Vaccination with dendritic cells pulsed with hepatitis C pseudo particles induces specific immune responses in mice

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AIM: To explore dendritic cells (DCs) multiple functions in immune modulation.

METHODS: We used bone-marrow derived dendritic cells from BALB/c mice pulsed with pseudo particles from the hepatitis C virus to vaccinate naive BALB/c mice. Hepatitis C virus (HCV) pseudo particles consist of the genotype 1b derived envelope proteins E1 and E2, covering a non-HCV core structure. Thus, not a single epitope, but the whole “viral surface” induces immunogenicity. For vaccination, mature and activated DC were injected subcutaneously twice.

RESULTS: Humoral and cellular immune responses measured by enzyme-linked immunosorbent assay and interferon-gamma enzyme-linked immunosorbent spot test showed antibody production as well as T-cells directed against HCV. Furthermore, T-cell responses confirmed two highly immunogenic regions in E1 and E2 outside the hypervariable region 1.

CONCLUSION: Our results indicate dendritic cells as a promising vaccination model for HCV infection that should be evaluated further.

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Key words: Dendritic cell; Hepatitis C; Pseudo particles; Immune responses; Vaccination

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INTRODUCTION

Despite many developments and improving results in treating hepatitis C virus (HCV) infection, chronic hepatitis C stays a severe medical health problem world wide with over 170 million people infected[1,2]. Even though treatment of chronic infected patients with pegylated interferon and ribavirin currently results in a sustained virological response in 40%-80%[3,4], there remains a large number of HCV positive patients, non-responders and relapsers. Until development of an effective vaccine, chronic hepatitis C remains one of the most important infectious diseases.
The main problem in developing such a vaccine is the limited understanding of the type of immune response that is necessary for viral clearance and the occurrence of various genotypes and quasispecies of HCV, evolving rapidly under selection pressure by the immune response\[32,33,37,38\]. So most likely a vaccine must induce a broad immune response to clear HCV infection\[10-14\]. And indeed, humoral and cellular immune responses to several of the viral proteins have been shown to be associated with clearance of HCV infection in experimental settings\[10-14\]. Infected people develop variant-specific neutralizing antibodies\[36\]. A main target of these antibodies is considered to be the hypervariable region 1 (HVR1) of the envelope glycoprotein E2\[7,11,14,17\] but also other regions in the envelope protein\[18,19\]. E2 covalently linked to E1, the second envelope glycoprotein of HCV, forms the virus envelope\[20\]. Chimpanzees immunized by recombinant E1 and E2 protein, synthesized in mammalian cells, showed protection against HCV challenge\[21,22\]. Anti-HVR1 antibodies even have some cross-reactive activity to different HVR1 sequences\[23\] and may persist up to 7 years\[24\]. Still, re-infection and viral persistence occurs even in the presence of these antibodies\[10,12\]. Besides this humoral immune response, cellular immune responses appear to be critical for HCV clearance. Development of an early class 1 restricted CD8\(^+\) cytotoxic T lymphocyte (CTL) response to HCV structural and non-structural proteins is associated with HCV clearance\[25,26\]. Human leukocyte antigen-A2, -A3 and -B7 restricted CTL responses have been identified to regions of HCV core, E1, nonstructural (NS)3, NS4 and NS5 proteins\[29,30\]. The additional inclusion of T helper epitopes has been shown to produce even stronger CD8\(^+\) responses\[10-12\]. It seems likely that an effective vaccine against HCV should therefore be capable of inducing a T helper cell, CTL and neutralizing antibody response in multiple major histocompatibility complex (MHC) types.

Considering that, key players could be dendritic cells (DCs). DC are the most potent type of antigen presenting cells and induce immune antiviral responses\[31-33\]. Found within the peripheral tissues and lymphoid organs, DC are perfectly suited to detect and capture pathogens. Their antigen presenting capability is crucial for generation of CD4\(^+\) T-cells priming B-cells for antibody production. By production of CD40 and interleukin-2 (IL-2), DC provide help to CD8\(^+\) cells. To fulfill their function, DC have to mature, normally triggered by exposure to viruses or other pathogens\[3\]. Interestingly DC from HCV carriers show impaired maturation, determined by absence of cell surface molecules\[14,19\], as well as reduced IL-2 production\[34\]. Loss of DC function is probably a direct consequence of HCV infection\[3\]. Ex vivo generated and matured DC therefore might be the most potent candidate for a cell based HCV vaccine. In fact, there are some promising results published for immunization with DC against the human immunodeficiency virus and HCV\[12,33,17,56\].

In sera of patients with chronic HCV infection antibodies directed towards E1 and E2 can be found as mentioned above\[39\]. Chimpanzees immunized with recombinant HCV E1 and E2 showed protection against HCV infection\[40\]. Peptide immunizations have been successful in producing humoral and cellular immune responses\[41,42\]. But peptides do not deliver a great number of epitopes and are not folded in the native protein form. To overcome these limitations virus like particles have been created, consisting of both envelope proteins E1 and E2 on an HCV core or retroviral core protein, the latter termed HCV pseudo particles (HCVpp)\[43-45\]. These particles mimic HCV virions in the best possible way and are therefore an interesting stimulant. In this study, we investigated if HCVpp are able to activate mature murine DCs ex vivo and if so, if vaccination with these DC induces specific immune responses against HCV in vivo.

