COMPARISON OF IMMUNOGENICITY OF HCV CORE AND ITS ALTERNATIVE READING FRAME PROTEIN IN MICE

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Background.

Hepatitis C virus (HCV) persists in up to 85% of infected individuals as a chronic infection characterized by liver infiltration of inflammatory cells that can lead to fibrosis, cirrhosis and hepatocellular carcinoma. There is no vaccine against HCV and available therapy is expensive and related with different side effects. HCV core protein represents an attractive target for an HCV vaccine. Besides the core protein, the 5 terminus of HCV genome encodes core+1/ARF protein. ARFP participates in HCV morphology or replication, it can be important in gene regulation and also it can affect immune response mechanisms.

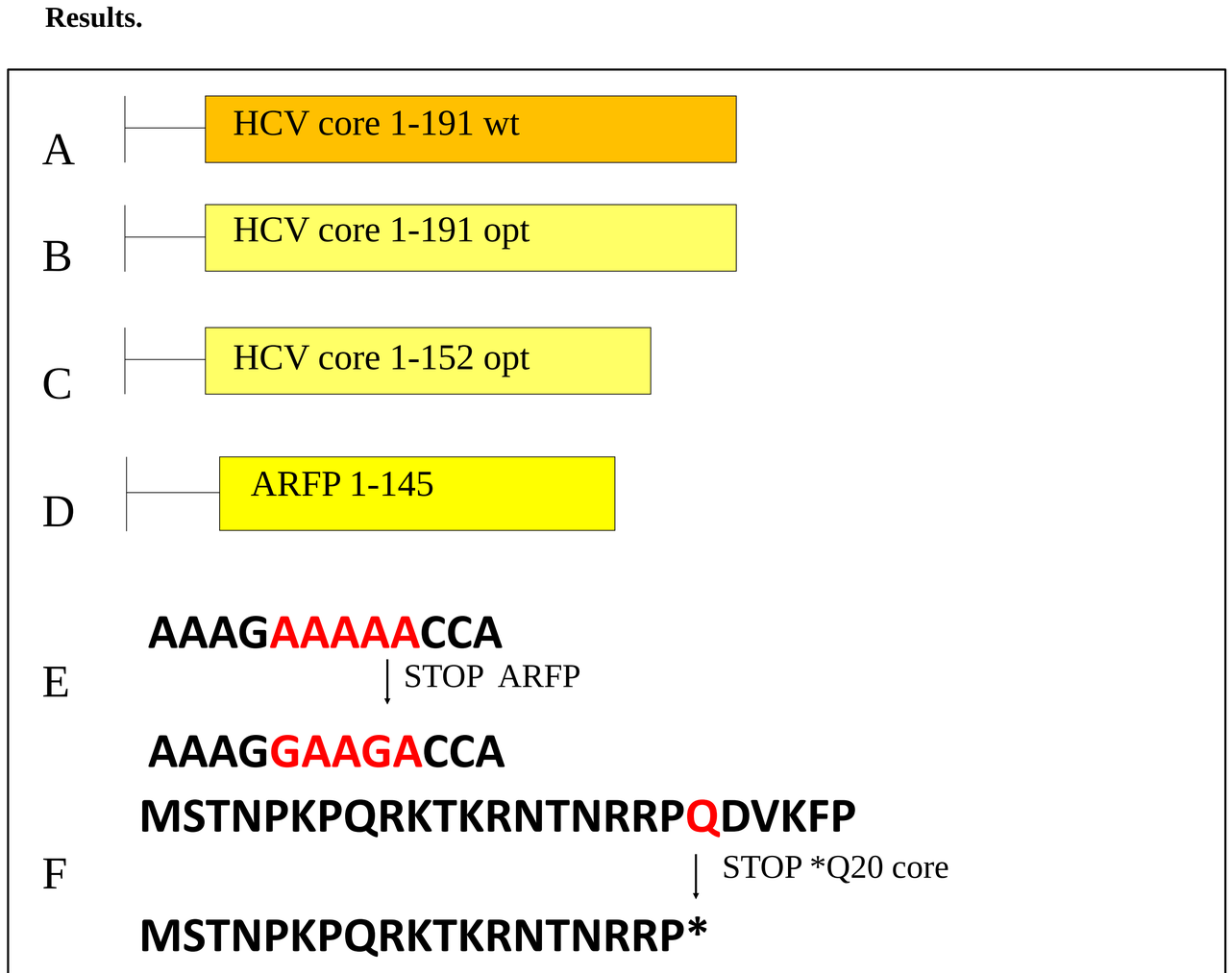
Aim.

The main aim was the study of immunogenicity of plasmids expressing proteins encoded by the 5 terminus of HVC RNA in DNA immunization.

