Immunization with DNA-launched RNA Replicon and Poxvirus Vaccine Candidates Expressing HCV Proteins Induce Potent Immune Responses

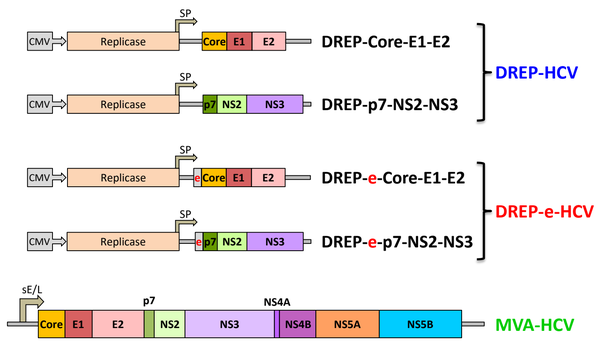
MARÍA Q. MARÍN[1], PATRICIA PÉREZ[1], CARLOS ÓSCAR S. SORZANO[2], KARL LJUNGBERG[3], PETER LILJESTRÖM[3], CARMEN E. GÓMEZ[1], MARIANO ESTEBAN[1] AND JUAN GARCÍA-ARRIAZA[1]

1 Department of Molecular and Cellular Biology, Centro Nacional de Biotecnología (CNB), Consejo Superior de Investigaciones Científicas (CSIC), Madrid, Spain. 2 Biocomputing Unit, Centro Nacional de Biotecnología (CNB), Consejo Superior de Investigaciones Científicas (CSIC), Madrid, Spain.

3 Department of Microbiology, Tumor and Cell Biology, Karolinska Institutet, Stockholm, Sweden.

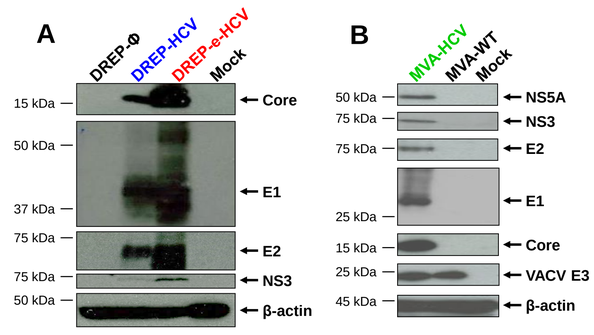
Hepatitis C virus (HCV) represents a major global health problem for which a vaccine is not available. We have previously described a vaccine against HCV based on the modified vaccinia virus Ankara (MVA) expressing the nearly full-length genome of HCV genotype 1a (MVA-HCV) that elicited mainly HCV-specific CD8$^+$ T-cell responses in mice. Here, to enhance the potency of MVA-HCV we combined it with two novel alphavirus-based DNA-launched self-replicating RNA replicons (DREP) vaccines expressing core-E1-E2-p7-NS2-NS3 HCV proteins (named DREP-HCV and DREP-e-HCV). DREP-e-HCV contains a translational enhancer that has been described to improve humoral responses against heterologous antigens. These DREP-HCV vaccine candidates efficiently trigger cellular apoptosis in transfected cells. Furthermore, when mice were immunized with the DREP vaccines as a prime followed by a MVA-HCV boost, the HCV-specific CD4$^+$ and CD8$^+$ T cell adaptive and memory immune responses were significantly improved compared to an MVA-HCV homologous prime/boost immunization, being DREP-e-HCV/MVA-HCV the most potent immunization regimen. HCV-specific CD4$^+$ and CD8$^+$ T cell responses were mainly directed against E1-E2 and NS2-NS3, respectively, and they were highly polyfunctional, with triple and quadruple cytokine-producer T cells. Additionally, DREP/MVA immunization regimen led to the generation of higher antibody levels against E2 protein than the MVA-HCV homologous immunization. Thus, our findings provided a potent immunization protocol triggering adaptive and memory HCV-specific T-cell and humoral immune responses, and based on an apoptosis-triggering DREP vaccine administrated as a prime followed by an MVA boost. These results support the consideration of DREP-HCV and MVA-HCV as immune efficient candidate vaccines against HCV.

