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

Stephanie C. Burke[1, 2], Lorna Carrasco-Medina[2], Curtis L. Cooper[1, 2, 3], Angela M. Crawley[1, 2]

1. University of Ottawa, Ottawa, Canada. 2. Ottawa Hospital Research Institute, Ottawa, Canada. 3. The Ottawa Hospital-General Campus, Ottawa, Canada.

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-7 is a cytokine critical for CD8⁺ T cell development, and has an important role in homeostasis, memory cell generation, and function. CD8⁺ T cell responses to IL-7 are also known to be impaired in other chronic infections such as HIV. CD8⁺ T cell 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 signalling 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 levels were measured by flow cytometry and immunobead assays, 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-infection is unknown
- There is no vaccine, treatment involves IFN-α, ribavirin and direct acting antivirals with variable efficiencies, with new protease inhibitors recently approved

Rationale

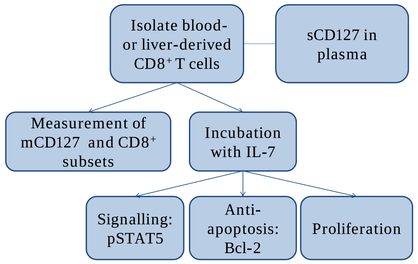
- CD8⁺ T cells are critical for HCV control and clearance (Shoukry *et al.* 2003; Li *et al.* 2005).
- HCV-specific CD8⁺ T cells are impaired in HCV infection - reduced perforin production, cytolytic function, ability to make IL-2, IFN-γ and TNFα in response to peptide stimulation (Rehermann 2007).
- IL-7 is necessary for T cell development, and important for homeostasis, memory cell generation, and function.
- In HIV 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 (Vranjkovic *et al.* 2007; Crawley *et al.* 2010; O'Connor *et al.* 2010; Vranjkovic *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: Control/healthy, HCV (untreated, uncontrolled HCV viremia), HIV-HCV (HCV untreated, HIV HAART treated, uncontrolled HCV viremia).



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Results

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

mCD127 Expression on CD8⁺ T Cell Subsets and Plasma Concentration of sCD127

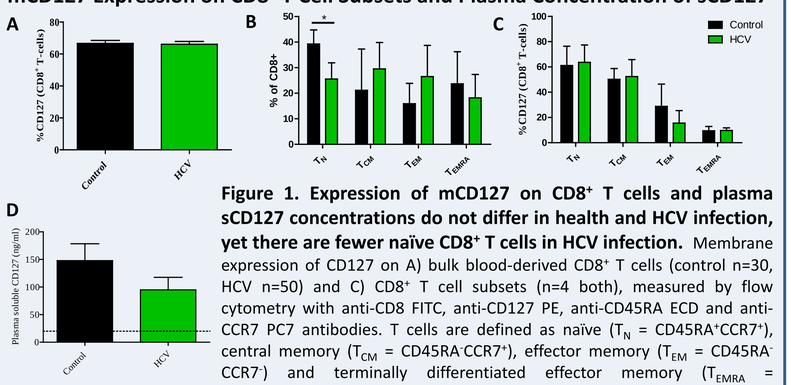


Figure 1. Expression of mCD127 on CD8⁺ T cells and plasma sCD127 concentrations do not differ in health and HCV infection, yet there are fewer naïve CD8⁺ T cells in HCV infection. Membrane expression of CD127 on A) bulk blood-derived CD8⁺ T cells (control n=30, HCV n=50) and C) CD8⁺ T cell subsets (n=4 both), measured by flow cytometry with anti-CD8 FITC, anti-CD127 PE, anti-CD45RA ECD and anti-CCR7 PC7 antibodies. T cells are defined as naïve (T_N = CD45RA⁺CCR7⁺), central memory (T_{CM} = CD45RA⁻CCR7⁺), effector memory (T_{EM} = CD45RA⁻CCR7⁻) and terminally differentiated effector memory (T_{EMRA} = CD45RA⁺CCR7⁻). B) The proportion of the naïve subset in HCV infection is significantly decreased (unpaired Student's *t*-test, p=0.02). D) Concentration of plasma sCD127 measured by immunobead assay, no significant difference between groups (control n=23, HCV n=17).

