Impaired IL-7 Activity of CD8+ T Cells in HCV Infection

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Abstract

**Background:** Effective immune responses against Hepatitis C virus (HCV) are dependent on CD8+ T cells, yet their function is impaired in chronic infection. The objective of the study is to determine how CD8+ T cell activity is impaired in chronic HCV infection, focusing on the responsiveness of CD8+ T cells to IL-7, IL-2, IL-7 is a cytokine critical for CD8+ T cell development, and has an important role in homeostasis, memory cell generation, and function. CD8+ T cells respond to IL-7 are also known to be impaired in other chronic infections such as HIV. CD8+ T cells responses to IL-7 are dependent on the expression of IL-7 receptor alpha on the cell membrane (mCD127), and CD8+ T cell impairment may be regulated in part by loss of mCD127, the concentration of soluble form of the receptor (sCD127), or deficiencies in IL-7 signaling in the cell. The hypothesis of this study is that HCV infection decreases CD8+ T cell activity, specifically IL-7 responsiveness.

**Methodology:** CD8+ T cells were isolated from healthy donors as well as individuals with untreated HCV infection from the Ottawa Hospital Viral Hepatitis Clinic. Expression of mCD127 on CD8+ T cells and plasma sCD127 concentrations were measured by flow cytometry and immuneassays, respectively. IL-7 induced-signalling (STAT5 phosphorylation), proliferation, and production of the anti-apoptotic molecule Bcl-2 were measured by flow cytometry.

**Results:** There was no significant difference in mCD127 expression on blood-derived bulk CD8+ T cells or plasma sCD127 levels between healthy and HCV+ individuals. IL-7 (0.1 and 1 ng/ml) induced STAT5 phosphorylation was significantly reduced (via Student’s t-test, p = 0.03 and 0.06, respectively) in CD8+ T cells from HCV+ individuals. Cell division of CD8+ T cells cultured with suboptimal T cell stimulator (PHA) was of lower magnitude in HCV infection than controls. Lastly, the production of Bcl-2 in response to IL-7 was significantly reduced as calculated by non-linear regression in CD8+ T cells of HCV+ individuals compared to healthy individuals.

**Conclusions:** These results suggest that HCV infection is characterized by decreased CD8+ T cell responsiveness to IL-7, independent of mCD127 expression. The mechanism of CD8+ T cell impairment may be through the IL-7 stimulated STAT5 pathway as phosphorylation of STAT5 and production of Bcl-2 were reduced in CD8+ T cells from chronic HCV. Identifying the mechanisms of CD8+ T cell impairment in HCV infection has implications in the design of novel treatments, namely cytokine directed immunotherapies.

**Hepatitis C Virus (HCV) Mono- and HIV-HCV Co-infection**

- HCV infection progresses to chronic infection in 50-80% of individuals, which can lead to liver fibrosis, cirrhosis and hepatocellular carcinoma
- HCV is the most common HIV co-morbidity
- HIV disease progression is worsened by HCV infection
- How HCV contributes to failing immune responses that lead to chronic disease progression in mono- and HIV-co-infected individuals
- There is no vaccine, treatment involves IFN-α and ribavirin direct acting antivirals with variable efficiencies, with new protease inhibitors recently approved

**Rationale**

- CD8+ T cells are critical for HCV control and clearance (Biely et al. 2016)
- HCV-specific CD8+ T cells are impaired in HCV infection - reduced perforin production, cytolytic function, ability to make IL-2, IFN-γ and TNFα in response to peptide stimulation (Kawate et al. 2015).
- IL-7 is necessary for T cell development, and important for homeostasis, memory cell generation, and function.
- In HCV infection, there is an increase in sCD127, a decrease in mCD127 expression on CD8+ T cells, and a decrease in CD8+ T cell response to IL-7 (Brockovec et al. 2007; CStory et al. 2010; Cimano et al. 2010; Versloot et al. 2011).

**Hypothesis**

Infection with HCV causes a reduction in CD8+ T cell activity in response to IL-7 in blood- and liver-derived CD8+ T cells, in HCV mono- and HIV-HCV co-infection.

**Experimental Design**

**Patient Population:** Healthy, HCV (untreated, untreated HCV viremia), HCV-HCV (HCV untreated, HCV HAART treated, untreated HCV viremia).

**Results**

**Blood-Derived CD8+ T Cells are Impaired in HCV Infection in Response to IL-7**

**Figure 1.** Expression of mCD127 on CD8+ T cells and plasma sCD127 concentrations do not differ in HCV infection. CD8+ T cells were stimulated with IL-7 for 15 minutes then stained with anti-pSTAT5 AlexaFlour 488 conjugated antibody and fixed and permeabilised. Mean fluorescence intensity (MFI) of pSTAT5 expression was measured by flow cytometry. CD8+ T cells from controls (n=14) and HCV infection (n=9) had a dose response to IL-7 (one way ANOVA and Dunnett-post test) and differed in the magnitude of their response (non-linear regression, p<0.001).

**Conclusions:**

- Cytokine signaling pathway could be one mechanism by which CD8+ T cells are impaired in HCV infection.
- Elucidates targets to boost CD8+ T cell function and increase HCV clearance.
- Add to knowledge about how HIV infection affects HCV infection and vice versa.

**References**