

Abnormal activation of the JAK/STAT pathway in CD4+ T cells of HIV infected patients (ANRS EP33 study)

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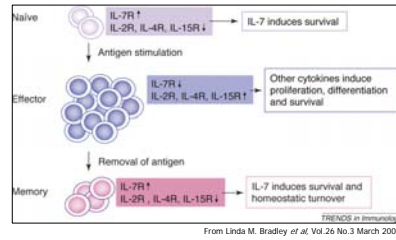
Background: IL-7 and IL-2 are the major cytokines that control CD4+ T cell homeostasis. Both cytokines activate the JAK/STAT5 pathway and induce the expression of the survival factor Bcl-2. Since HIV is known to perturb CD4+ T cell homeostasis, we set to investigate the effect of HIV infection on cytokine-dependent signal transduction.

Methods: PBMC from healthy blood donors (n=14) and HIV-infected patients with viral loads >10,000 copies/ml (n=14) were tested for responses to IL-7 and IL-2 by flow cytometry. The phosphorylation of STAT5 and the induction of Bcl-2 were measured in different CD4+ T cell subpopulations: conventional naive (Nv-c, CD45RA+ Foxp3-) conventional memory (Mem-c, CD45RA- Foxp3-), naive Tregs (TregN, CD45RA+ Foxp3+ CD25+) and memory Tregs (TregM, CD45RA- Foxp3+ CD25+). The expression of the IL-7 receptor (CD127, gamma-c), of the IL-2 receptor (CD25, CD122, gamma-b), and of the activation marker HLA-DR were assessed on the same subpopulations.

Results: Viremic patients showed increased HLA-DR expression in all CD4+ T cell compartments, consistent with generalized immune activation. Interestingly, basal levels of pSTAT5 were increased in Nv-c and Mem-c populations (p<0.05), suggesting a chronic activation of the JAK/STAT5 pathway in HIV infection. CD25 expression was decreased in TregM of viremic patients, but these cells phosphorylated STAT5 as efficiently as TregM from healthy donors in response to IL-2. Similarly, CD127 expression was decreased in the Mem-c population of viremic patients, while these cells phosphorylated STAT5 to higher levels in response to IL-7 stimulation. pSTAT5 induction correlated with CD127 expression in healthy donor CD4+ T cells. This correlation was maintained in patient CD4+ T cells, but with a steeper slope, indicative of an increased efficiency of downstream signaling events (slope = 8.38 vs 3.81; p<0.0001). In contrast to the pSTAT5 response, the induction of Bcl-2 by IL-7 was impaired in viremic patients as compared to healthy donors.

Conclusions: These data provide evidence for an abnormal activation of the JAK/STAT5 pathway in CD4+ T cells of viremic patients, which may contribute to the deleterious chronic immune activation characteristic of HIV infection. In contrast, Bcl-2 induction by IL-7 was defective in viremic patients, which likely compromises the survival capacity of CD4+ T cells. Thus, HIV perturbs cytokine responses at multiple levels, by simultaneously inducing activation pathways and blocking survival pathways.

1- Role of Interleukine-7 (IL-7) / Interleukine-2 (IL-2) in T cell homeostasis

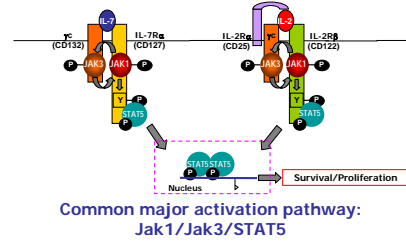


4- Patients Characteristics

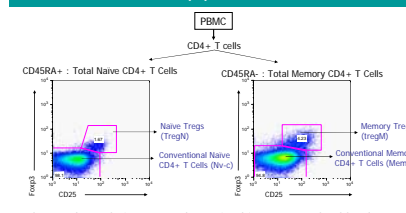
Patient Group	Age median (range)	CD4 count ¹ median (range)	Viral Load ² median (range)
HAART (n=14)	48 (30-59)	649 (411-1116)	<40 (<40-<40)
Viremic (n=14)	41 (29-63)	441 (150-646)	77860 (11260-877030)
Healthy Donor (n=14)	29 (24-47)	N/A	<40

¹ CD4+ T cells/mm³ plasma
² HIV RNA copies/ml plasma
Methods:
 1. Phenotyping: CD25, CD127, CD4, CD45RA, Foxp3, HLA-DR. Cells were permeabilized with the Foxp3 specific fix/permeabilizer (eBiosciences)
 2. STAT5 Phosphorylation: Labeling with the phospho-specific antibody for STAT5 pY694 (clone 47, BD biosciences) was assessed on methanol-permeabilized PBMC.
 3. Bcl-2 induction was measured after 3 days culture in the presence of IL-7. Cells were permeabilized with methanol and labeled with Bcl-2-FITC (clone 124, DAKO)

2- Common signaling pathway



5- Gating Strategy to characterize CD4+ T cells subpopulations

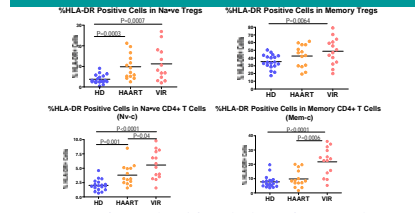


These subpopulations were determined in 3 groups: healthy donors (HD), HAART-treated patients (HAART), and viremic patients (VIR).