pSTAT5 Expression

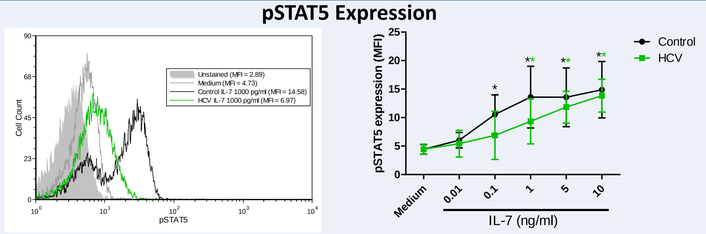


Figure 2. Expression of phosphorylated STAT5 in response to IL-7 stimulation is decreased in HCV infection. CD8⁺ T cells were stimulated with IL-7 for 15 minutes then stained with anti-pSTAT5 alexa fluor® 488 conjugated antibody by a standard fix and permeabilization protocol. Mean fluorescence intensity (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=0.005).

Cell Proliferation

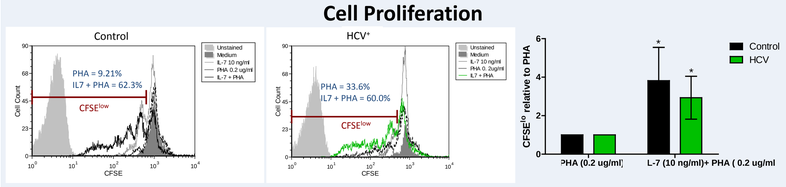


Figure 3. Proliferation of CD8⁺ T cells in health and HCV infection in response to PHA and IL-7, relative to PHA. CD8⁺ T cells were labelled with CFSE (Carboxyfluorescein succinimidyl ester) and incubated with IL-7 (10 ng/ml) and/or subumptimal amounts of PHA (phytohaemagglutinin, 0.2 ug/ml) for 5 days. Proliferation is the proportion of $CFSE^{low}$ cells by flow cytometry. CD8⁺ T cells from control and HCV infection had significant increases in proliferation due to IL-7 and PHA compared to PHA alone (Student's *t*-test, control p=0.002, n=8 and HCV p=0.003, n=8).

Bcl-2 Expression

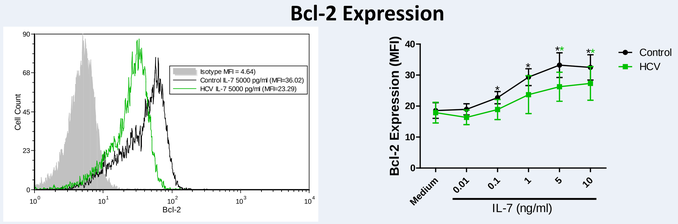


Figure 4. Bcl-2 production in response to IL-7 is decreased in HCV infection. CD8⁺ T cells were incubated with IL-7 (0.01-10 ng/ml) for 48 hours and stained with anti-Bcl-2 FITC conjugated antibody via a standard fix and permeabilization protocol. Mean fluorescence intensity (MFI) of Bcl-2 expression was measured by flow cytometry. Both CD8⁺ T cells from controls (n=8) and HCV infection (n=8) had a dose response to IL-7 (one way ANOVA and Dunnett post-test) and differed in the magnitude of their response to IL-7 (non-linear regression, p<0.0001).

Blood Derived CD8⁺ T Cells are Equivalently Impaired in HCV Mono- and HIV-HCV Co-infection in Response to IL-7

mCD127 Expression on CD8⁺ T Cells and Plasma sCD127 Concentrations

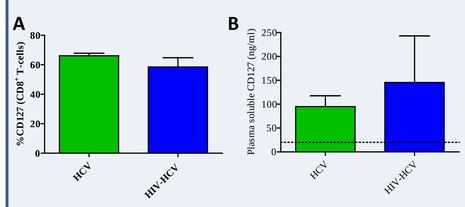


Figure 5. Expression of mCD127 on CD8⁺ T cells and plasma sCD127 concentrations do not differ in HCV mono- and HIV co-infection. A) Membrane expression of CD127 on bulk CD8⁺ T cells (HCV n=50, HIV-HCV n=9). B) Plasma sCD127 concentrations of HCV mono- and HIV-HCV co-infected groups (HCV n=17, HIV-HCV n=2). Protocols as in Figure 1.

