Impaired activity of $CD8^+$ T cells in HCV infection in response to IL-7

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HIV and HCV Co-infection
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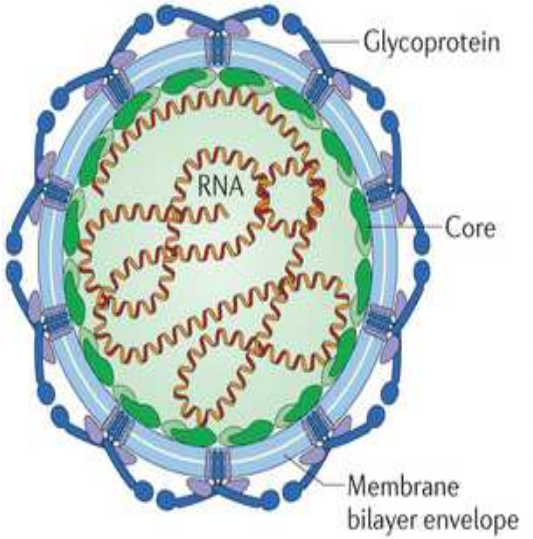
OHTN 2013 RESEARCH CONFERENCE
NOVEMBER 17-19, 2013
CHANGING THE COURSE OF THE HIV PREVENTION, ENGAGEMENT AND TREATMENT CASCADE

Hepatitis C Infection

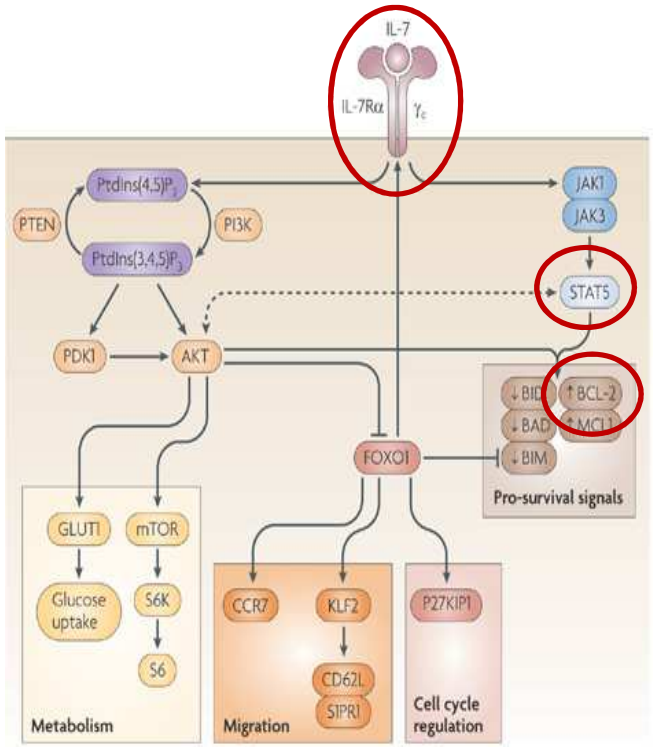
- Hepatitis C Virus (HCV)
  - Enveloped, single stranded, positive sense RNA virus
  - Liver tropic
- HCV Infection
  - Progresses to chronic infection in 50-80% individuals
    - Associated with liver fibrosis, cirrhosis and hepatocellular carcinoma
  - No vaccine, treatment involves IFN-α, ribavirin and DAAs