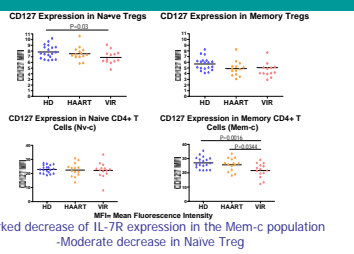
3- Involvement of the IL-2 and IL-7 systems in the immunopathology of HIV Infection

IL-7/IL-2 system in HIV infected patients:
 • Number of CD4+ T cells progressively decrease in HIV infection, in spite of an increase of IL-7 plasmatic level
 • Impairment of IL-2 production by HIV-specific CD4+ T Cells from patients
 • Impairment of Bcl-2 induction and proliferation of patient T cells in response to IL-7
Working Hypothesis: Chronic immune activation in HIV infection induces a dysfunction of the IL-2/IL-7 system, leading to perturbed CD4+ T cells homeostasis
 Defects in activation pathways dependent of IL-2 and IL-7

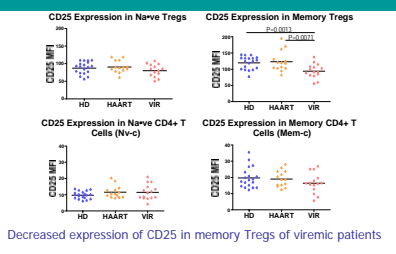
Increased expression of the activation marker HLA-DR in viremic patients, consistent with generalized immune activation



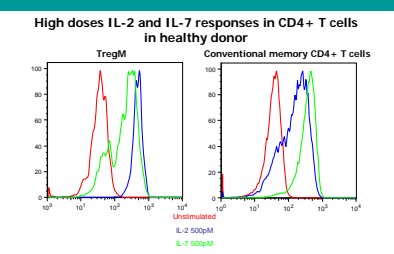
12- STAT5 phosphorylation in response to IL-2 and IL-7: Conclusions



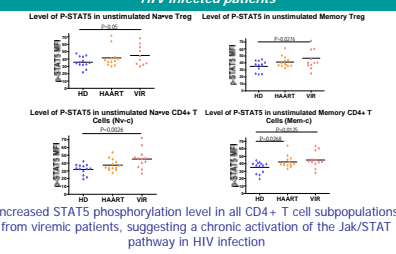
9- STAT5 phosphorylation in CD4+ T cells



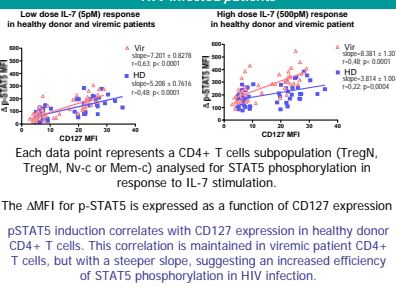
10- Increased Basal Levels of STAT5 Phosphorylation in HIV infected patients



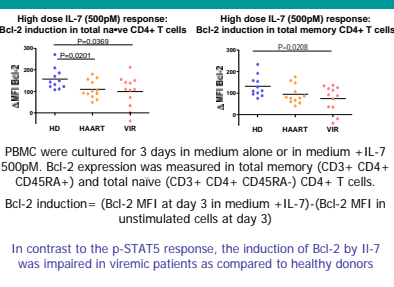
11- STAT5 phosphorylation in response to IL-2 and IL-7: Results



13- Increased responsiveness of the STAT5 pathway in HIV infected patients



14- Impairment of Bcl-2 induction by IL-7



15- Conclusions

These data provide evidence for an abnormal activation of the JAK/STAT pathway in CD4+ T cells of viremic patients, which may contribute to the deleterious chronic immune activation characteristic of HIV infection.
 In contrast, Bcl-2 induction by IL-7 was defective in HIV infected patients, which likely compromises the survival capacity of CD4+ T cells.
 HIV perturbs cytokine responses at multiple levels, by simultaneously inducing activation pathways and blocking survival pathways.

1. Efficient STAT5 phosphorylation in response to IL-2 or IL-7 in the CD4+ T cells memory compartment of viremic patients in spite of decreased receptor expression:
 - decreased CD127 expression in Mem-c CD4+ T cells
 - decreased CD25 expression in TregM

2. Higher responsiveness to IL-7 in the CD4+ T cells naive compartment in viremic patients

