

IL-7 Mediated T-Cell Homeostasis in Long Term Survivors of Perinatal HIV-1 Infection

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Abstract

Background: Survival of HIV-1 infected infants into adulthood has become increasingly common with the availability of combination antiretroviral therapy (ART). We previously reported that these long term survivors generally have CD4+ T cell counts, thymus volume, and T cell receptor recombination excision circle (TREC) values that are similar to uninfected young adults, even though most had developed significant immunodeficiency prior to the availability of ART. However, these subjects displayed surprisingly vigorous persisting cellular immune responses against HIV-1 in the absence of detectable viremia, suggesting substantial ongoing HIV-1 replication below the limits of detection.

Methods: We examined 20 adolescent and adult survivors of HIV-1 infection (AAS) acquired perinatally (n = 18) or by transfusion in infancy (n = 2) for evidence of ongoing viral replication. 16 (80%) had prior CDC class B or C disease. All were receiving ART with viremia < 400 copies/mL for at least 1 year. Thymic size was determined by volumetric computed tomography analysis. Percentages of CD4+, CD8+, HLA-DR+/CD38+/CD8+, and CD45RA+/CD27+/CD4+ T cells were assessed by flow cytometry. PBMC TREC and HIV-1 DNA levels were quantified by real time PCR. Plasma IL-7 levels were determined by ELISA. 18 seronegative subjects served as controls.

Results: The AAS were slightly younger than the control subjects (mean age 18±1.4 versus 20±1.4 years) but had similar thymus volume (20.2±13.1 versus 15.4±6.09 mL, p>0.05). CD4+ T cell percentages were higher in the control group (37.1% versus 25.3%, p = 0.0007), but the AAS had mean total and naive CD4+ T cells and TRECs that were statistically indistinguishable from the control group. The AAS had a higher fraction of activated CD8+ T cells than controls, and high HIV-1 DNA viral load (mean = 3.7 logs per million CD4+ T cells) despite undetectable plasma viral RNA. There was a significant inverse correlation between plasma IL-7 levels and the total (r = -0.52, p = 0.02) and naive (r = -0.47, p = 0.037) peripheral blood CD4+ T cell concentrations in the AAS group. In contrast, IL-7 levels were unrelated to T cell numbers in the control group. Lymphocyte proliferation responses to phytohemagglutinin stimulation and candida and tetanus antigens were significantly lower in the AAS compared to controls (p = 0.05, 0.01, 0.40).

Study Participants

Study Participants. We examined thymic volume and lymphocyte parameters in a subset of 25 HIV infected patients followed at the Adolescent Medicine Clinic at Children's Hospital Los Angeles (CHLA), the Clinical Immunology Service at CHLA, and the Care 4 Families Clinic at UCLA (C4F). These patients all had evidence of HIV infection since childhood by antibody methods, and had plasma HIV RNA levels of 10,000 copies/ml for at least 3 months (median 3.2 years), while receiving multidrug antiretroviral therapy. At the time of enrollment, protease inhibitor combinations included lopinavir/ritonavir (LPV/R) combined with amprenavir, saquinavir, or atazanavir, and ritonavir with atazanavir.

We also recruited healthy adolescents and young adults (n = 18) by advertising in the Los Angeles area, and confirmed that they were HIV uninfected by standard ELISA antibody screening methods.

Informed consent for study participation was obtained in accordance with approvals from the Institutional Review Board at each institution.

Laboratory Methods

Cell Staining and Flow cytometry. Whole blood cell staining was performed. Flow cytometric analysis was performed using FACSCalibur flow cytometer and the results were analyzed using CellQuest Software (BD Biosciences).

IL7 assays Plasma IL7 was measured by ELISA.

Quantitation of T cell receptor recombination excision circles (TREC). TREC were quantified by real time PCR. A standard curve was established with 25 copies up to 1,000,000 copies of a plasmid containing the signal joint TREC fragment (provided by D. Douek). Cellular DNA was quantified by amplifying CCR5 sequences. Using an estimate of 8 µg of DNA per million cells, TREC numbers are reported as TREC/million PBMC (or million purified CD4+ T cells).

HIV Viral Loads. HIV plasma RNA concentrations were measured at UCLA and CHLA using Amplicor HIV-1 Monitor testing, with a limit of sensitivity of 50 copies/ml (Roche Diagnostics, Indianapolis, ID). HIV DNA copy numbers per million CD4+ T cells were quantified by Real time PCR.

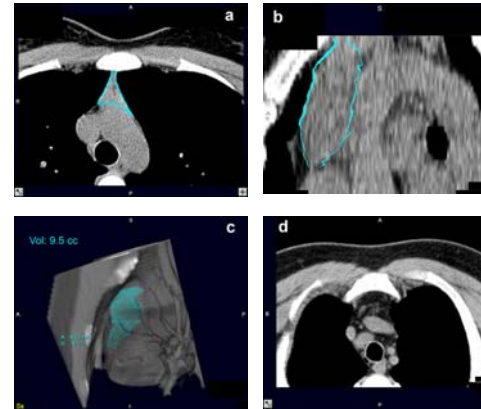
Volume of the thymus Non-contrast helical CT studies of the chest were performed with 3 mm collimation extending from the thoracic inlet to the lung bases. Two radiologists, blinded to the identity and HIV disease status of the subject, determined the superior and inferior margins of the thymus. The contours of the thymus were identified by anatomical landmarks and outlined in all CT slices with a free-hand region-of-interest (ROI) tool using the Vitrea 2 software, running on a special 3D imaging workstation. Surface rendering and Vitrea 2 built-in volume calculation were subsequently performed.

