Repression of HIV Transcription in Memory CD4⁺ T Cells by Blimp-1

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ABSTRACT

Objective:
A barrier to eradicating HIV infection is targeting and eliminating latently infected memory CD4⁺ T cells, which are the main reservoir of latent virus. Our goal is to determine if T cell specific transcription factors restrict HIV transcription in specific T cell subsets to establish and maintain HIV latency. The transcription factor B Lymphocyte-Induced Maturation Protein 1 (Blimp-1) is expressed in B and T cells and upregulated in patients chronically infected with HIV. Blimp-1 inhibits IL-2 production which is regulated by a transcriptional element that shares similar properties to the HIV long terminal repeat (LTR). We hypothesize that Blimp-1 binds to the HIV LTR, inhibits HIV transcription and contributes to HIV latency.

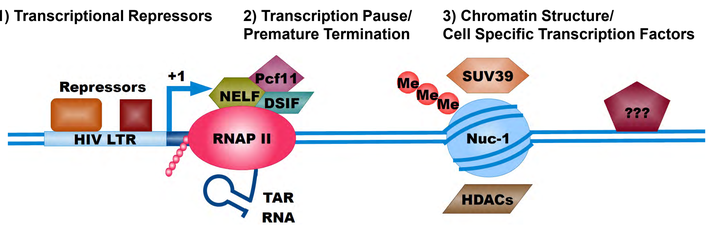
Methods:
We used T cell lines and primary human CD4⁺ T cells to study the role of Blimp-1 in HIV transcription. HIV replication was monitored using luciferase reporters, RT-PCR, and HIV p24 ELISA assays. Binding of Blimp-1 to the HIV LTR was determined using chromatin immunoprecipitation assay. Blimp-1 expression was diminished using shRNA. The levels of Blimp-1 in different T cell subsets were measured with flow cytometry and immunoblots.

Results:
We show that Blimp-1 is expressed in primary peripheral blood CD4⁺ T cells and is further induced by T cell activation. However, infecting CD4⁺ T cells with HIV inhibits the induction of Blimp-1. Importantly, Blimp-1 is differentially expressed in T cell populations and in particular is highly expressed in memory CD4⁺ T cells which do not support robust HIV replication. Blimp-1 binds downstream of the HIV 5'-LTR in resting primary CD4⁺ T cells and represses Tat-dependent HIV transcription. Upon T cell activation with anti-CD3 and anti-CD28 antibodies Blimp-1 is released from the HIV LTR and this correlates with significant increase in HIV transcription in cells expressing Blimp-1, but not in Blimp-1-depleted cells.

Conclusions:
We propose a model in which Blimp-1 acts as an HIV restriction factor in memory CD4⁺ T cells through binding downstream of the HIV LTR and remodeling chromatin. Intrinsic inhibitors of HIV transcription, such as Blimp-1, may provide novel therapeutic targets for purging latent virus from specific cells.

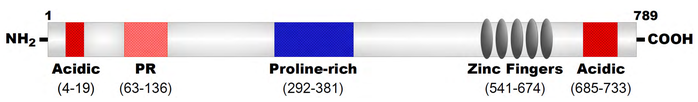
BACKGROUND

HIV Latency

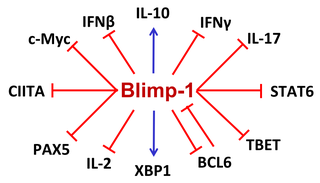


- During initiation of HIV transcription RNA Polymerase (RNAP II) is recruited to the HIV LTR and transcribes nascent TAR RNA.
- Transcriptional repressors bind to the HIV LTR resulting in paused RNAP II.
- Negative elongation factor (NELF) is required for promoter proximal pausing and interacts with Pcf11, a transcription termination factor shown to mediate premature transcription termination.
- Methyltransferases from the SUV39 family and histone deacetylases (HDACs) remodel nucleosome 1 (Nuc-1) making the chromatin less accessible to the transcriptional machinery.

B Lymphocyte-Induced Maturation Protein 1 (Blimp-1)



- DNA binding transcription factor encoded by Prdm1 gene.

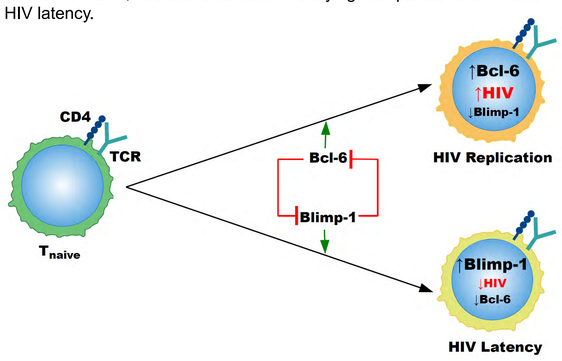


- Key regulator of effector and memory differentiation of B cells, CD4⁺ T cell, and CD8⁺ T cells.