Lindenbach & Rice (2013). *Nature Reviews Microbiology* 11, 688–700

IL-7 and CD8$^+$ T cells in Health and Infection

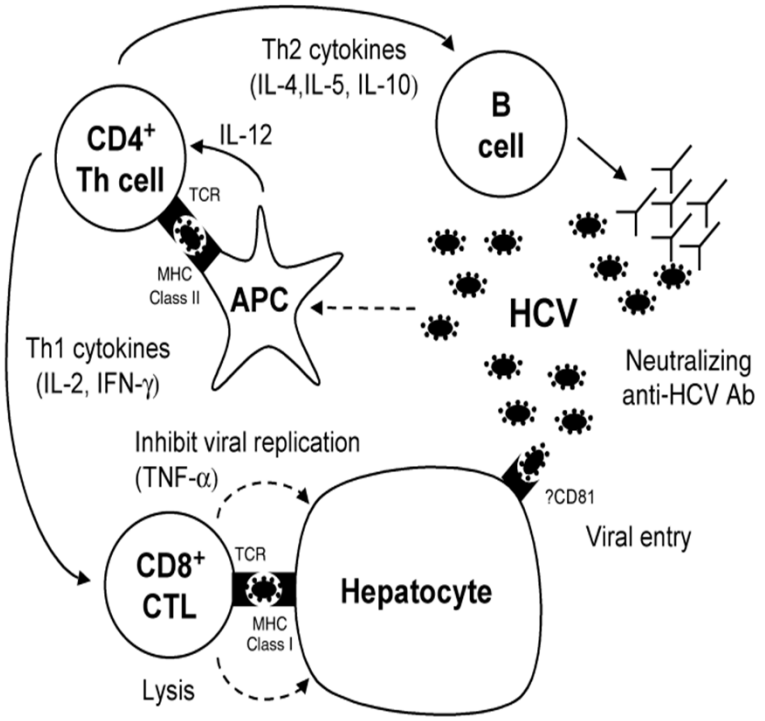
- CD8$^+$ T cells
  - Cytolytic T cell
    - cytokine and chemokine secretion
    - release of apoptotic granules
- Interleukin 7 (IL-7)
  - T cell development, homeostasis, memory cell generation, and function
  - IL-7 receptor – common IL-2 receptor γ chain (γ_c, CD132) and IL-7 receptor α (CD127)



Takeda & Jameson (2009). *Nature Reviews Immunology* **9**, 823-832

CD8$^+$ T cells in HCV Infection

- Critical for clearance of HCV infection (Li *et al.* 2005; Shoukry *et al.* 2003)
- Impaired in chronic HCV infection (Rehermann 2007)
  - Reduced perforin production, CTL function, ability to make IL-2, IFN-γ and TNFα



Freeman A J, *et al. Immunol. & Cell Biol.* (2001) **79**, 515–536

HIV-HCV Co-infection

- 25% of HIV infected individuals also have HCV (CDC 2013)
- HIV infection alters HCV prognosis
  - Decreased spontaneous resolution
  - Faster progression to liver disease
  - Higher recurrence rates (Pol et al. 1998; Kim et al. 2006)
- Little known about effect of HIV on HCV

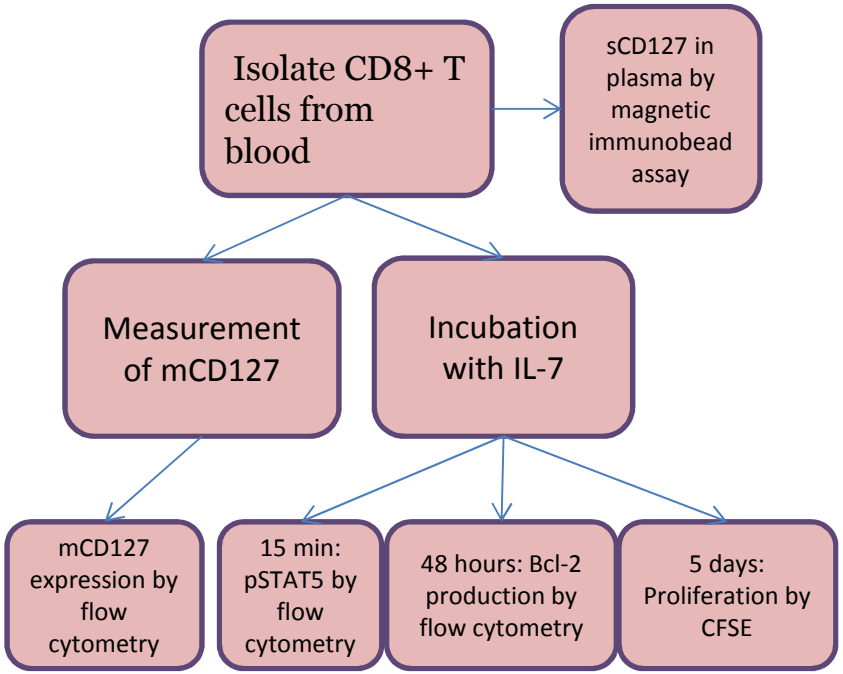
Hypothesis and Objectives

- Hypothesis
  - Infection with HCV causes a reduction in $CD8^+$ T cell activity in response to IL-7 in blood derived $CD8^+$ T cells, in both mono-HCV infection and co-infection with HIV that is HAART controlled
- Objectives
  - Determine expression of membrane CD127 (mCD127) and levels of soluble CD127 (sCD127)
  - Determine activity of $CD8^+$ T cells in response to IL-7 in health and infection, specifically
    - Phosphorylation of STAT5
    - Proliferation
    - Production of anti-apoptotic Bcl-2 protein

