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Chronic Exposure to Type-I IFN under Lymphopenic Conditions Alters CD4 T Cell Homeostasis

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ABSTRACT

Background: HIV infection and the associated chronic immune activation alter T cell homeostasis leading to CD4 T cell depletion and CD8 T cell expansion. While the acute CD4 depletion observed in the initial phase of HIV infection is likely due to direct cytopatic effects of the virus, the mechanism/s underlying the steady decline of the CD4 T cell pool during the chronic phase of infection are not totally understood. We hypothesized that the homeostatic response to HIV-induced CD4 T cell depletion occurs in an inflammatory environment rich in Type-I IFNs and driven by HIV replication and that combination of these two distinct forces: lead to a form of immune T cell activation that results in a decline in the CD4 T cell pool and an increase in the CD8 T cells.

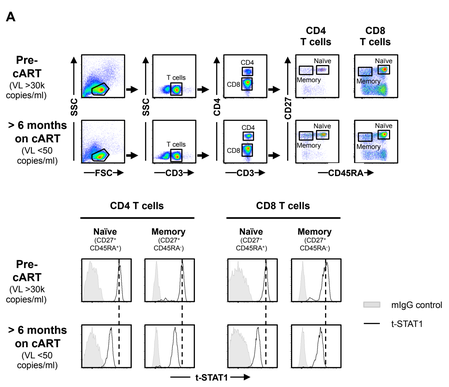
Methodology: We analyzed the in vitro effects of IL-7 and IFN-α to increase the total expression levels of the Signal Transducer and Activator of Transcription (t-STATs) in CD4 and CD8 T cells from healthy controls (n=12). We also analyzed the relationships between CD4 T cell counts and tota levels of STAT-1 (t-STAT-1) in HIV infected patients (n=53). To examine the combine effects of lymphopenia and IFN-α in vivo we developed an animal model in which lymphocytes were adoptively transferred into lymphopenic mice that were receiving chronically administered IFN-α.

Results: IL-7 in vitro upregulated the expression levels of t-STAT-1, -2, -3 and enhanced T cell responsiveness to IFN-α as measured by phosphorylation of STAT-1. In patients with HIV infection and suppressed viremia <50 copies/ml, levels t-STAT1 were inversely correlated with the degree of lymphopenia (R= -0.52, p< 0.01 and R= -0.34, p< 0.01) in CD4 and CD8 T cells respectively. In addition, a positive correlation between STAT-1 and IL-7 serum levels was observed in CD4 (R= 0.38, p= 0.01) but not in CD8 T cells. In a murine model, lymphopenia and chronic treatment with IFN-α led to diminished survival of CD4 T cells and an expansion of CD8 T cells, thus recapitulating the alterations of the homeostasis of these pools observed in patients with HIV infection.

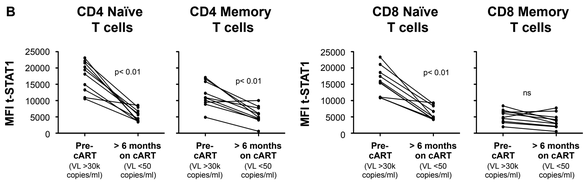
Conclusions: IL-7 in vitro and lymphopenia in vivo can modulate T cell responsiveness to Type I IFNs, suggesting a synergistic interaction of these cytokines. Therefore, the persistent stimulation of this pathway in the setting of a lymhopenic host, such as chronic untreated HIV infected patients could be deleterious for CD4 T cell homeostasis and may contribute to the aberrant immune activation and eventual depletion of the CD4 T cells. The analysis of these pathways may contribute to the development of new strategies to reverse the dysregulation of the T cell pools seen in patients with HIV infection.

RESULTS

Figure 1. Upregulation of t-STAT1 in T cells from viremic HIV-infected patients.



