



HIV Infection Leads to Increased Immune Activation by Two Distinct Pathways that Differentially Affect CD4 and CD8 T Cells

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Introduction

HIV infection is characterized by a brisk immune activation that is felt to play an important role in the CD4 depletion and dysfunction of patients with AIDS. Several hypotheses explaining the cause(s) of immune activation have been postulated. The mechanisms are not completely understood, and while closely associated with changes in viral load are likely only partially explained by direct effect of the virus. Inflammatory cytokines with bystander activation, antigen-specific T cell receptor engagement as well as homeostatic pathways may be important in this setting. Whatever pathway(s) are involved they must explain the depletion of CD4⁺ T cells in a setting of expansion of CD8⁺ T cells.

Aim

In the current study we tested the hypothesis that the immune activation in HIV infection is the net product of 2 distinct pathways: the inflammatory response to HIV infection and the homeostatic response to CD4 depletion.

Materials and Methods

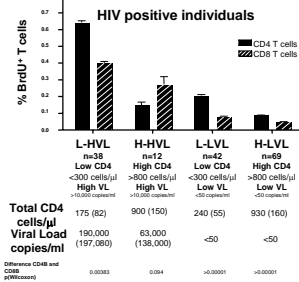
PATIENTS: Patients enrolled in NIAID intramural HIV clinical research studies had routine measurement of cell turnover/immune activation. Among the measurements was spontaneous incorporation of BrdU. During this time a total of 341 patients and 373 controls underwent a total of 5240 and 1453 evaluations respectively. Eliminating patients on IL-2 or IFN- α decreased the number of patients to 283 and 3725 respectively. The 283-patient sample was divided based on CD4 T cell counts Low: CD4<300 cells/ml and High: CD4>800 cells/ml. These groups (Low and High CD4 T cell counts) were further divided by the Viral Load (VL) into Low VL: >51 copies/ml and High VL: >10,000. The four categories of patients (L-LVL, H-LVL, L-HVL and H-HVL) included 161 patients.

CYTOKINES: Serum samples from selected patients were tested for levels of gamma-common cytokines (IL-2, 7 and 15) and for levels of inflammatory cytokines TNF-alpha, IL-5, etc.

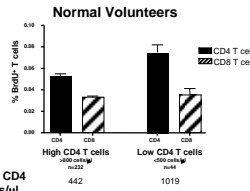
pSTAT-5: the *in vivo* effects of gamma-common cytokines were determined by measuring levels of STAT5 phosphorylation in PMBCs by flow cytometry.

Results

The proliferation of CD4 T cells is primarily driven by CD4 T cell depletion while the proliferation of CD8 T cells is primarily driven by viral load



Ex vivo BrdU labeling in HIVpos individuals: The result shows an increase of proliferating CD4 T cells in response to CD4 T cell depletion in those patients with low CD4 T cell counts that is maintained after viremia is suppressed to <50copies/ml. CD8 T cell proliferation is mainly driven by viremia.



Ex vivo BrdU labeling of CD4 and CD8 T cells from normal volunteers: Only CD4 T cell proliferation is influenced by CD4 T cell counts.

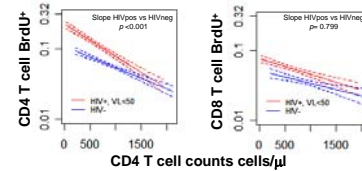
CD4 T cell counts and viral load both contribute to CD4 T cell proliferation while viral load is the main determinant of CD8 T cell proliferation in HIV infected individuals

Model	r ²	Coeff of CD4 (95% CI)	Coeff of VL (95% CI)	p-value Univariate vs Multivariate
CD4	Bivariate analysis	0.375 (p<0.0001)	0.1666 (p=0.0004)	<0.0001
	Univariate analysis	0.302 (p<0.0001)	0.15411 (p=0.0001)	<0.0001
	Univariate analysis	0.302 (p<0.0001)	0.17238 (p=0.0001)	<0.0001
CD8	Bivariate analysis	0.188 (p<0.0001)	0.2072 (p=0.0004)	<0.0001
	Univariate analysis	0.188 (p<0.0001)	0.21768 (p=0.0001)	<0.0001
	Univariate analysis	0.188 (p<0.0001)	0.24284 (p=0.0001)	<0.0001

Multivariate analysis: both viral load and CD4 T cell depletion contribute to CD4 T cell proliferation. CD8 T cell proliferation is mainly driven by the viral load.

