

HAART Therapy in HIV+ patients increases IL-7 responsiveness of peripheral T cells ex vivo

Amanda Leone, Ginger Lehrman, Mamta Jain, Philip Kaiser, Louis Picker, Francois Villinger and Donald Sodora
Dept. of Internal Medicine, University of Texas Southwestern Medical Center, Dallas TX 75390,

Abstract

Background:

HAART suppresses viral loads to undetectable levels and increases peripheral CD4 T cell counts in most HIV patients, however peripheral CD4+ T-cells remain depleted in some patients despite having reduced viral loads. Interleukin-7 (IL-7) is a T-cell homeostatic cytokine involved in production and maintenance of peripheral T-cells. To determine the utility of IL-7 as an immunotherapeutic, we assessed the impact of HAART treatment on the ability of peripheral CD4 cells to proliferate in response to IL-7 ex vivo.

Methods:

Peripheral blood mononuclear cells (PBMCs) were isolated from whole blood and stimulated 6 days with IL-7 (10ng/mL) and anti-CD3 antibody (5ug/mL) or IL-7 alone (100ng/mL). CD4+ cells were phenotypically assessed via flow cytometry (CD28, CD95 and CD127) and proliferation was assessed using a CFSE dilution assay. IL-7 signaling was assessed by Western blot probing for phosphorylated STAT5 after IL-7 or interferon- α (IFN α) (100u/ml) and IL-7 treatment.

Results:

Ex vivo IL-7 (100ng/mL) stimulation induced efficient CD4+ T-cell proliferation from uninfected patients, on average 34.6% of the CD4 T cells in culture proliferated. However CD4+ T-cells from HIV+ patients did not proliferate as robustly (16.7%). HAART-treated HIV+ patients exhibited an increased proliferative capacity (27.4%). Similarly, low dose IL-7 (10ng/mL) and anti-CD3 treatment resulted in decreased responsiveness of CD4+ T cells from HIV+ patients compared to uninfected and HAART-treated patients. The absence of any significant alteration of IL7R surface expression in the three cohorts indicated that reduced IL-7 responsiveness was not the result of decreased receptor availability. We hypothesized that other cytokines may be impacting IL-7 responsiveness and undertook an assessment of the antiviral cytokine IFN α . Pretreatment of CD4+ T cells with IFN α did not impact CD4+ T cell proliferation nor STAT5 activation in the IL-7 treated cultures. Therefore, the presence of IFN α would not be predicted to abrogate the potential benefits of IL-7 immune therapy in vivo, however the exact cause of the decreased IL-7 responsiveness in HIV+ untreated patients remains unknown.

Conclusions:

HAART treatment increases the functional efficacy of IL-7 in HIV+ patients. These findings suggest that IL-7 has the potential to be an effective immunotherapeutic to increase CD4+ T cell levels if administered to patients that are virologically responsive to HAART.

Introduction

Human immunodeficiency virus (HIV) infection is characterized by peripheral CD4 T-cell depletion and immune dysfunction leading to opportunistic infections and death. Highly active antiretroviral therapy (HAART) suppresses the viral load in many patients but in some cases fails to reconstitute the depleted CD4 T-cell compartment. Patients in this category have a poorer long term prognosis than patients in which the CD4+ T-cell number increases concurrent with the decrease in viral load. Interleukin-7 (IL-7) is a key peripheral T-cell homeostatic cytokine. It is critical for the expansion and maintenance of the peripheral T-cell pool. Our long term goal is to assess the potential of IL-7 as an immunotherapeutic to reverse the CD4+ T-cell decline in the context of an HIV infection. In the non-human primate model of HIV infection, simian immunodeficiency virus (SIV) of Rhesus Macaques, IL-7 administration has successfully increased the number of peripheral T-cells in the absence of antiretroviral therapy with minimal effect on viral load. Untreated HIV infection is associated with aberrant pro-inflammatory cytokine production, including elevated IFN α . Thus, the interaction of IFN α and IL-7 in the context of an HAART treated HIV infection is potentially important. Our goal in this study was to assess proliferation and intracellular signaling events in peripheral T-cells from uninfected and HIV+ patients following IL-7 as well as IFN α stimulation ex-vivo.