Mature B-cell —Blimp-1→ Plasma cell
CD4⁺ T cell —Blimp-1→ Non-follicular helper cell
CD8⁺ T cell —Blimp-1→ Cytotoxic T cell

HYPOTHESIS

Blimp-1 binds to the HIV LTR, recruits chromatin modifying complexes and inhibits HIV transcription contributing to HIV latency.



RESULTS

Blimp-1 is Induced by T Cell Activation

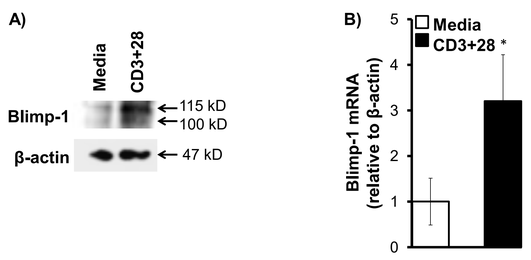


Fig 1. CD4⁺ cells were activated with anti-CD3 and anti-CD28 antibodies for 24H. Expression of Blimp-1 was measured by **(A)** western blot analysis and **(B)** qRT-PCR using β-actin as a housekeeping gene. The two bands detected by immunoblots correspond as reported by Dr. Tirosh, *et al.* (FEBS Lett, 2011) to the unmodified (~100 kD) and sumoylated (~115kD) form of Blimp-1. *p < 0.05 (Student's t test).

HIV Infection Decreases Blimp-1 Levels in CD4⁺ T Cells

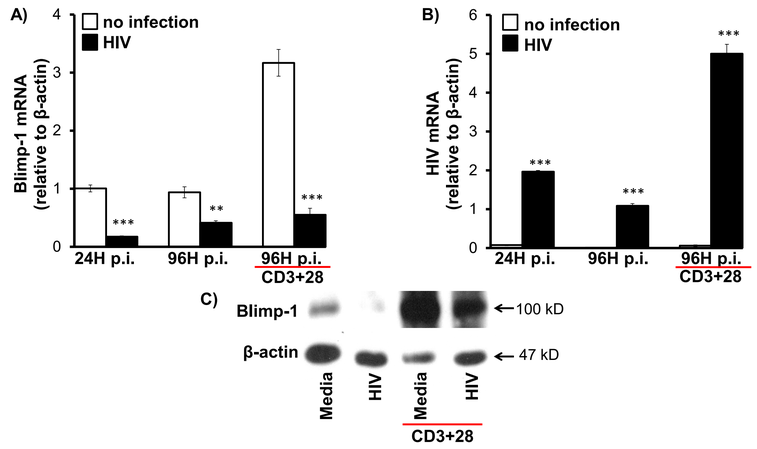


Fig 2. Primary CD4⁺ cells were infected with HIV via spinoculation. 72H post-infection cells were activated with anti-CD3 and anti-CD28 antibodies for 24H. qRT-PCR using β-actin as a housekeeping gene and western blot analysis were used to measure the expression of Blimp-1 **(A** & **C)** and HIV **(B)**. **p < 0.01 and ***p < 0.001 (Student's t test).

Blimp-1 Represses Tat-Dependent HIV Transcription

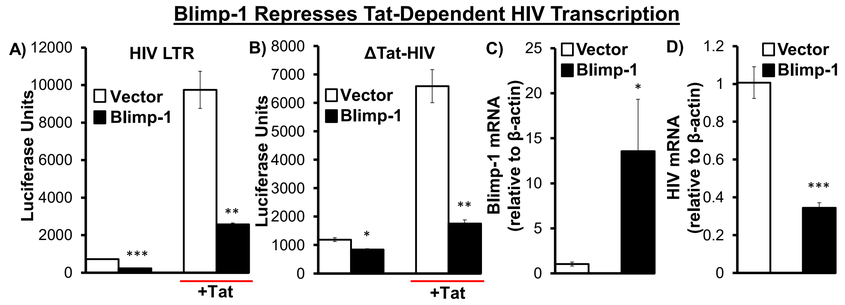
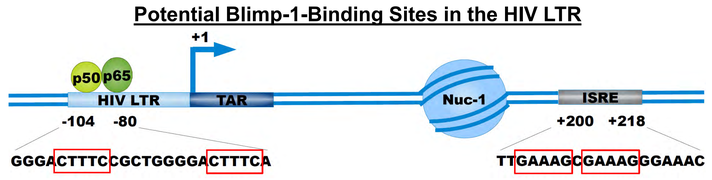