pSTAT5 Expression

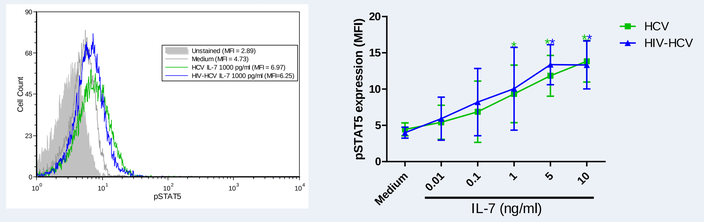


Figure 6. Expression of phosphorylated STAT5 in response to IL-7 stimulation is the same in HCV and HIV-HCV infection. CD8⁺ T cells from both HCV mono- (n=9) and HIV-HCV co-infection (n=4) had a dose response to IL-7 (one way ANOVA and Dunnett-post test), and did not differ significantly (non-linear regression). Protocol as in Figure 2.

Cell Proliferation

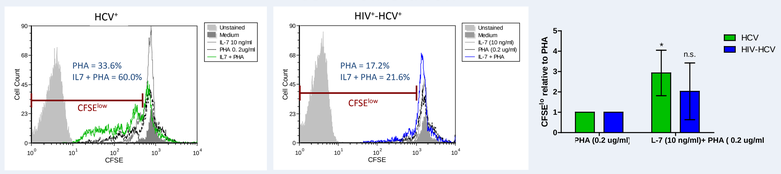


Figure 7. Proliferation of CD8⁺ T cells is reduced in HIV-HCV co-infection compared to mono-HCV infection in response to PHA and IL-7, relative to PHA. CD8⁺ T cells from HIV-HCV co-infection did not significantly proliferate in response to IL-7 and PHA, compared to PHA alone, as HCV did (n=3). Protocol as in Figure 3.

Bcl-2 Expression

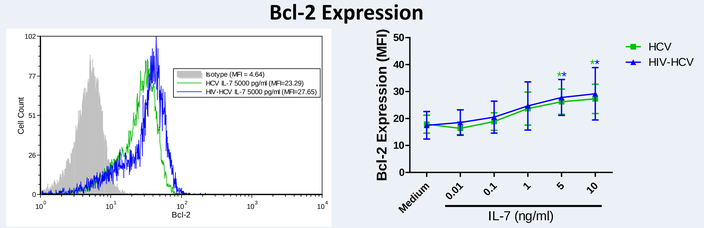


Figure 8. Bcl-2 production in response to IL-7 is the same in HCV and HIV-HCV co-infection. CD8⁺ T cells from HCV mono- (n=8) and HIV-HCV co-infection (n=3) had a dose response to IL-7 (one way ANOVA and Dunnett post-test) but did not differ in the magnitude of their response to IL-7 (non-linear regression). Protocol as in Figure 4.

Blood-Derived and Liver-Derived CD8⁺ T Cells Differ in their Subsets and Expression of mCD127 in HCV Infection

mCD127 Expression on CD8⁺ T Cell Subtypes

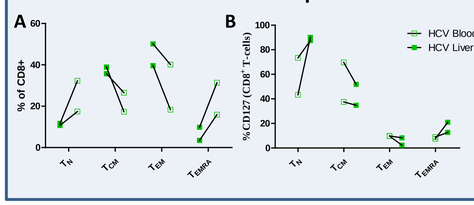


Figure 9. Proportions of CD8⁺ T cell subsets differ in blood and liver in the same HCV⁺ individual. A) Phenotype of CD8⁺ T cells in PBMCs or liver infiltrating lymphocytes. B) Membrane expression of CD127 on CD8⁺ T cell subtypes (n=2). Protocols followed as in Figure 1.

Conclusion

CD8⁺ T cells in HCV mono- and HIV-HCV co-infection have decreased activity as measured by IL-7 responsiveness independent of mCD127 expression, indicating a potential cell intrinsic mechanism of impairment. This is in contrast to what is seen in other chronic infections, such as HIV.

Relevance

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

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