Figure 1: Multiple Roles of IL-7:

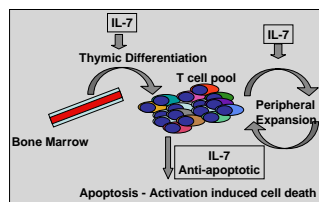
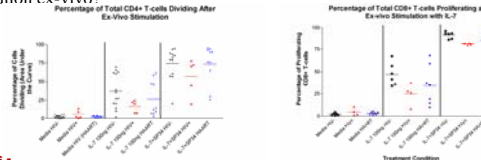


Figure 2 – Proliferation of Peripheral T-cells after IL-7 stimulation ex-vivo

Question: Does HIV infection effect the proliferative response of peripheral T-cells to IL-7 stimulation ex-vivo?



Results:

- Following IL-7 stimulation CD4+ T-cells from untreated HIV+ patients were among the poorest responders compared to CD4+ T-cells from uninfected or HAART treated HIV+ patients.
- CD8+ T-cells from untreated HIV+ patients proliferated at significantly lower levels following IL-7 stimulation than CD8+ T-cells from their counterparts from uninfected or HAART treated patients.
- In some cohorts patients can be divided into "good responders" and "poor responders" in response to IL-7 alone (100ng/mL).

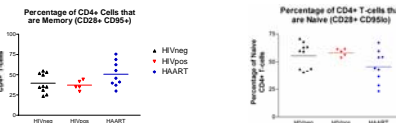
IL-7 receptor (IL-7R) Expression on CD4+ and CD8+ T-cells in Uninfected, HIV+ and HAART patients

Question: Does decreased IL-7R expression account for the decreased proliferations observed in untreated HIV+ patients? Not Likely.

Studies have demonstrated that CD8+ T cell surface expression of the IL-7R is decreased in HIV+ patients and SIV+ macaques following infection. The receptor downregulation is partially restored by HAART. (Paandim et al. *Journal of Immunology* 2005, 174: 2900-290; Reith et al. *AIDS* 2005, 19:2077-2086; Patwa et al. *Journal of Infectious Disease* 2006, 193:879-87; MacPherson et al. *J Acquir Immune Defic Syndr* 2001, 28(5):454-457; Cole et al. *Clinical and Experimental Immunology* 2006, 143:398-403) Studies of CD4+ T-cells have indicated that the IL-7R is only slightly decreased following infection (~80% IL-7R+ uninfected vs ~70% IL-7R+ HIV+). Additional studies have shown that peripheral CD4+ T-cells do respond to IL-7 stimulation in HAART treated patients expressing similarly reduced levels of IL-7R. (Cole et al. *J Acquir Immune Defic Syndr* 2006, 00:1-9; Koesters et al. *Eur. J Immunol* 2006, 36:336-344; Monzack et al. *Virology* 2006, doi:10.1016/j.virus.2006.07.031; Read et al. *J Acquir Immune Defic Syndr* 2006, 42(5):537-543)

Figure 3 – Peripheral Naïve and Memory Phenotypes in Uninfected, HIV+ and HAART Treated Patients

Question: Are the differences in proliferation observed between the cohorts a result of an intrinsic difference in the makeup of the T-cell populations?

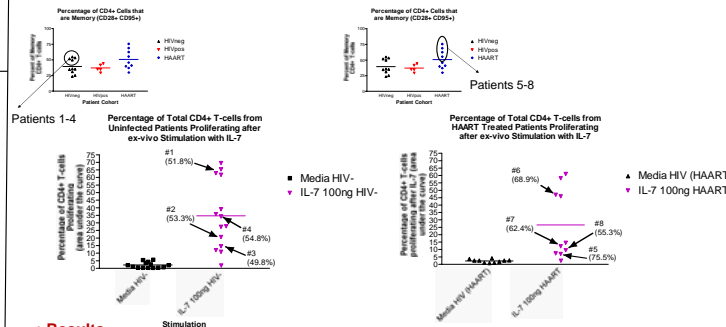


Results:

- Phenotypic changes between uninfected and untreated HIV+ patients do not account for reduced proliferation in response to IL-7.