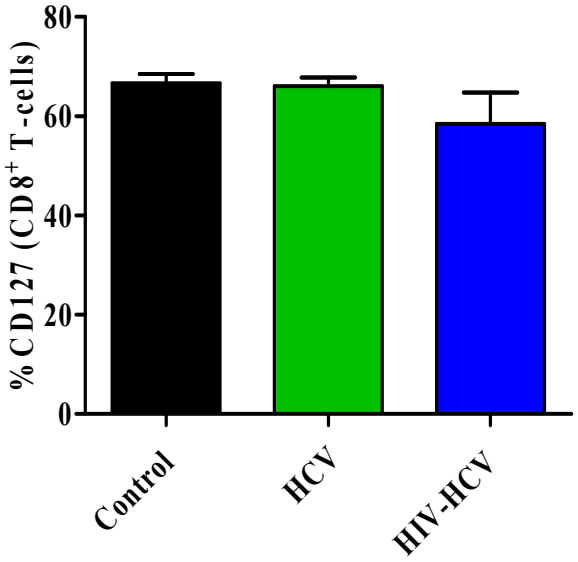
Methods

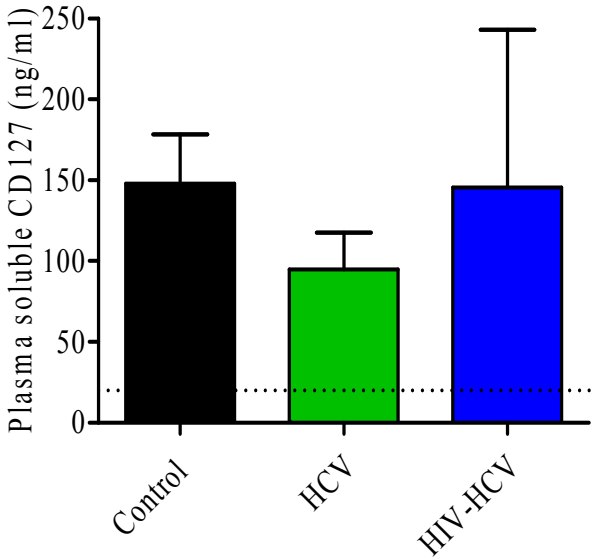
Study Subjects:

- Control/healthy
- HCV – 60, untreated, uncontrolled HCV viremia ($1.2x10^4$ – 4.4×10^8 IU/ml)
- HIV-HCV – 10, HCV untreated, HIV HAART treated, uncontrolled HCV viremia ($6.3x10^4$ – $7.8x10^7$ IU/ml)



