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Cell-mediated immune responses directed against hepatitis C virus (HCV) alternate reading frame protein (ARFP) are undetectable during acute infection

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Keywords

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Conflicts of interest

None.

Hepatitis C virus (HCV) alternate reading frame protein (ARFP) is produced as a result of alternate translational decoding of the core protein gene.^{1,2} ARFP induces production of pro-inflammatory cytokines and fibrogenic chemokines, suggesting a role in hepatic injury.^{3–10} ARFP has been reported to co-localize in the mitochondria and differential mitochondrial targeting may influence hepatocyte apoptosis and viral clearance.¹¹ However, biological functions of ARFP are difficult to dissociate from those of RNA structures located within the core-ARFP coding region.¹² Humoral and cell-mediated ARFP-specific immune responses are present during chronic HCV infection, confirming its expression *in vivo*.^{13–15} Because it is located in the 5' region of HCV and its expression is presumably independent of viral and host proteases, ARFP could be expressed early following infection.¹⁶ This is a key point because immunodominance and epitope hierarchy in antiviral responses are strongly influenced by the timing of viral gene expression.¹⁷ Spontaneous resolution of HCV infection occurs in 20–30% of cases and is associated with broad, multi-specific and sustained CD8⁺ and CD4⁺ T cell responses.^{18–21} However, the kinetics of ARFP-specific cell-mediated immunity and its implication in HCV clearance remain unclear. To address this question, IFN- γ ELISpot was used to examine ARFP-specific cell-mediated immune responses in a group of subjects with acute HCV infection ($n = 30$) who either spontaneously cleared the virus ($n = 8$) or progressed to chronic infection ($n = 22$) recruited through the Montreal Acute Hep C cohort.²² Cryopreserved peripheral blood mononuclear cells (PBMCs) collected before infection, during the acute phase (1–3 months), and following resolution or establishment of chronic infection (>6 months) were used. ELISpot assays were performed as previously described²³ using antigenic peptides, 15–19 amino acids in length with 11–12 residues overlap, divided in 4 pools of 25–46 peptides corresponding to HCV core, NS3 (1027–1339), NS3 (1340–1658) and ARFP proteins (BEI Resources Repository, Manassas, VA). Consistent with previous reports,^{18–21} NS3-specific T cells were detected at high frequency in the majority of study subjects, and were not significantly different between spontaneous resolvers and chronics (Fig. 1 and data not shown). In contrast, ARFP-specific T cell responses were not observed in any of the study subjects, irrespective of the outcome of acute HCV infection and in spite of the fact that all patients exhibited detectable levels of ARFP-specific antibodies in serum (data not shown). These results strongly suggest that ARFP-specific cell-mediated immune responses that involve IFN- γ production either develop late or are of low magnitude during acute HCV infection, and that they play no major role in spontaneous viral clearance. It was recently reported that ARFP expression can be suppressed by core protein,²⁴ and that ARFP binds the proteasome $\alpha 3$ subunit and is degraded by an ubiquitin-independent pathway.²⁵ These results confirm that ARFP is short-lived and are consistent with the low frequency of ARFP-specific T cells observed in our study group. Overall, data presented herein indicate that resolution of acute HCV infection is not associated with the presence of significant ARFP-specific cell-mediated immune responses. Interestingly, this also suggests that enhancement of these responses through immunization against ARFP could potentially lead to heightened rates of spontaneous viral clearance.

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Fig. 1.

HCV-specific cell-mediated immune responses in peripheral blood mononuclear cells from subjects acutely infected with HCV. IFN- γ production was quantified in individuals acutely infected with HCV followed by spontaneous viral clearance (gray symbols) or persistent infection (black symbols) using ELISpot following *in vitro* stimulation with pooled overlapping peptide panels representing HCV core protein (26 peptides), NS3 pool 1 (residues 1027–1339) (45 peptides), NS3 pool 2 (residues 1340–1658) (46 peptides), and ARFP (25 peptides). 2×10^5 cryopreserved PBMCs were stimulated in duplicates with HCV peptide pools at a final concentration of 3 $\mu\text{g}/\text{ml}$ of each peptide for 36 h as previously described.²³ Specific spot forming units (SFU) were calculated as (mean number of spots in test wells-mean number of spots in media control wells) and normalized to SFU/ 10^6 PBMCs. A response was scored positive if greater than 50 SFU/ 10^6 PBMCs. Peptides were based on the sequence of HCV-1a (H77) or HCV-3a (K3a/650), depending on the infecting viral genotype. Statistical analysis was performed using the Kruskal–Wallis test with Dunn’s post test (GraphPad Prism 4, GraphPad Software, San Diego, CA; * $p < 0.05$; ** $p < 0.01$).