**MATERIALS AND METHODS**

**Cells and culture conditions**

293T cells and Hub-7 cells were maintained in Dulbecco’s Modified Eagle Medium (Invitrogen, Karlsruhe, Germany) supplemented with 10% fetal calf serum (FCS) (Invitrogen, Karlsruhe, Germany), 2 mmol/L L-glutamine (Invitrogen, Karlsruhe, Germany), 100 units/mL penicillin and 100 units/mL streptomycin (Invitrogen, Karlsruhe, Germany). Bone marrow derived dendritic cells were maintained in RPMI 1640 medium (Invitrogen, Karlsruhe, Germany) supplemented with 10% FCS, 2 mmol/L L-glutamine, 100 U/mL Penicillin, 100 mg/mL streptomycin and 50 μmol/L 2-mercaptoethanol (Invitrogen, Karlsruhe, Germany).

**Preparation of HCVpp**

Pseudoparticles were generated as described previously\[44\]. Briefly, 293T cells were transfected using a calcium phosphate-based transfection kit with three expression vectors encoding an envelope glycoprotein, viral core components, and a viral genome containing a green fluorescent protein marker gene. The plasmids used in our laboratory are the following: Cytoomegalovirus (CMV)-Gag-Pol murine leukemia virus (MLV) packaging construct, encoding the MLV gag and pol genes; the MLV-green fluorescent protein (GFP) plasmid, encoding an MLV-based transfer vector containing a CMV-GFP internal transcriptional unit and the plasmid phCMV-E1E2-HCV, encoding the HCV E1 and E2 glycoproteins of Con1-isolate, genotype 1b. After 40-46 h supernatants containing pseudoparticles were filtered through 0.45 μm pore size membrane and for further concentration a centrifugation with Amicon Ultra-15 centrifugal filter units (MWCO 100K) was done. Infectious titers of the concentrated supernatants were then determined by infection of Huh7 cells as previously described\[45\]. The infectious titers, expressed as transducing units per milliliter, were deduced from the transduction efficien-
cies, determined as the percentage of GFP-positive cells measured by fluorescence activated cell sorting (FACS) analysis[46].

**Generation of bone-marrow derived dendritic cells**

For preparation of bone-marrow derived DC, we used 6-10 wk old BALB/c mice (Charles River Breeding Laboratories, Sulzfeld, Germany). They were maintained under barrier-sustained conditions and handled according to international guidelines. After sacrificing the animals, the tibia and femur bones were used to prepare bone marrow cells. With minor adaptations, cultivation of bone marrow cells was done following the Inaba protocol[47]. On day 7 we pooled non-adherent and loosely adherent cells. The isolated cell suspensions were either taken for FACS analysis or plated into 12 well culture plates (Greiner Laborteknich, Kremsmuenster, Germany) at a density of 1.5 x 10^6 cells per well in one ml of complete bone marrow-derived dendritic cell (BMDC) medium.

**FACS analysis of BMdDC**

Flow cytometry analysis for measuring the expression of different surface molecules was performed with a FACScalibur® cytometer and data was analysed with Cell Quest Pro software (Beckton Dickinson, Franklin Lakes, United States). For staining, 2 x 10^6 cells were incubated in staining buffer [phosphate buffer solution (PBS) and 0.5% body surface area, Invitrogen, Paisley, United Kingdom] with either 1 µL of specific antibodies or the corresponding isotype control (APC anti-mouse CCR-7 (Biozol, Eching, Germany), R-PE anti-mouse CD 11c, FITC anti-mouse CD 86, FITC anti-mouse MHC II (1-Ad) (all from BD Bioscience, Heidelberg, Germany) for 30 min on ice in the dark. Stained cells were pelleted for 3 min at 2000 r/min (Biofuge pico; Kendro, Hanau, Germany) and were washed twice with staining buffer. A negative control with unstained cells was run first to determine the baseline fluorescence. Checking for unspecific binding, marker setting was done with isotype controls. For instrument settings and compensation of R-PE and FITC, samples stained with individual fluorescent probes were used.

**Activation of BMdDC**

On day 7, FACS analysis revealed 60%-70% mature DC, which were placed in a 12-well culture plate with a concentration of 1.5 x 10^6 cells per mL. Twenty-four hours later, the DC were activated by adding nothing (negative control), HCVpp (7.5 x 10^6), 1 µg/mL E. coli lipopolysaccharide (LPS) (Sigma, St. Louis, MO), or HCVpp together with LPS into the culture medium. LPS is a known co-stimulatory factor for DC[48]. Cells were harvested on day 9 (24 h after activation) and washed extensively. Activation of DC was measured by FACS analysis. For immunization, 1.0 x 10^6 DC were collected in 75 µL of 0.9% sodium chloride per mouse.