Expression of mCD127 and plasma sCD127 levels

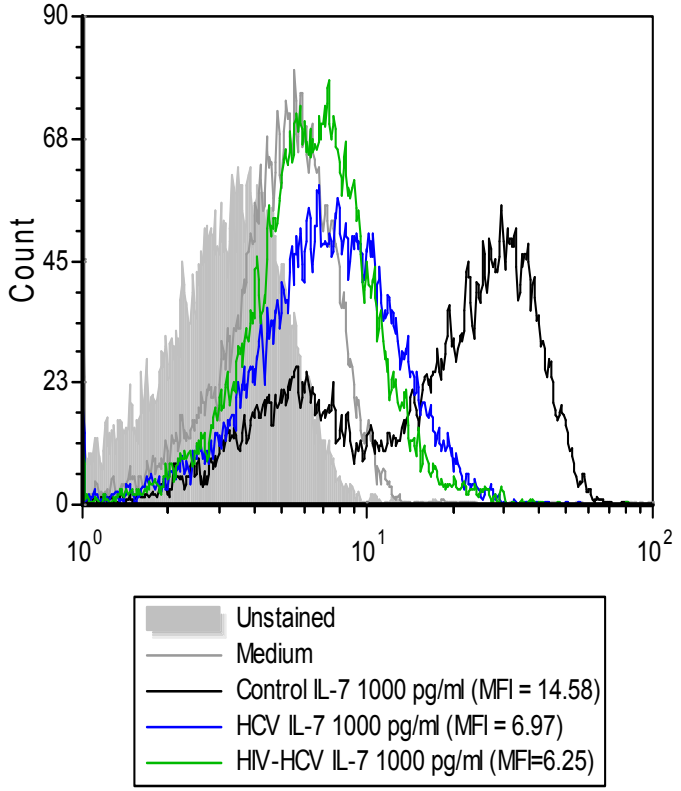


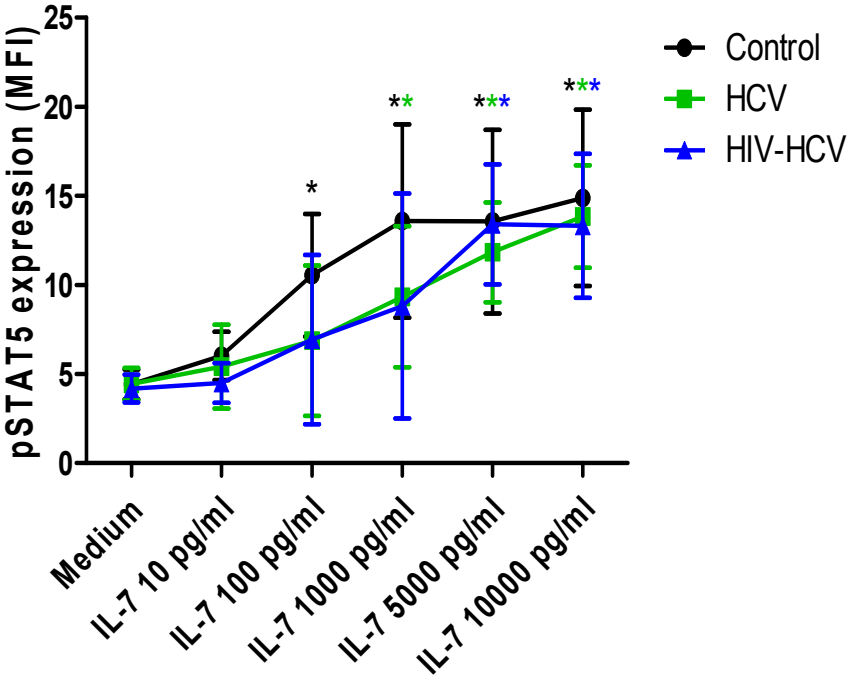


Experimental Detail:

- *Ex vivo* measurement of mCD127 expression by staining with anti-CD127 PE conjugated antibody and reading by flow cytometry
- Level of sCD127 in plasma measured directly by immunobead assay read via luminex