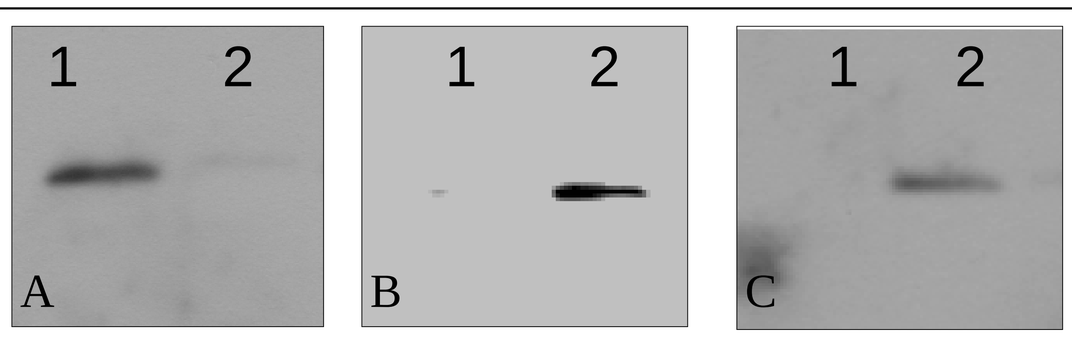
Methods.

Plasmids carrying fragments encoding HCV core and its alternative reading frame protein were obtained. Eukaryotic expression of HCV core and ARFP variants were tested in HEK293 cells, and expression level was defined by Western blotting with polyclonal rabbit anti-HCV or anti-ARFP antibodies. Mice were immunized by two injections with 40 μg plasmids or empty vector intradermally with four days interval; injections were followed by electroporation (BEX, Japan). Cellular immune responses were analyzed by IFN-γ/IL2 Fluorospot after stimulation with proteins and antigen-derived peptides. Specific antibodies were assessed by ELISA.

Results.



Schematic presentation of DNA immunogens used in current work. A – HCV core wild type, B- HCV core optimized for eukaryotic expression,C – truncated HCV optimized core, D – full lenght ARFP (HCV core gene reading frame is shifted +1 nt), E - core with prohibited ARFP expression, F – plasmid with prohibited HCV core expression but expressing ARFP.



Eukariotic expression of ARF protein. Western blot with (A) anti-βactin antibodies; (B) rabbit polyclonal anti-ARFP antibodies; (C) rabbit polyclonal anti-HCV core antibodies 1 – full lenght ARFP expressed in HEK293 cells, 2 – ARF protein expressed in *E. coli* (1-10 aa HCV core + 11-145 aa ARFP).