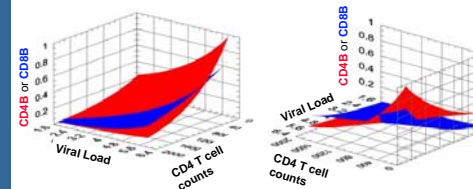
Results

Ongoing viral replication maintains higher levels of proliferation in both CD4 and CD8 T cells



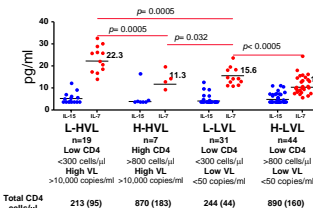
Comparisons between HIVneg and HIVpos (<50 copies/ml) volunteers with CD4 T cell counts: 203 to 1938 cells/ml. Convergence of CD4 slopes indicates a similar rate of turnover at high CD4 T cell count; there was no convergence of the CD8 slopes.

Contribution of CD4 levels and viral load to CD4 and CD8 T cell proliferation in HIVpos individuals



Modeling CD4 and CD8 T cell proliferation: the model explores the behavior of CD4 and CD8 T cells in response to viral load and CD4 T cell depletion

Increased CD4 T cell proliferation in response to CD4 T cell depletion is driven by IL-7



Detection of cytokines: Significant increases of IL-7 were detected in the serum of HIVpos individuals with low CD4 T cell counts

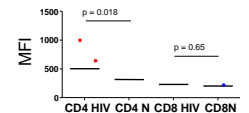
Results

Increased circulating IL-7 is driven mainly by the homeostatic response to CD4 T cell depletion

Cytokine	Analysis	p ² (p value)	Regression coefficients (95% CI)		p-value univariate vs bivariate
			CD4 count/ml	VL (copies/ml)	
Over 1000 subjects with VL<500 copies/ml					
IL-7	Bivariate	0.0001	0.0001	0.0001	0.0001
	Univariate	0.0001	0.0001	0.0001	0.0001
	Bivariate	0.0001	0.0001	0.0001	0.0001
	Univariate	0.0001	0.0001	0.0001	0.0001
IFN γ	Bivariate	0.0001	0.0001	0.0001	0.0001
	Univariate	0.0001	0.0001	0.0001	0.0001
	Bivariate	0.0001	0.0001	0.0001	0.0001
	Univariate	0.0001	0.0001	0.0001	0.0001
TNF α	Bivariate	0.0001	0.0001	0.0001	0.0001
	Univariate	0.0001	0.0001	0.0001	0.0001
	Bivariate	0.0001	0.0001	0.0001	0.0001
	Univariate	0.0001	0.0001	0.0001	0.0001

Multivariate analysis: the multivariate analysis shows serum levels IL-7 is mainly determined by CD4 T cell counts; not by HIV RNA levels.

Phosphorylated STAT-5 (pSTAT-5) is increased in CD4 T cells From HIVpos individuals



Detection of pSTAT-5: Significant increases in MFI of phosphorylated STAT-5 was detected in CD4 T cells from HIVpos individuals compared to normal volunteers

Conclusions

- CD4 and CD8 T cell proliferation are differentially regulated in HIVpos and control individuals
- CD4 T cell proliferation in HIV infected individuals is driven by both CD4 T cell depletion and viral load
- CD8 T cell proliferation is mainly driven by the viremia
- IL-7 drives CD4 T cell proliferation
- Increased intracellular levels of pSTAT-5 support an increased role *in vivo* for γ -common cytokine(s) in HIV infected individuals

Acknowledgements

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