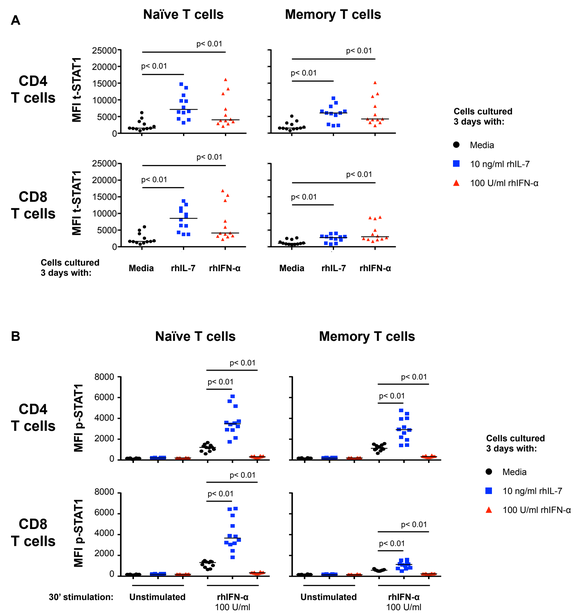
Viral load	Total Patients (n=11) >30,000 copies/ml	<50 copies/ml
HIV RNA (copies/ml) [median (IQR)]	76,081 (49,209-16,0298)	<50
Months viral load < 50 copies/ml [median (IQR)]	-	11 (9-20)
CD4 count (cells/µl) [median (IQR)]	198 (121-240)	264 (243-295)
CD8 count (cells/µl) [median (IQR)]	992 (611-1680)	601 (470-1,053)



PBMCs from a longitudinal cohort of HIV-infected individuals (n=11) were analyzed by flow cytometry before and after suppressing viremia with cART for intracellular expression of t-STAT1. **(A)** Gating strategy to assess t-STAT1 expression in T cell subsets, using CD27 and CD45RA as markers of naïve ($CD45RA^+$ $CD27^+$) and memory ($CD45RA^-$ $CD27^+$) CD4 ($CD3^+$ $CD4^+$) or CD8 ($CD3^+$ $CD4^-$) T cells. The table describes the patient's characteristics at these two time points. **(B)** The Median Fluorescence Intensity (MFI) of t-STAT1 in the different T cell subsets was compared before and after treatment and viremia suppressed to <50 copies/ml using a Wilcoxon signed-rank test.

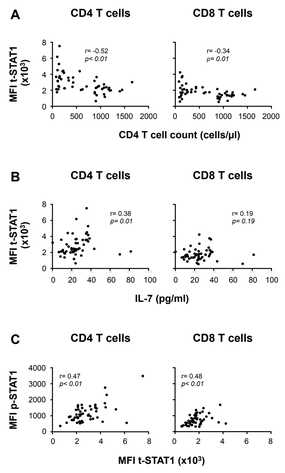
RESULTS

Figure 2. *In vitro* culture with IL-7 increases IFN-α-induced STAT1 activation.



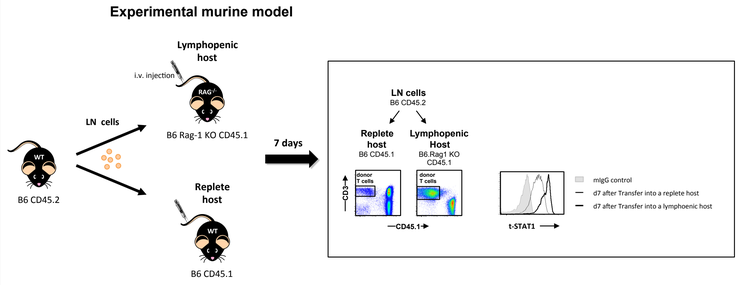
PBMCs from healthy donors (n=12) were cultured 3 days in media alone (black symbols), rhIL-7 (10 ng/ml; blue symbols) and rhIFN-α (100 U/ml; red symbols). After 3 days of culture, the cells were harvested, washed and rested overnight. Rested cells were stimulated *in vitro* with rhIFN-α (100 U/ml) for 30 minutes and analyzed for intracellular expression of t-STAT1 **(A)** and phosphorylated STAT1 **(B)** in naïve and memory T cell subsets. A Wilcoxon signed-rank test was performed for comparisons of the MFI of t-STAT1 and p-STAT1 between groups.

Figure 3. Levels of t-STAT1 in HIV-infected patients undergoing cART are associated with CD4 lymphopenia and IL-7 serum levels.



PBMCs from HIV-infected patients with viremia suppressed to <50 copies/ml for more than 6 months on cART (n=53) were analyzed for t-STAT1 and p-STAT1 levels in total CD4 and CD8 T cell populations. Serums from the same patients were tested by ELISA for IL-7 levels. **(A)** Relationship between t-STAT1 levels and CD4 T cell counts. **(B)** Relationship between t-STAT1 expression and IL-7 serum levels. **(C)** Relationship between STAT1 phosphorylation after *in vitro* stimulation with IFN-α and t-STAT1 levels. The correlations between the levels of t-STAT1, p-STAT1, IL-7 and CD4 T cell counts were analyzed with non-parametric Spearman test.