**Vaccination schedule of the mice**

Different groups of 8 mice each were subcutaneously injected with vaccines on day 1 and day 15 (Table 1). In all experiments HCVpp were used at a concentration of 7.5 x 10^6/mL after amicon filtration. Two weeks after the second vaccination, sera and spleen cells were collected for immunological analysis. Sera were centrifuged at 13 000 r/min for 30 min and plasma was collected and stored at -80 °C.

**Isolation of splenocytes**

Collected spleens were ground on ice in complete RPMI medium (Invitrogen, Paisley, United Kingdom), centrifuged (1000 r/min, 5 min) and resuspended in PBS twice. The purified cells were treated with erythrocyte lysis buffer, centrifuged, washed and resuspended in complete RPMI medium. The isolated cells were counted with a Neubauer chamber. Splenocytes were used for immunological analysis at a concentration of 3 x 10^6 cells per mL.

**Immunological analysis**

Interferon-gamma (IFN-γ) enzyme-linked immunosorbent spot test (ELISPOT) assay was performed using 3 x 10^5 splenocytes per well (96 well plates, Millipore, Bedford, United States) from each vaccination group precoated with anti-IFN-γ antibodies (5 µg/mL; Beckton Dickinson, Franklin Lakes, United States). HCV specific T-cell responses were examined after stimulation with either HCVpp or overlapping peptides from PepSet™ (Mimotopes, Clayton Victoria, Australia) of the E1 and E2 Protein of the Con1 HCV sequence which was used for HCVpp production. Altogether, 69 peptides (24 covering the E1 protein and 45 covering the E2 protein) consisting of 20 amino acids each with an offset of 8, were pooled by 7 peptides for better handling as described earlier[49]. As a positive control, concanavalin A (1 µg/mL, Sigma, St. Louis, United States) was added. Following standard protocol IFN-γ spot-forming cells (SFC) were counted by a computer-based image analyser (Zeiss-Vision, Eching, Germany). All results were expressed as mean SFC/3 x 10^5 splenocytes of quadruplicate measurements.

**Table 1 Different vaccination groups with 8 mice each**

| No. | Vaccine | Purpose |
|-----|---------|---------|
| 1   | Sodium chloride | Negative control |
| 2   | DC 293T-supernatant, concen-+ LPS | Negative control to rule out immunogenic components of Amicon filter and 293T supernatant |
| 3   | HCVpp | Immunogenic impact of HCVpp |
| 4   | DC activated with HCVpp | Treatment group without adjuvant |
| 5   | DC activated with HCVpp + LPS | Treatment group with adjuvant |

DC: Dendritic cell; HCVpp: Hepatitis C virus pseudo particles; LPS: Lipopolysaccharide.
used to determine the levels of anti-HCV-immunoglobulin in the sera of the different immunization groups. Following standard protocol 96-well microtiter plates (Millipore, Bedford, United States) were coated with either HCVpp or PepSet™ (Mimotopes, Clayton Victoria, Australia) containing biotinylated overlapping peptides (offset by 8) of the E1 and E2 Protein of the Con1 HCV sequence (Table 2). Again the 69 peptides were pooled by 7 peptides. Mouse serum was added in a 1:100 dilution. Colour development, using an HRP conjugated goat anti-mouse-IgG antibody (Santa Cruz Biotech, San Diego, United States), was read in an automated reader at 450 nm (Microplate Reader 2001, Whittaker Bioproduction, Walkersville, United States).

### RESULTS

**Dendritic cells are strongly activated by HCVpp**

As shown in Figure 1, CD11c positive DC that were used in our study were activated by HCVpp in vitro. In the FACS analysis non-activated DC expressed CD86 in 10% and CCR7 in 10%. After 24 h of incubation with HCVpp and the costimulatory factor LPS, HCVpp only, or LPS alone CD86 rates were increased to 41%, 40% and 10% and CCR7 in 10%. After 24 h of incubation with HCVpp and the costimulatory factor LPS, HCVpp only, or LPS alone CD86 rates were increased to 41%, 40% and 10%, respectively. Thus, DC were strongly activated. Regarding the more specific migration marker CCR7, incubation with LPS resulted in low levels of expression (12%). In contrast, priming with HCVpp or HCVpp and LPS resulted in a stronger upregulation. Here, CCR7 was found in 25% and 39%, respectively. In all our experiments CCR7 receptor expression was more enhanced whenever we used HCVpp for activation of the DC.

**DC treatment is well tolerated in mice**

We immunized different groups of BALB/c mice, each consisting of 8 animals (Table 1). For immunization details see Materials and Methods section. All mice were checked daily. Treatment was well tolerated and no mouse was considered ill, lost weight or died.

**BALB/c mice immunized with DC pulsed with HCVpp can induce antibody response**

Humoral immune responses against HCVpp were assessed by ELISA. Antibody titers were highest in groups of mice vaccinated with HCVpp pulsed DC. However, only slightly significant antibody binding could be demonstrated in the overlapping peptides (PepSets™) spanning the E1 and E2 protein of the HCV Con 1 isolate (Figure 2). Mice vaccinated with HCVpp showed lower antibody binding of the overlapping peptides compared to mice vaccinated with DC pulsed HCVpp. However, using HCVpp as read-out antigen, the measured antibody binding was comparably high in HCVpp pulsed DC and HCVpp vaccinated animals. Overall, highly significant antibody binding (P < 0.001) was only observed with HCVpp as read-out antigen, indicating the importance of correct three dimensional folding.