1 Design of vaccine candidates against HCV



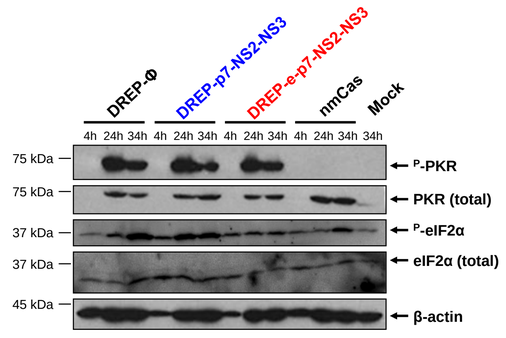
Scheme of the DREP-based and MVA-HCV hepatitis C vaccines. We constructed four novel DREP-based HCV vaccines that contains the alphavirus replicase placed under the control of the cytomegalovirus (CMV) promoter, and the HCV genes (either Core-E1-E2 or p7-NS2-NS3; genotype 1a) placed under the control of the alphavirus subgenomic promoter (SP). DREP-e-HCV vectors contains a translational enhancer (e) upstream HCV proteins. MVA-HCV is a poxvirus modified vaccinia virus Ankara (MVA) vector, previously generated, that express all HCV proteins under the control of a viral synthetic early/late (sE/L) promoter.

2 Expression of HCV proteins



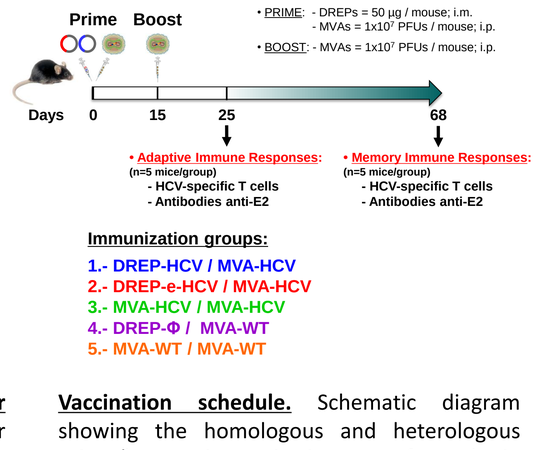
DREP-HCV, DREP-e-HCV and MVA-HCV correctly express HCV proteins. **A)** Human 293T cells were mock-transfected or transfected with DREP-HCV (mixture of DREP-C-E1-E2 + DREP-p7-NS2-NS3), DREP-e-HCV (mixture of DREP-e-C-E1-E2 + DREP-e-p7-NS2-NS3) or empty DREP-Ø. At 48 h post-transfection, cells were harvested and HCV proteins were detected by Western Blot. **B)** DF-1 cells were mock-infected or infected with MVA-HCV and MVA-WT. At 24 h post-infection, cells were harvested and HCV proteins were detected by Western Blot.