Statistical Analysis. Analyses were conducted using SAS 8.2 (SAS, Inc., Cary, NC) software to examine data from the TREC assays on cell lysates from PBMC. Continuous variables were compared using a two-sided Wilcoxon Rank Sign Test. Categorical variables were compared using Fisher's exact test. Univariate analyses utilized SAS PROC CORR to calculate simple descriptive statistics and Pearson correlation coefficients for selected laboratory marker results. Ordinal logistic regression was used to analyze categorical outcomes.

Study Participants

Variable	Control Group (n=18)	HIV Infected (n=20)	P Value
Age	20.6 ± 1.3	17.6 ± 2.5	0.0006
Height (m)	1.66 ± 0.08	1.60 ± 0.11	0.03
Weight (kg)	66.8 ± 15.0	55.5 ± 9.7	0.012
Body Mass Index (wt/ht ²)	23.9 ± 4.7	21.6 ± 3.2	NS
CDC Classification :		N=1,A=3, B=6,C=10	
Current ARV therapy			
PI*		14	
NNRTI		2	
PI + NNRTI		3	
PI + enfuvirtide		1	

Thymic volume by CT



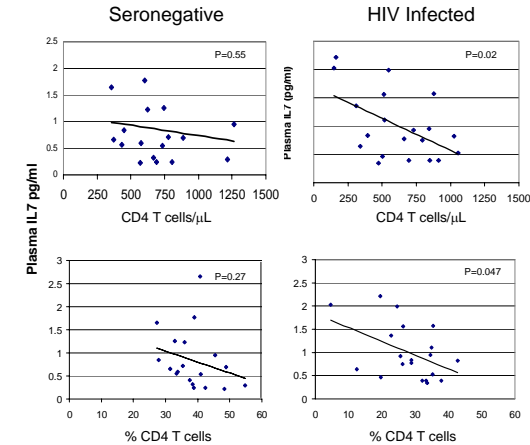
- Axial CT section from uninfected subject. Thymus outlined in blue.
- Ovoid thymus in control subject
- Example of reconstructed image with volume calculation.
- Example of thymus with fatty infiltration

Immunological and virological parameters

Variable	Control Group (n=18)	HIV Infected Youth (n=20)	P Value
% CD4+	38.7 ± 7.4	28.0 ± 9.1	0.0007
CD4+ cells/ml	693 ± 248	618 ± 270	NS
% Naive CD4+ cells	44.2 ± 12.7	52.2 ± 14.4	NS
Naive CD4+ cells/µl	320 ± 163	345 ± 196	NS
% CD8+	25.7 ± 6.8	37.1 ± 10.2	0.0005
CD8+ cells/µl	435 ± 68	852 ± 387	0.0018
% Activated CD8+	0.64 ± 0.55	1.59 ± 2.12	NS
Thymic Index*: 0	0	0	
1	1	3	
2	11	6	
3	6	10	NS
4	0	1	
5	0	0	
Thymic volume	15.8 ± 6.3	20.5 ± 13.2	NS
TREC/million PBMC	10,400 ± 5520	11,700 ± 8200	NS
Log ₁₀ HIV DNA copies /million CD4+ T cells		3.69	

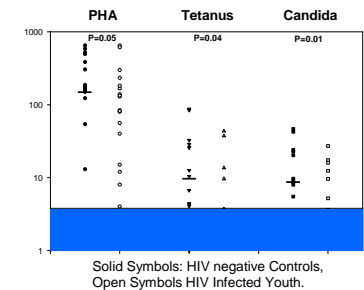
*McCune JM, et al. High prevalence of thymic tissue in adults with HIV-1 infection. J Clin Invest 1998;101:2301-8.

IL7 vs CD4 T cell Parameters



Upper panels: IL7 versus Absolute CD4+ T cell number
Lower Panels: IL7 versus % CD4 T cells/ml blood.

Lymphocyte Proliferation Assay DNA



Solid Symbols: HIV negative Controls,
Open Symbols HIV Infected Youth.

Summary and Conclusions

IL-7 mediated homeostatic mechanisms may be involved in the maintenance of thymopoiesis and T cell populations in adult survivors of HIV-1 infection in infancy, in whom incomplete suppression of HIV-1 replication appears common. The observed correlation of IL-7 to T cell levels suggests limitations in IL-7 and T cell regeneration in these subjects is limiting under the stress of ongoing viral replication. Intensified therapy may be needed in these AAS to optimize immunological function.