**ELISPOT demonstrates a specific T-cell response in BALB/c mice immunized with DC previously incubated with HCVpp**

Specific T-cell responses were assessed by IFN-γ-ELISPOT analysis. Results are shown as mean SFC/3 × 10⁵ splenocytes (Figure 3). T-cells from mice vaccinated with DC pulsed by the combination of HCVpp and LPS showed the highest amount of IFN-γ when stimulated with the peptide pools or HCVpp itself. The responses were significantly higher compared to T-cells from mice vaccinated with HCVpp only. As expected, the two negative control groups (mice vaccinated with saline or 293T supernatant) showed the lowest number of spots. Peak results with SFC/3 × 10⁵ splenocytes above 80 were seen in cells stimulated with pools 3 and 7 in the analysis. Pool 3 (85.8 spots/well) represents amino acids 312-379 of the E1 protein and pool 7 (86.3 spots/well) represents amino acids 544-611 of the E2 protein. In contrast to the ELISA assay, there was no significant difference between the read-out antigens used (peptide pools vs HCVpp).

**DISCUSSION**

The hepatitis C virus inhibits intracellular interferon pathways, impairs DC activation and T-cell responses[34-36,50]. In addition, it induces a state of T-cell exhaustion and selects escape variants with mutations in immunodominant T-cell epitopes[51]. This is especially important since...
Figure 1  Fluorescence activated cell sorting analysis of bone-marrow derived dendritic cell of BALB/c mice, identified by expression of CD11c, after 7 d of maturation, followed by incubation with different agents. Column one shows the negative control, columns two and three show dendritic cell (DC) incubated with only lipopolysaccharide (LPS) or hepatitis C virus pseudo particles (HCVpp) with the co-stimulator LPS, respectively, and column four shows DC incubated with HCVpp only. Expression of the surface markers CD86 and CCR7 after pulsion of the DC with HCVpp and/or LPS is shown in percent.

Figure 2  Induction of anti-E1 and anti-E2 antibodies following s.c. immunization of BALB/c mice. All animals specifically vaccinated developed specific antibodies. Highest antibody titer were observed in the two groups of mice which received the dendritic cell (DC) based vaccines. The negative control with phosphate buffered saline showed only very little unspecific binding. PepSets™ Pools 1-9 spanning the E1 and E2 protein of the hepatitis C virus (HCV) Con1 isolate showed considerably lower binding activity in the treatment groups, whereas the negative control groups did not show any differences between the different antigens. Through serial dilutions OD was calculated for HCV pseudo particles (HCVpp) group to be OD 1755, for the DC + HCVpp group OD 2013 and for the DC + HCVpp+ lipopolysaccharide (LPS) group OD 1944. For significance, NaCl groups were compared with DC + HCVpp groups. Pool 1-3 covers most of the E1 protein, pool 4 comprises the last 24 amino acids of the E1 protein and the first 32 amino acids of the E2 protein, and pool 5-9 enclose the rest of the E2 protein. NaCl: Saline; 293T: Cell culture supernatant of 293T-cells. Results are given as means of quadruplicate measurements of eight mice each group. aP < 0.05, bP < 0.001.

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The clearance of HCV infection requires strong and broadly cross-reactive T-cell and neutralizing antibody responses\(^{[52-54]}\). It has been shown that the development of a multi-specific T-cell response during acute HCV infection is associated with spontaneous clearance of infection\(^{[55]}\). A successful immune response against HCV is therefore based on sufficient innate and adaptive immune responses.

Several approaches in HCV vaccine development have been studied. In chimpanzees it has been shown that the inclusion of structural HCV proteins was more significantly associated with protective immune responses compared to vaccines based on non-structural proteins of HCV\(^{[56]}\). Immunization with recombinant HCV E1 and E2 glycoproteins has been shown to prevent development of chronic infection in chimpanzees\(^{[57,58]}\). Distinct epitopes in certain regions of the E1 and E2 protein have been shown to drive the production of neutralizing antibodies\(^{[59]}\).

We have shown recently that \textit{in vitro} activation of DC followed by immunization with these DC leads to the induction of strong and specific antibody and T-cell responses in the hepatitis B context\(^{[60]}\). For HCV it has been shown that activation of DC by the core or the NS3 protein leads to maturation and stimulation of T-cells\(^{[61]}\). In addition, it was shown that DC function was restored in chronic HCV infected patients by the use of IL-10 inhibitors\(^{[62]}\). Thus, re-activation of DC may be an important tool in fighting HCV infection. We activated DC derived from BALB/c mice with HCVpp and were able to induce HCV specific antibodies and T-cells after immunization of mice with these DC. HCVpp were chosen to activate the DC for several reasons. They contain the E1 and E2 proteins and present them as closely to mature virions as possible. Due to that, neutralizing epitopes of the E1 and E2 proteins are potentially presented in the natural three-dimensional fashion. We intended to improve the immune responses using this approach. Immunization with recombinant E1 and E2 proteins as well as synthetic peptides led to limited humoral and cell mediated responses due to the limited number of viral epitopes and the inclusion of incorrectly folded recombinant proteins.