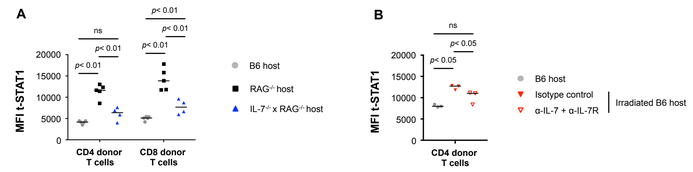
Figure 4. *in vivo* upregulation of t-STAT1



Lymphoreplete B6 CD45.1 (n=5) and lymphopenic B6.Rag1 KO CD45.1 ($RAG^{-/-}$, n=5) mice were injected intravenously (i.v.) with 9 x 10^6 of lymph node (LN) cells isolated from congenic B6 CD45.2 mice. Mice were killed on day 5 after transfer. The levels of t-STAT1 donor T cells were evaluated by flow cytometry in LNs.

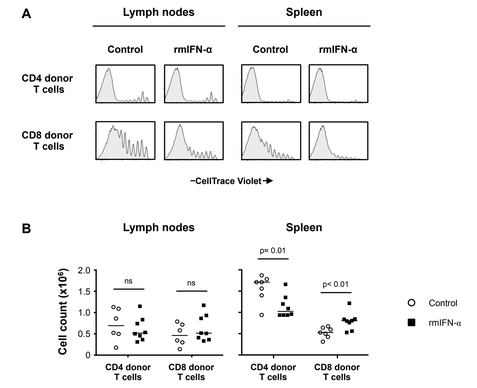
RESULTS

Figure 5. *In vivo* lymphopenia-induced t-STAT1 upregulation in T cells is partially dependent on IL-7



(A) Lymphoreplete B6 CD45.1 (n=5; gray symbols), lymphopenic $RAG^{-/-}$ (n=5; black symbols) and $IL\text{-}7^{-/-}$ x $RAG^{-/-}$ (n=4; blue symbol) mice were adoptively transferred and analyzed as described in Figure 4. The MFIs of t-STAT1 in CD4 and CD8 donor T cells in the different hosts were compared using a nonparametric Mann-Whitney test. Data are from one representative experiment out of three, including an average of 4 mice per group. **(B)** Non irradiated (n=3, gray symbols) and irradiated B6 CD45.1 mice were adoptively transferred as described above. Irradiated B6 hosts were treated with either anti-IL-7 and anti-IL-7R (α-IL-7 + α-IL-7R; n=3, open red symbols) or the respective isotype matched control (n=3, filled red symbols) mAbs. 5 days after transfer, mice were analyzed as described above and the MFI of t-STAT1 in donor T cells were compared between groups using a nonparametric Mann-Whitney test. Data are from one representative experiments out of three, including 3 mice per group.

Figure 6. Continuous exposure to Type-I IFN under lymphopenic conditions leads to decreased CD4 T cell counts and CD8 T cell expansion.



Lymphopenic $RAG^{-/-}$ mice transferred with CellTrace Violet-labeled LN cells from congenic B6 mice were treated with either rmIFN-α (10,000 U per mouse, daily; n=8) or a matched control (n=7). Mice were killed on day 35 after transfer and the presence of donor T cells in different organs was analyzed. **(A)** Levels of CellTrace Violet fluorescence on gated $CD45.2^+$ $CD3^+$ $CD4^+$ and $CD8^+$ donor lymphocytes in LNs and spleen are shown. **(B)** Absolute numbers of $CD4^+$ and $CD8^+$ donor T cells in the lymphoid organs were enumerated following the treatment with rmIFN-α (black symbols) and a matched control (open symbols). A Wilcoxon signed-rank test was performed for comparisons of CD4 and CD8 T cell counts between groups. Data from two representative experiments out of four, including an average of 4 mice per group, are presented.

CONCLUSIONS

- **Naïve and Memory CD4 T cells and Naïve CD8 T cells from viremic HIV infected individuals expressed high level of t-STAT1 that decreased after treatment.**
- ***In vitro* culture with IL-7 and IFN-α induced high expression of t-STAT1 in Naive and Memory CD4 T cells and Naive CD8 T cells from healthy donors.**
- **T cells cultured with IL-7 became more responsive to IFN-α and showed increased phosphorylation of STAT1.**
- ***In vivo* exposure to lymphopenic conditions enhances t-STAT1 expression and IFN-α responsiveness in T cells.**
- **Continuous exposure to Type-I IFN under lymphopenic conditions led to enhanced CD8 donor T cell expansion, and decreased CD4 donor T cell counts in the spleen.**

ACKNOWLEDGEMENTS

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