3 Induction of apoptosis *in vitro*



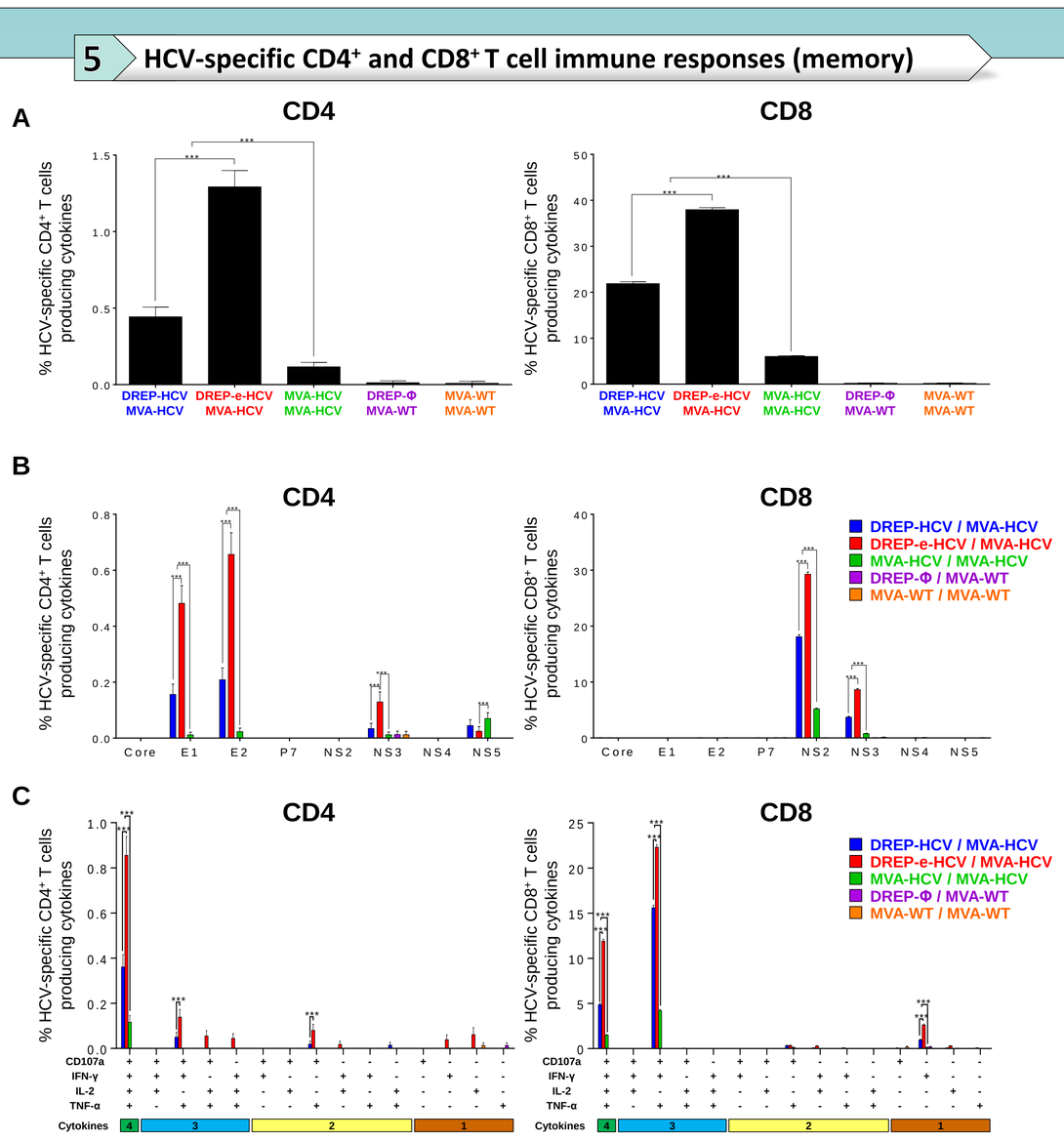
DREP-HCV and DREP-e-HCV vectors trigger cellular apoptosis. 293T cells were mock-transfected or transfected with DREP-Ø, DREP-p7-NS2-NS3 or DREP-e-p7-NS2-NS3 vectors. Plasmid nmCas, not containing the alphavirus replicase and with a similar size to DREPs, was used as a negative control. At 4, 24 and 34 h post-transfection cells were harvested, lysed with Laemli1X-β-mercaptoethanol and p-PKR and p-eIF2α apoptosis-related proteins were detected by Western Blot. Rabbit anti-β-actin antibody was used as a loading control.

4 Immunization schedule



Vaccination schedule. Schematic diagram showing the homologous and heterologous prime/boost immunizations performed in C57BL/6 mice (n=10 mice/group), to study the immunogenicity of DREP-HCV and DREP-e-HCV vaccine candidates. Immunization groups are indicated, together with the time points where animals were immunized and sacrificed to analyze the adaptive and memory HCV-specific T cell and humoral immune responses.