DC were chosen since we and others showed that they can be used to strongly induce immune responses which exceed the responses achieved by immunization with proteins or peptides only\(^{[63]}\). There are many challenges to face using DC as a therapeutic vaccine. The DC must be in the correct maturation state to be sufficiently activated, which may be different regarding the focused target\(^{[64]}\). Early used DC were immunogenic, but suboptimal with regard to their lymph-node homing ability and T-cell stimulatory potential\(^{[65]}\). Besides the maturation state the inflammatory cytokine milieu and the area of origin (plasmacytoid or myeloid) seems to play an important role. Furthermore, a challenge is the site of injection. In some studies intradermally or subcutaneous injected DC only migrated at low levels to the lymph nodes\(^{[66]}\). Reaching the lymph-node DC must show full ability to produce bioactive cytokines to properly activate T- and B-cells\(^{[67]}\). Many DC-based vaccines do not work due to these hurdles and the challenge is to find the right approach for the specific target.

In the present study, we were able to demonstrate that mouse DC can efficiently be activated \textit{in vitro} using HCVpp. Reinjection of these DC into BALB/c mice led to humoral and cellular immune responses, demonstrating that in the HCV context \textit{in vitro} activation of DC induces immune responses. These data lead to the hypothesis that impaired DC function/activation of HCV patients could be restored \textit{in vitro}. This hypothesis is supported by the fact that immunization of the mice with...
HCVpp only resulted in less antibody and significantly less IFN-γ production compared to immunization with HCVpp pulsed DC. Interestingly, in vitro co-stimulation with LPS led to enhanced T-cell responses but not to enhanced antibody production. The reason for this difference remains elusive. It may be due to a stronger cross-talk between DC and T-cells compared to B-cells. Furthermore, we could not detect significant specific binding of antibodies to recombinant HCV peptides in our experiments. We believe this is due to the lack of a three dimensional read-out using overlapping peptides and not folded proteins. This is supported by the fact that the use of HCVpp as read-out antigen resulted in very strong specific antibody binding. However, HCVpp priming of DC only resulted in slightly higher HCV specific antibody production.

We found relatively few differences between the overlapping peptide pools used for read-out. This is interesting, since we expected to find strong differences between the hypervariable regions in the E proteins compared to other regions. The homogeneity observed in our model suggests that sufficient broad-range immune responses were induced and therefore DC vaccination may be more suitable to potentially match the emergence of escape variants during HCV infection. Moreover, the HCVpp could be engineered for different sub- and quasispecies and thereby even widen the immune response after pulsion of DC.

When analyzing T-cell responses, we found two peaks in the immune response. These peaks were seen in pools 3 and 7 of the used overlapping peptides, corresponding to amino acids (aa) 312-379 of the E1 protein and 544-611 of the E2 protein, respectively (Table 2). This is consistent with the described regions aa174-337 and aa527-560, outside the hypervariable region 1[1,11,17], that have been shown to act as targets of especial interest for the natural immune system fighting HCV infection[18,19].

In conclusion, the data presented in the present study demonstrates that vaccination with HCVpp pulsed DC strongly enhances immune reactions against the structural proteins of HCV in mice. Both specific antibody production and T-cell immunity were enhanced. Furthermore, our data confirms that aa312-379 and aa544-611 of the HCV polyprotein are interesting and strong immunodominant sequences within the structural proteins of HCV. We believe that use of DC as a cellular based therapy is of great interest and should be evaluated further to sufficiently fight chronic HCV infection.