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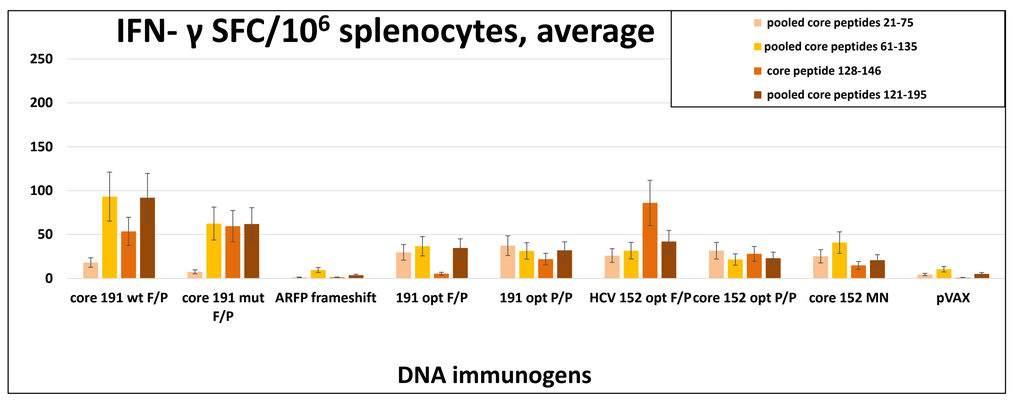
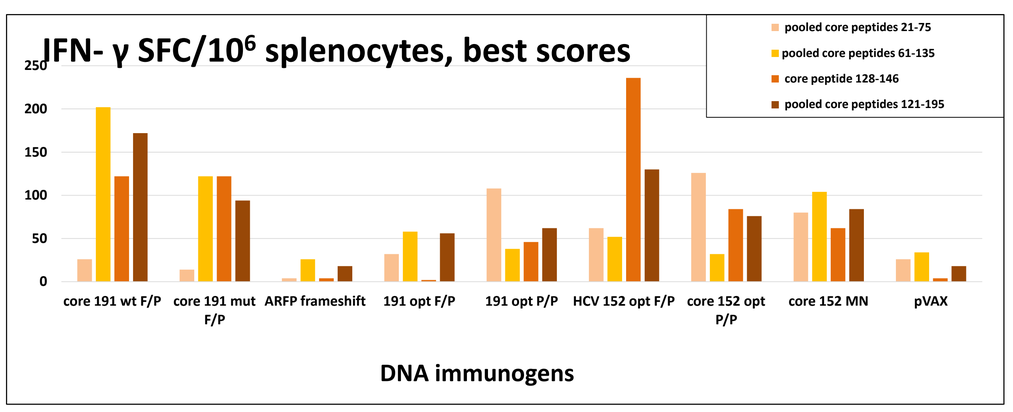
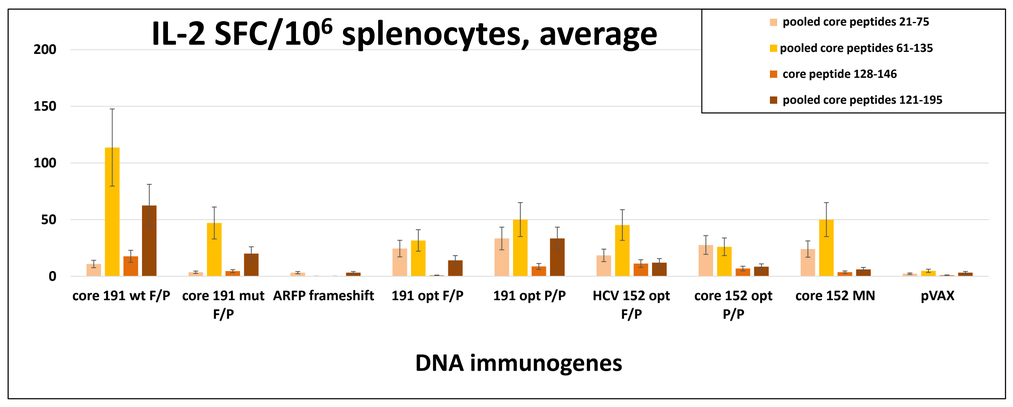
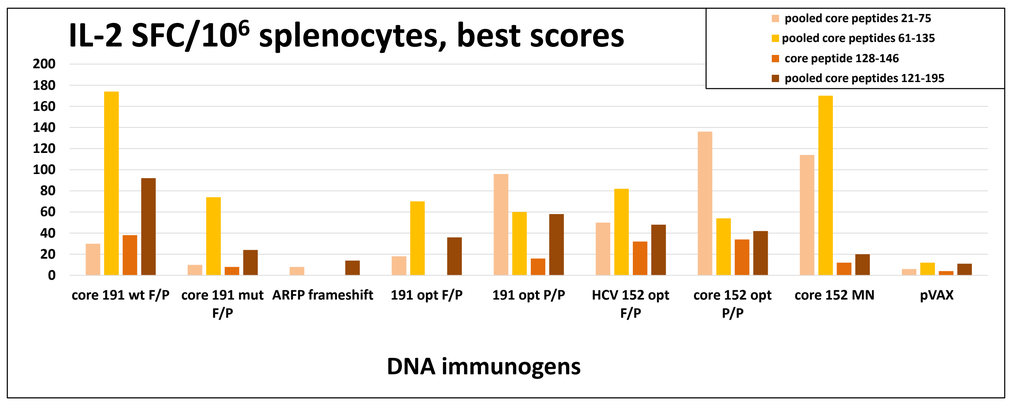
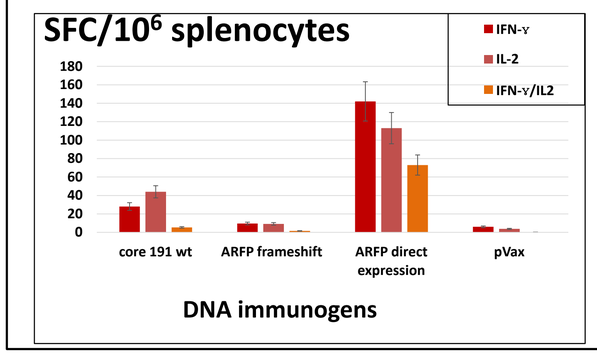








Anti-HCV core immune response induced by DNA immunization using different electrodes: F/P – fork-plate tweezers, P/P – plate-plate tweezers, MN – multineedle array. Isolated splenocytes were stimulated by HCV core derived peptides, stimulation indexes are shown.



Anti-HCV ARFP immune response induced by DNA immunization. Isolated splenocytes were stimulated by HCV ARFP-6His recombinant protein, stimulation indexes are shown.

Conclusions.

✓ ARF protein apparantly do not accumulate in suffiecient quantity in cells to be detected by Western blot.

✓Anti-ARFP immune response is not competing with that against HCV core, and cannot explain low immunogenicity of the latter in DNA-immunization performed with the virus-derived genes.