Phosphorylation of STAT5 by CD8+ T cells in response to IL-7

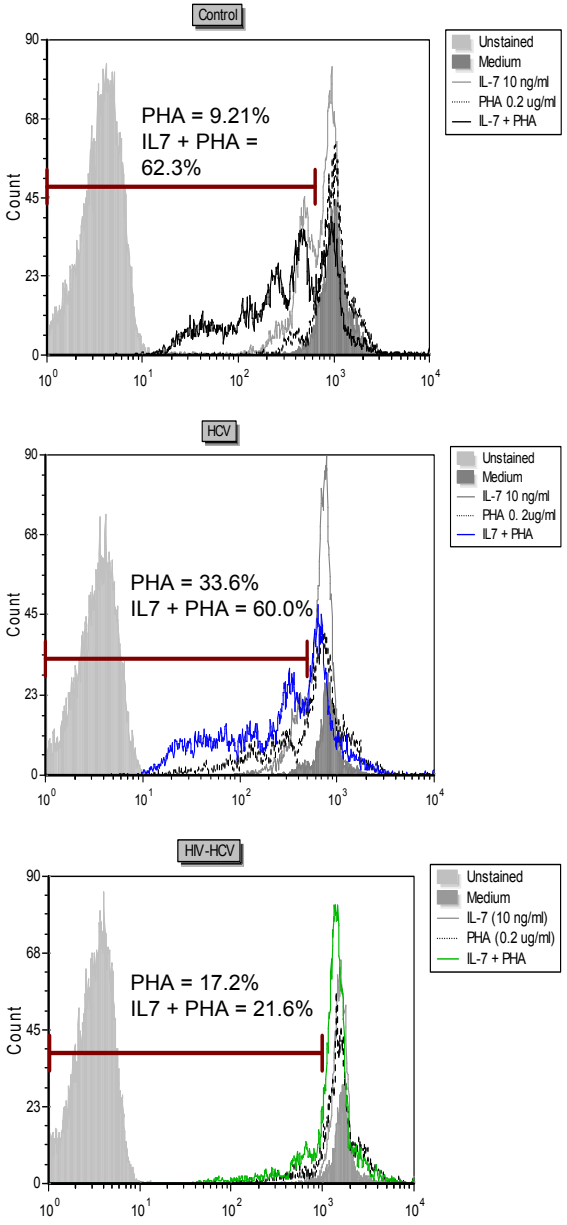


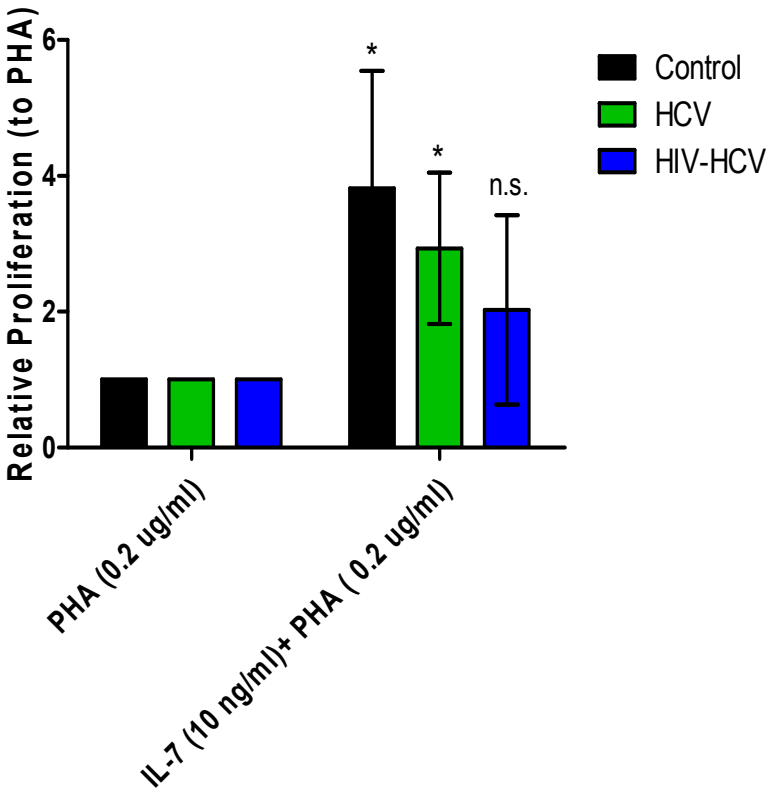


Experimental Detail:

- 15 min incubation with IL-7 (10-10,000 pg/ml)
- Stained with anti-pSTAT5 Alexafluor® 488 conjugated antibody
- Mean Fluorescence Intensity (MFI) measured by flow cytometry