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8 Arvin AM, Greenberg HB. New viral vaccines. Virology 2006; 344: 240-249
9 Houghton M, Abrignani S. Prospects for a vaccine against the hepatitis C virus. Nature 2005; 436: 961-966
10 Diepolder HM, Gerlach JT, Zachoval R, Hoffman RM, Jung MC, Wierenga EA, Scholz S, Santantonio T, Houghton M, Southwood S, Sette A, Pape GR. Immunodominant CD4+ T-cell epitope within nonstructural protein 3 in acute hepatitis C virus infection. J Virol 1997; 71: 6011-6019
11 Farci P, Shimoda A, Wong D, Cabezon T, De Gioannidis D, Strazzera A, Shimizu Y, Shapiro M, Alter HJ, Purcell RH. Prevention of hepatitis C virus infection in chimpanzees by hyperimmune serum against the hypervariable region 1 of the envelope 2 protein. Proc Natl Acad Sci USA 1996; 93: 15394-15398
12 Lamonaca V, Missale G, Urbani S, Pilli M, Boni C, Mori C, Sette A, Massari M, Southwood S, Bertoni R, Valli A, Fiacchini F, Ferrari C. Conserved hepatitis C virus sequences are highly immunogenic for CD4+ T cells: implications for vaccine development. Hepatology 1999; 30: 1088-1098
13 Rehermann B, Chang KM, McHutchison JG, Kokka R, Houghton M, Chisari FV. Quantitative analysis of the periphenotypic glycoprotein T lymphocyte response in patients with chronic hepatitis C virus infection. J Clin Invest 1996; 98: 1432-1440
14 Wedemeyer H, He XS, Nascimbeni M, Davis AR, Greenberg HB, Hoofnagle JH, Liang TJ, Alter H, Rehermann B. Impaired effector function of hepatitis C virus-specific CD8+ T cells in chronic hepatitis C virus infection. J Immunol 2002; 169: 3447-3454
15 Shimizu YK, Hijikata M, Iwamoto A, Alter HJ, Purcell RH, Yoshikura H. Neutralizing antibodies against hepatitis C virus and the emergence of neutralization escape mutant viruses. J Virol 1994; 68: 1494-1500
16 Puntoriero G, Meola A, Lahm A, Zucchelli S, Ercole BB, Tafi R, Pezzanera M, Mondelli MU, Cortese R, Tramontano A, Galfre G, Nicosa A. Towards a solution for hepatitis C virus hyper variability: mimotopes of the hypervariable region 1 can induce antibodies cross-reacting with a large number of viral variants. EMBO J 1998; 17: 3521-3533
17 Zibert A, Kraas W, Meisel H, Jung G, Roggendorf M. Epitope mapping of antibodies directed against hypervariable region 1 in acute self-limiting and chronic infections due to hepatitis C virus. J Virol 1997; 71: 1423-1427
18 Lee JW, Kim K, Jung SH, Lee KJ, Choi EC, Sung YC, Kang CY. Identification of a domain containing B-cell epitopes in hepatitis C virus E2 glycoprotein by using mouse monoclonal antibodies. J Virol 1999; 73: 11-18
19 Nakano I, Maertens G, Major ME, Vitvitski L, Dubuisson J, Fourniller A, De Martynoff G, Trepo C, Inchauspé G. Immunization with dendritic cells loaded with heat-inactivated from hepatitis C virus infected individuals. J Virol 1999; 73: 11429-11437
20 Dubuisson J, Rice CM. Hepatitis C virus glycoprotein folding: disulfide bond formation and association with calnexin. J Virol 1996; 70: 778-786
21 Houghton M. Strategies and prospects for vaccination against the hepatitis C viruses.Curr Top Microbial Immunol 2000; 242: 327-339
22 Rosa D, Campagnoli S, Morett C, Guenzi E, Couzens L, Chin M, Dong C, Weiner AJ, Lau JY, Choo QL, Chien D, Pileri P, Houghton M, Abrignani S. A quantitative test to estimate neutralizing antibodies to the hepatitis C virus: cytofluorimetric assessment of envelope glycoprotein 2 binding to target cells. Proc Natl Acad Sci USA 1994; 91: 1759-1763
23 Scarselli E, Cerino A, Espósito G, Silini E, Mondelli MU, Traboni C. Occurrence of antibodies reactive with more than one variant of the putative envelope glycoprotein (gp70) hypervariable region 1 in viremic hepatitis C virus-infected patients. J Virol 1995; 69: 4407-4412
24 Bichr S, Rende-Fournier R, Vona G, Yamamoto AM, Depla E, Maertens G, Bréchet C. Detection of neutralizing antibodies to hepatitis C virus using a biliary cell infection model. J Gen Virol 2002; 83: 1673-1678
25 Esumi M, Zhou YH, Tanoue T, Tomoguri T, Hayasaka I. In vivo and in vitro evidence that cross-reactive antibodies to C-terminus of hypervariable region 1 do not neutralize heterologous hepatitis C virus. Vaccine 2002; 20: 3095-3103
26 Farci P, Alter HJ, Govindarajan S, Wong DC, Engle R, Lesniewski RR, Mushahwar IK, Desai SM, Miller RH, Ogata N. Lack of protective immunity against reinfection with hepatitis C virus. Science 1992; 258: 135-140
27 Cooper S, Erickson AL, Adams EJ, Kansopon J, Weiner AJ, Chien DV, Houghton M, Parham P, Walker CM. Analysis of a successful immune response against hepatitis C virus. Immunity 1999; 10: 439-449
28 Lechner F, Wong DK, Dunbar PR, Chapman R, Chung RT, Dohrenwend P, Robbins G, Phillips R, Klennerman P, Walker BD. Analysis of successful immune responses in persons infected with hepatitis C virus. J Exp Med 2000; 191: 1499-1512
29 Cerny A, McHutchison JG,Pasquinelli C, Brown ME, Brothers MA, Grabbe C, Frey C, Szyngler P, Houghton M, Chisari FV. Cytoytic T lymphocyte response to hepatitis C virus-derived peptides containing the HLA A2.1 binding motif. J Clin Invest 1995; 95: 521-530
30 Chang KM, Gruener NH, Southwood S, Sidney J, Pape GR, Chisari FV, Selle A. Identification of HLA-A3- and B7-restricted CTL response to hepatitis C virus in patients with acute and chronic hepatitis C virus. J Immunol 1999; 162: 1156-1164
31 Bhardwaj N, Bender A, Gonzalez N, Bui LK, Garrett MC, Steinman RM. Stimulation of human anti-viral CD8+ cytolytic T lymphocytes by dendritic cells. Adv Exp Med Biol 1995; 378: 375-379
32 Yu H, Huang H, Xiang J, Babuik LA, van Drunen Littel-van den Hurk S. Dendritic cells pulsed with hepatitis C virus NS3 protein induce immune responses and protection from infection with recombinant vaccinia virus expressing NS5. J Gen Virol 2006; 87: 1-10
33 Encke J, Findeelke J, Geib J, Piffet E, Stremmel W. Prophylactic and therapeutic vaccination with dendritic cells against hepatitis C virus infection. Clin Exp Immunol 2005; 142: 362-369
34 Sette A, Fatmi A, Zoulim F, Zarski JP, Trepo C, Inchauspé G. Impaired allostimulatory function of dendritic cells in chronic hepatitis C infection. Gastroenterology 2001; 120: 512-524
35 Kanto T, Hayashi N, Takehara T, Tatsumi T, Kuzushita N, Ito A, Sasaki Y, Kasahara A, Hori M. Impaired allostimulatory capacity of peripheral blood dendritic cells recovered from hepatitis C virus-infected individuals. J Immunol 1999; 162: 5584-5591
36 Sarohe P, Lasarte JJ, Casares N, Lopez-Diaz de Cerio A, Baixeras E, Labarga P, Garcia N, Borrás-Cuesta F, Prieto J. Abnormal priming of CD4+ T cells by dendritic cells expressing hepatitis C virus core and E1 proteins. J Virol 2002; 76: 5082-5070
37 Garcia F, Lejeune M, Climent N, Giel C, Alcamí J, Morente V, Alós L, Ruiz A, Setoain J, Fumero A, Castro P, López A, Cruca A, Piera C, Florence E, Pereira A, Libois A, Gonzalez N, Guila M, Caballero M, Lométa F, Joseph J, Miró JM, Pumarola T, Plana M, Gatell JM, Gallart T. Therapeutic immunization with dendritic cells loaded with heat-inactivated autologous HIV-1 in patients with chronic HIV-1 infection. J Infect Dis 2005; 191: 1680-1685
38 Van Guik ER, Fonsaerts P, Heyndrickx L, Vereecken K, Moerman M, Dunbar PR, Coldeboudens R, Van den Bosch G, Van Bockstaele DR, Van Tendeloo VF, Allard S, Verrier B, Marañón C, Hoffel G, Hosmalin A, Berneman ZN, Vanham G. Efficient stimulation of HIV-1-specific T cells using dendritic cells electroporated with mRNA encoding autologous
HIV-1 Gag and Env proteins. *Blood* 2006; 107: 1818-1827