Figure 4 – The Percentage of CD4+ Memory Cells (CD95+) in Unstimulated PBMCs from Uninfected and HAART patients does not predict IL-7 responsiveness ex-vivo

Question: Does the percentage of memory cells account for the difference in responsiveness of the total CD4+ T-cells to IL-7 stimulation ex-vivo?



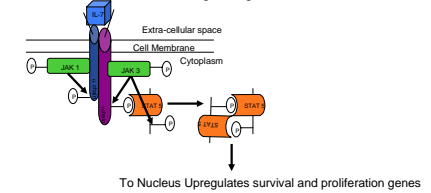
Results:

- In both the uninfected and HAART patients an increased percentage of CD4+ memory T-cells does not predict whether the patient will be highly responsive to IL-7 stimulation ex-vivo.

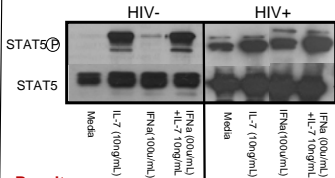
Figure 5 – Effect of Co-treatment with IFN α and IL-7 on proliferation and apoptosis of HIV- PBMCs

Question: Impaired proliferation in both CD4+ and CD8+ T-cells suggest that the virus induced indirect effects are impairing T-cell responses. Is the ability of IL-7 to phosphorylate STAT5 impaired in peripheral T-cells from HIV infected patients?

A) IL-7R associated JAK/STAT signaling



Assessment of IL-7 induced activation of STAT5 downstream of the IL-7R in peripheral T-cells from uninfected and HIV+ patients. Impact of coadministration of IFN α



Materials and Methods:

PBMCs were isolated from uninfected patients and treated with IL-7 (10ng/mL), IFN α (100u/mL) or the two in combination for 30 minutes at 37C. The cells were then lysed and the lysate quantified and electrophoresed through a 10% SDS-PAGE gel. STAT5 in both its unphosphorylated and phosphorylated form was detected by western blot using monoclonal antibodies.

Results:

Preliminary analyses indicate that IL-7 increases the amount on phosphorylated STAT5 in T-cells from both uninfected and untreated HIV+ patients following ex-vivo stimulation.

Summary

- In summary, HIV infection impairs proliferative response to IL-7 when left untreated. HAART treatment restores a robust proliferative response in some patients.
- In uninfected and HAART treated patients, there appears to be two distinct response phenotypes to IL-7 stimulation ex-vivo.
- The impaired proliferative response in untreated HIV infection does not appear to be due to:
 - IL-7 receptor levels
 - T cell phenotypic changes (memory/naive)
 - Impaired STAT5 phosphorylation

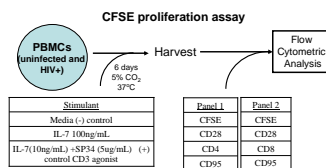
Conclusions

- The decreased proliferative response to ex-vivo IL-7 stimulation during HIV infection could not be attributed to any discernable intrinsic phenotypic change in the peripheral T-cells. When compared to the uninfected patients, HIV+ patients had similar percentages of naive and memory CD4+ T-cells and have been shown previously to express a functional IL-7R. The similarly impaired proliferation observed in CD8+ T-cells suggests that the impairment is a result of indirect rather than direct effects of the virus. Patients can recover IL-7 responsiveness in CD4+ and CD8+ T cells following HAART.
- Future studies are focusing on investigation of the signaling events following IL-7 stimulation of T cells from uninfected and HIV-infected patients.

IL-7 as a potential immune therapeutic?

- These results suggest that IL-7 could be an effective immunotherapeutic agent to help recover the depleted CD4+ T-cell compartment in the context of a HAART treated HIV infection.
- Patients that are most likely to benefit from IL-7 therapy may be those patients that respond to HAART by suppressing viral load but fail to increase their CD4+ T-cell count. Further investigation is needed to identify the factors that may contribute to the efficacy of an IL-7 therapy.

Experimental Design to Assess IL-7 induced proliferation in peripheral T-cells:



Method - Proliferation of peripheral blood mononuclear cells (PBMCs) was assessed using a CFSE dilution assay over 6 days. CD4+ T-cells were phenotypically identified using flow cytometry (CD4, CD28 and CD95). Cells were stimulated with media alone, IL-7 (100ng/mL) or IL-7 (10ng/mL)+SP34(5ug/mL).