5 HCV-specific CD4$^+$ and CD8$^+$ T cell immune responses (memory)

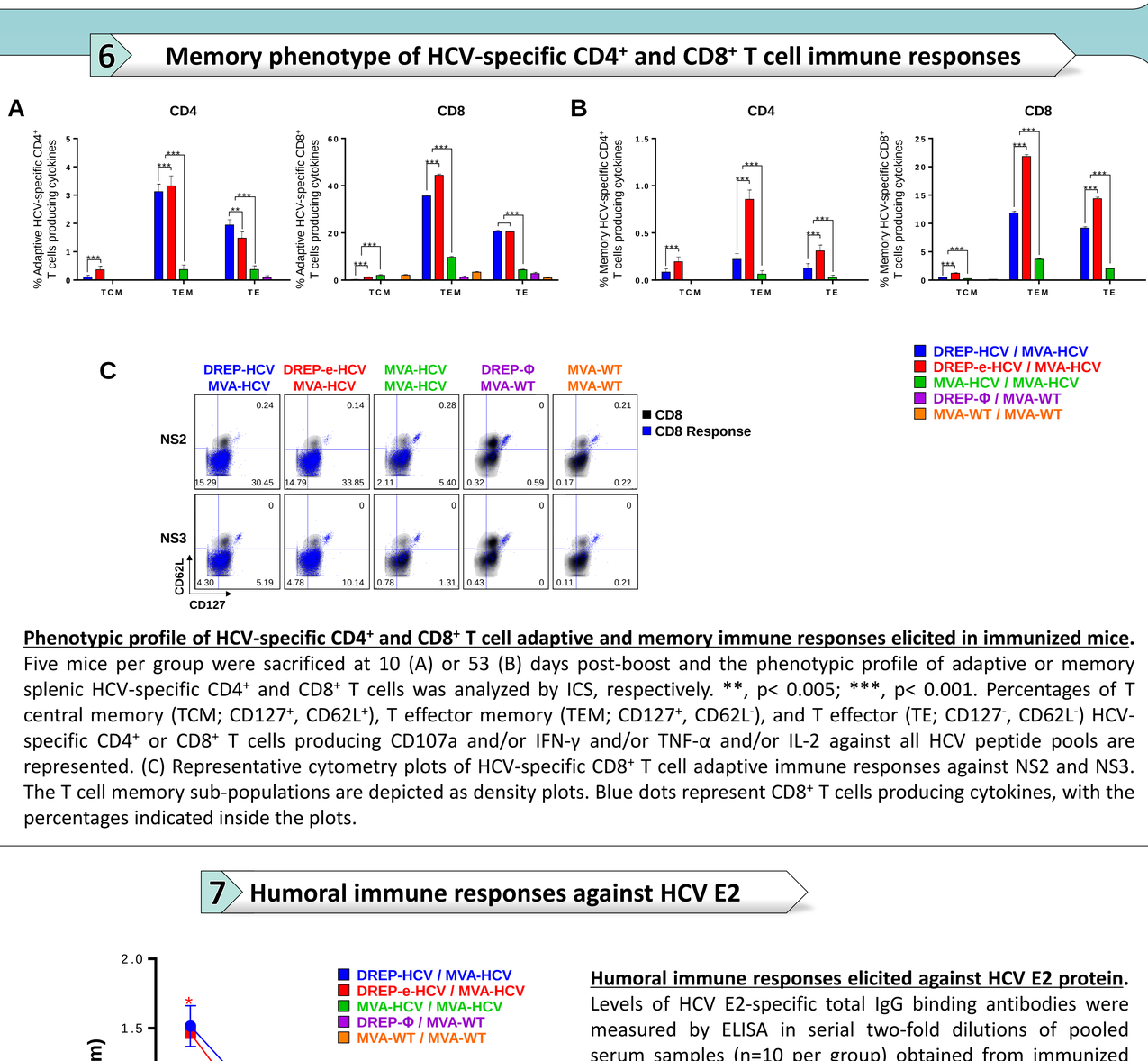


A) Magnitude of total HCV-specific CD4$^+$ and CD8$^+$ T cell memory immune responses. Splenocytes were obtained at 53 days post-boost from five immunized mice per group and HCV-specific CD4$^+$ and CD8$^+$ T cell memory immune responses elicited against all HCV peptide pools were measured by ICS. (***, p< 0.001).