Proliferation of CD8$^+$ T cells in response to IL-7

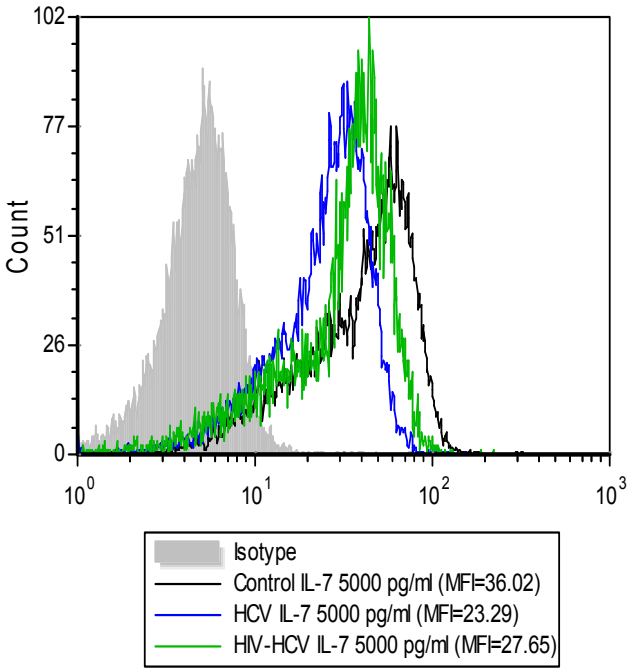


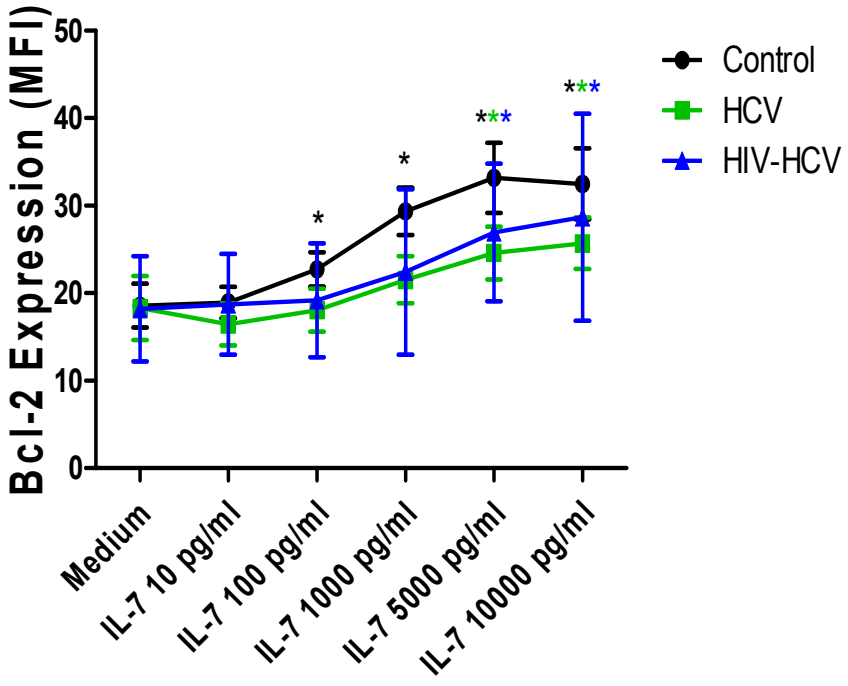


Experimental Detail:

- CD8$^+$ T cells labeled with CFSE
- 5 day incubation with IL-7 (10,000 pg/ml) and/or PHA (0.2 ug/ml)
- Proliferation measured by flow cytometry , proliferation = CFSE^{low}

Production of Bcl-2 by CD8$^+$ T cells in response to IL-7





Experimental Detail:

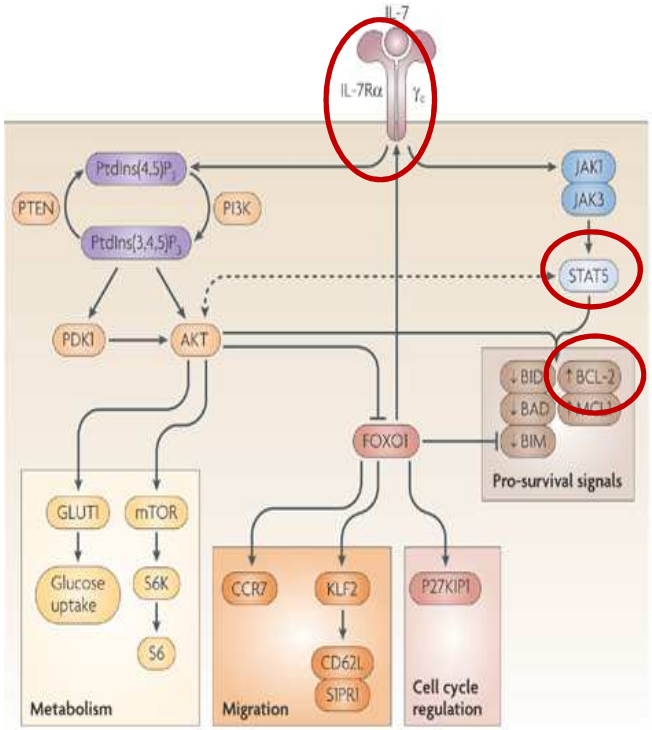
- 48 hour incubation with IL-7 (10-10,000 pg/ml)
- Stained with anti-Bcl-2 FITC conjugated antibody
- MFI measured by flow cytometry

Results in Summary and Discussion

- No change in mCD127 expression on bulk CD8$^+$ T cells nor sCD127 levels in plasma → defect in IL-7 responsiveness is inherent to the cell

- Phosphorylation of STAT5 and production of Bcl-2 in response to IL-7 are reduced in HCV and HIV-HCV co-infection compared to controls → STAT5 pathway dependent?

- Proliferation of CD8$^+$ T cells in response to IL-7 and PHA is reduced in proportion to proliferation in response to PHA alone in HCV infection compared to control



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Conclusion

- $CD8^+$ T cells in HCV and HIV-HCV infection have decreased IL-7 responsiveness that is independent of mCD127 expression

Relevance to HCV Infection

- IL-7 signaling pathway could be one mechanism by which $CD8^+$ T cells are impaired in HCV infection
- May elucidate targets that can be used to boost $CD8^+$ T cell function and increase HCV clearance
- Add to knowledge about how HIV infection affects HCV infection

Acknowledgements

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