Lechner S, Rispeter K, Meisel H, Kraas W, Jung G, Roggen dorff M, Zibert A. Antibodies directed to envelope proteins of hepatitis C virus outside of hypervariable region 1. *Virology* 1998; 243: 313-321

Choo QL, Kuo G, Ralston R, Weiner A, Chien D, Van Nest G, Han J, Berger K, Thudium K, Kuo C. Vaccination of chimpanzees against infection by the hepatitis C virus. *Proc Natl Acad Sci USA* 1994; 91: 1294-1298

Oseroff C, Sette A, Wentworth P, Celis E, Maewal A, Dah lberg C, Fikes J, Kubo RT, Chesnut RW, Grey HM, Alexander J. Pools of ligand HTL-CTL constructs prime for multiple HBV and HCV CTL epitope responses. *Vaccine* 1998; 16: 823-833

Laumans B, Braunischweiger I, Schweitzer S, Jung G, In chauspé G, Sauerbruch T, Spengler U. Lipidation of T helper sequences from hepatitis C virus core significantly enhances T-cell activity in vitro. *Immunology* 2001; 102: 460-465

Baumert TF, Ito S, Wong DT, Liang TJ. Hepatitis C virus structural proteins assemble into viruslike particles in insect cells. *J Virol* 1998; 72: 3827-3836

Murata K, Lechmann M, Qiao M, Gunji T, Alter HJ. Immune response to hepatitis C virus-like particles protects mice from recombinant hepatitis C virus-vaccinia infection. *Proc Natl Acad Sci USA* 2003; 100: 6753-6758

Bartosch B, Dubuisson J, Cosset FL. Infectious hepatitis C virus pseudo-particles containing functional E1-E2 envelope protein complexes. *J Exp Med* 2003; 197: 633-642

Sandrin V, Boson B, Salmon P, Gay W, Nègre D, Le Grand R. Expression of hepatitis C virus core protein in mammalian cells: a modified RD114 envelope glycoprotein show increased stability in sera and augmented transduction of primary lymphocytes and CD34+ cells derived from human and nonhuman primates. *Blood* 2002; 100: 823-832

Inaba K, Inaba M, Romani N, Aya H, Deguchi M, Ikehara S, Muramatsu S, Steinman RM. Generation of large numbers of dendritic cells from mouse bone marrow cultures supplemented with granulocyte/macrophage colony-stimulating factor. *J Exp Med* 1992; 176: 1693-1702