B) Breadth of HCV-specific CD4$^+$ and CD8$^+$ T cell memory immune responses. Percentages of Core-, E1-, E2-, p7-, NS2-, NS3-, NS4- or NS5-specific CD4$^+$ and CD8$^+$ T cells. Frequencies represent the sum of the percentages of CD4$^+$ or CD8$^+$ T cells producing CD107a and/or IFN-γ and/or TNF-α and/or IL-2 against each HCV peptide pool. (***, p< 0.001).

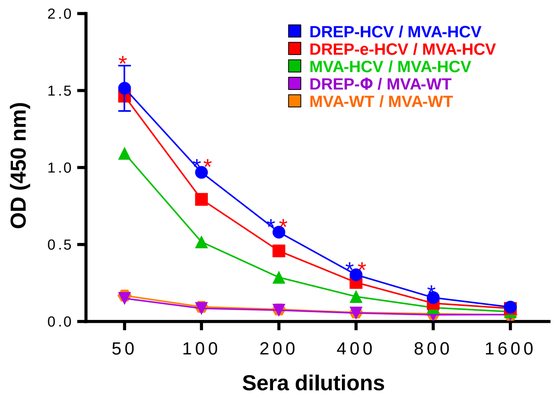
C) Polyfunctionality of total HCV-specific CD4$^+$ and CD8$^+$ T cell memory immune responses. All possible combinations of the responses are shown on the X axis while the percentages of CD4$^+$ or CD8$^+$ T cells producing CD107a and/or IFN-γ and/or TNF-α and/or IL-2 against all the HCV peptide pools are shown on the Y axis. (***, p< 0.001).

6 Memory phenotype of HCV-specific CD4$^+$ and CD8$^+$ T cell immune responses



Phenotypic profile of HCV-specific CD4$^+$ and CD8$^+$ T cell adaptive and memory immune responses elicited in immunized mice. Five mice per group were sacrificed at 10 (A) or 53 (B) days post-boost and the phenotypic profile of adaptive or memory splenic HCV-specific CD4$^+$ and CD8$^+$ T cells was analyzed by ICS, respectively. **, p< 0.005; ***, p< 0.001. Percentages of T central memory (TCM; CD127$^+$, CD62L$^+$), T effector memory (TEM; CD127$^+$, CD62L$^-$), and T effector (TE; CD127$^-$, CD62L$^-$) HCV-specific CD4$^+$ or CD8$^+$ T cells producing CD107a and/or IFN-γ and/or TNF-α and/or IL-2 against all HCV peptide pools are represented. (C) Representative cytometry plots of HCV-specific CD8$^+$ T cell adaptive immune responses against NS2 and NS3. The T cell memory sub-populations are depicted as density plots. Blue dots represent CD8$^+$ T cells producing cytokines, with the percentages indicated inside the plots.

7 Humoral immune responses against HCV E2



Humoral immune responses elicited against HCV E2 protein. Levels of HCV E2-specific total IgG binding antibodies were measured by ELISA in serial two-fold dilutions of pooled serum samples (n=10 per group) obtained from immunized mice at 10 days post-boost. Absorbance values were measured at 450 nm. The mean and standard deviations are indicated. Statistical significance shows the comparison of DREP-HCV/MVA-HCV (blue) and DREP-e-HCV/MVA-HCV (red) versus MVA-HCV/MVA-HCV . (*, p<0.05).

Conclusions

- We successfully generated novel DREP-based HCV vaccine candidates (DREP-HCV and DREP-e-HCV).
- DREP vectors efficiently promoted cellular apoptosis in transfected cells.
- Mice immunized with DREP-based HCV/MVA-HCV prime/boost immunization protocols induced a significant enhancement in adaptive and memory HCV-specific CD4$^+$ and CD8$^+$ T cell immune responses compared to MVA-HCV/MVA-HCV; being DREP-e-HCV/MVA-HCV the most potent regimen.
- HCV-specific CD4$^+$ and CD8$^+$ T cell immune responses were broad and directed mainly against E1-E2 and NS2-NS3, respectively.
- The elicited T cell immune responses were highly polyfunctional and have a T effector memory phenotype.
- The heterologous immunization schedule elicited high anti-E2 antibodies, with no differences between both DREP-based HCV vaccines.
- These findings reinforce the combined use of DREP-based vectors and MVA-HCV as promising vaccines against hepatitis C.