle isapltvtse altvaaaapl
121 mvagntltmq sqapltvhds klsiatqgpl tvsegklalq tsgpltttds stltitaspp
181 lttatgslgi dlkepiytqn gklglkygap lhvtddlntl tvatgpgvti nntslqtkvt
241 galgfdsqgn mqlnvagglr idsqnrrlil dvsypfdaqn qlnlrlgqgp lfinsahnld
301 inynkglylf tasnnskkle vnlstakglm fdataiaina gdglefgspn apntnplktk
361 ighglefdsn kamvpklgtg lsfdstgait vgnknndklt lwttpapspn crlnaekdak
421 ltlvltkcgs qilatvsvla vkgslapisg tvqsahliir fdengvllnn sfldpeywnf
481 rngdltegta ytnavgfmpn lsaypkshgk taksnivsqv ylngdktkpv tltitlngtq
541 etgdttpsay smsfswdwsg hnyineifat ssytfsyiaq e
```

25. Protein pIVa2 [Human adenovirus 5], ACCESSION: AP_000201

```
  1 metrgrrpaa lqhqqdqpqa hpgqraarsa plhrdpdyad edpapverhd pgpsgraptt
 61 avqrkppqpa krgdmldrda veqvtelwdr lellgqtlks mptadglkpl knfaslqell
121 slggerllad lvrenmrvrd mlnevapllr ddgscsslny qlhpvigviy gptgcgksql
181 lrnllssqli sptpetvffi apqvdmipps elkawemqic egnyapgpdg tiipqsgtlr
241 prfvkmaydd lilehnydvs dprnifaqaa argpiaiimd ecmenlgghk gvskffhafp
301 sklhdkfpkc tgytvlvvlh nmnprrdmag nianlkiqsk mhlisprmhp sqlnrfvnty
361 tkglplaisl llkdifrhha qrscydwiiy nttpqhealq wcylhprdgl mpmylniqsh
421 lyhvlekihr tlndrdrwsr ayrarktpk
```

26. Protein pIX [Human adenovirus 5], ACCESSION: AP_000200

```
  1 mstnsfdgsi vssylttrmp pwagvrqnvm gssidgrpvl pansttltye tvsgtpleta
 61 asaaasaaaa targivtdfa flsplassaa srssarddkl tallaqldsl trelnvvsqq
121 lldlrqqvsa lkassppnav
```

FIG. 25I

27. U exon protein [Human adenovirus 5], ACCESSION: ABW72885

```
  1 mkivgadgqe qeetdipfrl wrkfaarrkl qyqsweegke vllnkldrnl ltdfkafaar
 61 fssrprpski fgtslseais gegngqsgrg aarnhprart rcgatspnhg grvvpvpvaa
121 aspgapkkad eaayrvrgrg rlitrragaa htqpaaidlg ggfghcaqee keapfsqara
181 paitrgnrgq rgrkrrcgat nggfqqptga nqawqrr
```

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CA2016/000015

A. CLASSIFICATION OF SUBJECT MATTER
IPC: ***A61K 39/29*** (2006.01), ***A61K 39/00*** (2006.01), ***A61P 31/14*** (2006.01), ***A61P 37/04*** (2006.01)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
All IPCs

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic database(s) consulted during the international search (name of database(s) and, where practicable, search terms used)
Questel-Orbit, Scopus, CIPO Library Discovery Tool, Keywords: Adenovirus, HCV, Vaccine, dendritic, Authors: Argawal, Kumar and Singh.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US8142794 B2, (Emini, E.A. et al.), 27 March 2012 (27-03-2012). Abstract, column 24, lines 12-32 and examples 5, 6, 13 and 14.	1-6, 8, 12-18, 22-26
X	Fattori, E. et al., "Efficient immunization of rhesus macaques with an HCV candidate vaccine by heterologous priming–boosting with novel adenoviral vectors based on different serotypes", Gene Therapy, 23 March 2006 (23-03-2006) (online), Vol. 13, pp. 1088–1096. doi:10.1038/sj.gt.3302754. The whole document.	1-6, 8, 12-18, 22-26
X	Zabaleta, A. et al., "*Vaccination Against Hepatitis C Virus With Dendritic Cells Transduced With an Adenovirus Encoding NS3 Protein*". Molecular Therapy, 9 October 2007 (09-10-2007) (online), Vol. 16, No. 1, pp. 210-217, doi:10.1038/sj.mt.6300333. The whole document.	19-21, 22-26

☐ Further documents are listed in the continuation of Box C. ☑ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier application or patent but published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 31 March 2016 (31-03-2016)	Date of mailing of the international search report 05 April 2016 (05-04-2016)
Name and mailing address of the ISA/CA Canadian Intellectual Property Office Place du Portage I, C114 - 1st Floor, Box PCT 50 Victoria Street Gatineau, Quebec K1A 0C9 Facsimile No.: 819-953-2476	Authorized officer Ali Abdallah (819) 639-7686

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CA2016/000015

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of the first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☑ Claim Nos.: 1-26
because they relate to subject matter not required to be searched by this Authority, namely:

Claims 1-26 are directed to a method for treatment of the human or animal body by surgery or therapy, which the International Searching Authority is not required to search under PCT Rule 39.1(iv). However, this Authority has carried out a search based on the alleged effect or purpose/use of an immunogenic composition comprising an adenoviral nucleic acid or an adenovirus polypeptide, or dendritic cells expressing one or more adenoviral protein for inducing an immune response in an individual to a hepatitis C virus antigen.

2. ☐ Claim Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claim Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claim Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/CA2016/000015

Patent Document Cited in Search Report	Publication Date	Patent Family Member(s)	Publication Date
US8142794B2	27 March 2012 (27-03-2012)	US2009233992A1	17 September 2009 (17-09-2009)
		AT467680T	15 May 2010 (15-05-2010)
		AU2002337840B2	09 August 2007 (09-08-2007)
		CA2461380A1	17 April 2003 (17-04-2003)
		CA2461380C	22 March 2011 (22-03-2011)
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		CA2718802C	26 November 2013 (26-11-2013)
		CN1582337A	16 February 2005 (16-02-2005)
		CN1582337B	14 December 2011 (14-12-2011)
		CN1880457A	20 December 2006 (20-12-2006)
		CN1880457B	26 May 2010 (26-05-2010)
		CN101988071A	23 March 2011 (23-03-2011)
		CY1110238T1	14 January 2015 (14-01-2015)
		DE60236364D1	24 June 2010 (24-06-2010)
		DK1436397T3	09 August 2010 (09-08-2010)
		EP1436397A2	14 July 2004 (14-07-2004)
		EP1436397A4	16 February 2005 (16-02-2005)
		EP1436397B1	12 May 2010 (12-05-2010)
		EP2172552A2	07 April 2010 (07-04-2010)
		EP2172552A3	21 July 2010 (21-07-2010)
		ES2344253T3	23 August 2010 (23-08-2010)
		JP2005505286A	24 February 2005 (24-02-2005)
		JP4475561B2	09 June 2010 (09-06-2010)
		JP2009183295A	20 August 2009 (20-08-2009)
		JP4479973B2	09 June 2010 (09-06-2010)
		PT1436397E	15 July 2010 (15-07-2010)
		SI1436397T1	30 September 2010 (30-09-2010)
		US2004247615A1	09 December 2004 (09-12-2004)
		US7598362B2	06 October 2009 (06-10-2009)
		US2010129901A1	27 May 2010 (27-05-2010)
		US8530234B2	10 September 2013 (10-09-2013)
		WO03031588A2	17 April 2003 (17-04-2003)
		WO03031588A3	30 October 2003 (30-10-2003)