Zanoni I, Ostuni R, Capuano G, Collini M, Caccia M, Ron chi AE, Rocchetti M, Minguozzi F, Foti M, Chirico G, Costa B, Zaza A, Ricciardi-Castagnoli P, Granucci F. CD14 regulates the dendritic cell life cycle after LPS exposure through NFAT activation. *Nat Med* 2009; 15: 264-268

Suneetha PV, Schlaphoff W, Wang C, Stegmann KA, Fytili P, Sarin SK, Manns MP, Cornberg M, Wedemeyer H. Effect of peptide pools on effector functions of antigen-specific CD8+ T cells. *J Immunol Methods* 2009; 342: 33-48

Bowie A, Kiss-Toth E, Symons JA, Smith GL, Dower SK, O’Neill LA. A46R and A52R from vaccinia virus are antagonists of host IL-1 and toll-like receptor signaling. *Proc Natl Acad Sci USA* 2007; 104: 10162-10167

Torresi J, Johnson D, Wedemeyer H. Progress in the development of preventive and therapeutic vaccines for hepatitis C virus. *J Hepatol* 2011; 54: 1273-1285

Lauer GM, Barnes E, Lucas M, Timm J, Ouchi K, Kim AJ, Day CL, Robbins GK, Casson DR, Reiser M, Dusheiko G, Allen TM, Chung RT, Walker BD, Klenerman P. High resolution analysis of cellular immune responses in resolved and persistent hepatitis C virus infection. *Gastroenterology* 2004; 127: 924-936

Pestka JM, Zeisel MB, Bläser E, Schürmann P, Bartosch B, Cosset FL, Patel AH, Meisel H, Baumert J, Viazov S, Rispeter K, Blum HE, Roggen dorff M, Baumert TF. Rapid induction of virus-neutralizing antibodies and viral clearance in a single-source outbreak of hepatitis C. *Proc Natl Acad Sci USA* 2007; 104: 6025-6030

Schulze Wieseh H, Lauer GM, Day CL, Kim AY, Ouchi K, Duncan JE, Wierol AG, Timm J, Jones AM, Mothe B, Allen TM, McGovern B, Lewis-Ximenez L, Sidney J, Sette A, Chung RT, Walker BD. Broad repertoire of the CD4+ Th cell response in spontaneously controlled hepatitis C virus infection includes dominant and highly promiscuous epitopes. *J Immunol* 2005; 175: 3603-3613

Spada E, Mele A, Bertoz A, Ruggeri L, Ferrigno L, Garbu gia AR, Perrone MP, Girelli C, Del Porto P, Fico S, Fonde celli E, Mondelli MU, Amoroso P, Cortese R, Nicosia A, Vitelli A, Folgori A. Multispecific T cell response and negative HCV RNA tests during acute HCV infection are early prognostic factors of spontaneous clearance. *Gut* 2004; 53: 1673-1681

Dahari H, Feinstein SM, Major ME. Meta-analysis of hepa titis C virus vaccine efficacy in chimpanzees indicates an importance for structural proteins. *Gastroenterology* 2010; 139: 965-974

Grollo L, Torresi J, Drummer H, Zeng W, Williamson N, Jackson DC. Exploiting information inherent in binding sites of virus-specific antibodies: design of an HCV vaccine candidate cross-reactive with multiple genotypes. *Antitox Ther* 2006; 11: 1005-1014

Meunier JC, Russell RS, Goossens V, Priem S, Walter H, Depla E, Union A, Faulk KN, Bukh J, Emerson SU, Purcell RH. Isolation and characterization of broadly neutralizing human monoclonal antibodies to the el glycoprotein of hepatitis C virus. *J Virol* 2008; 82: 966-973

Farag MM, Hoyler B, Encke J, Stremmel W, Weigand K. Dendritic cells can effectively be pulsed by HBVsvp and induce specific immune reactions in mice. *Vaccine* 2010; 28: 200-206

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

Landi A, Yu H, Babiuk LA, van Drunen Littel-van den Hurk S. Human dendritic cells expressing hepatitis C virus core protein display transcriptional and functional changes consistent with maturation. *J Virol Hepat* 2011; 18: 700-713

Diaz-Valdés N, Manterola L, Belisúe V, Riezú-Boj JJ, Larrea E, Echeverría I, Llopis D, López-Sagasta J, Lerat H, Pawlotsky JM, Prieto J, Lasarte JJ, Borrás-Cuesta F, Sarobe P. Improved dendritic cell-based immunization against hepatitis C virus using peptide inhibitors of interleukin 10. *Hepatology* 2011; 53: 23-31

Onaitis M, Kalady MF, Pruitt S, Tyler DS. Dendritic cell gene therapy. *Surg Oncol Clin N Am* 2002; 11: 645-660

Kalinski P, Urban J, Narang R, Berk E, Wieckowski E, Muthuswamy R. Dendritic cell-based therapeutic cancer vaccines: what we have and what we need. *Future Oncol* 2009; 5: 379-390

Rinaldo CR. Dendritic cell-based human immunodeficiency virus vaccine. *J Intern Med* 2009; 265: 138-158

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