Fig 3. 293T cells were transfected with viral clones HIV LTR-LUC containing the TAR element (**A**) or ΔTat-HIV-LUC (**B**) and vector control or Blimp-1 in the absence or presence of Tat. Luciferase assay was performed 48H post-transfection to measure HIV transcription. **(C)** Primary CD4⁺ cells were activated with PMA+PHA for 16H and then infected with HIV. 16H post-infection cells were transduced with Vector and Blimp-1. Expression of Blimp-1**(C)** and HIV **(D)** was measured by qRT-PCR using β-actin as a housekeeping gene. *p < 0.05 , **p < 0.01 and ***p < 0.001 (Student's t test).

Potential Blimp-1-Binding Sites in the HIV LTR



Blimp-1 Binds the Proviral LTR

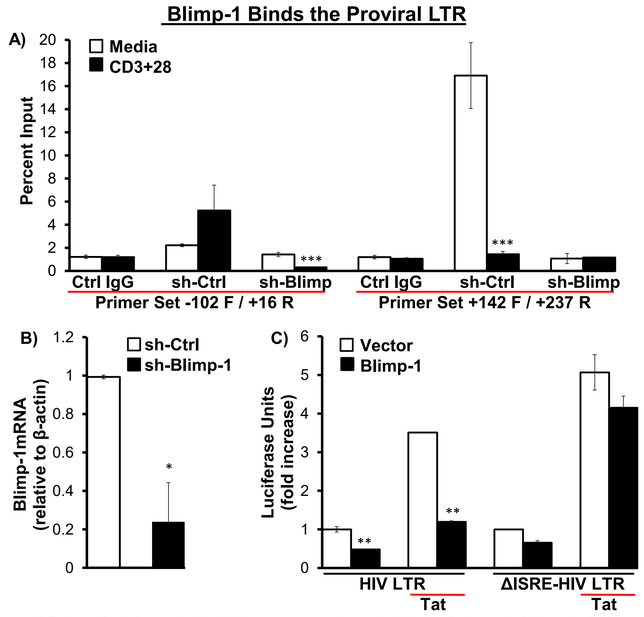


Fig 4. Primary CD4⁺ cells infected with HIV were transduced with sh-Ctrl or sh-Blimp-1. 72H post-knock down cells were activated with anti-CD3 and anti-CD28 antibodies for 24H. **(A)** ChIP analysis used anti-Blimp-1 or anti-rabbit antibody and -102F/+16R or +142F/+237R HIV primer sets. **(B)** qRT-PCR analysis of Blimp-1 mRNA using β-actin as a housekeeping gene. **(C)** 293T cells were transfected with HIV LTR-LUC or ΔISRE-HIV LTR-LUC and control vector or Blimp-1 in the absence or presence of Tat. Luciferase assay was performed 48H post-transfection to measure HIV transcription. *p < 0.05, **p < 0.01 and ***p < 0.001 (Student's t test).

Blimp-1 Represses HIV Transcription in CD4⁺ T Cells

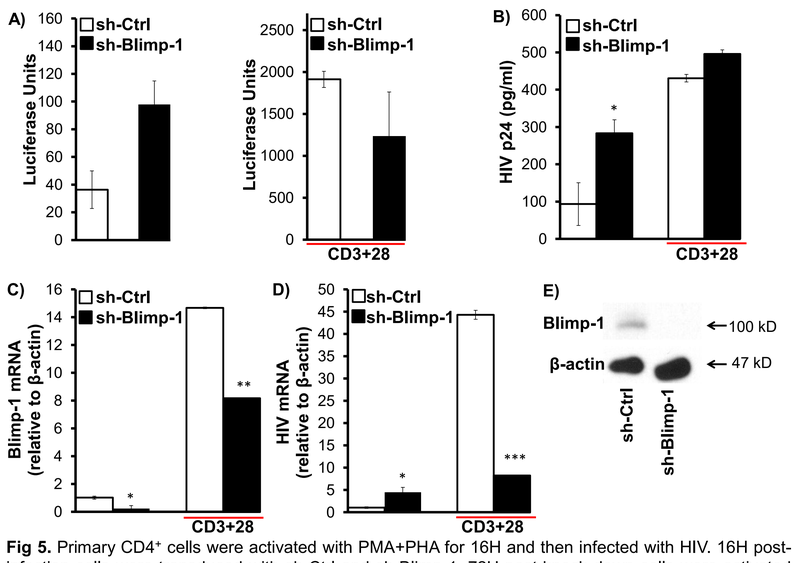
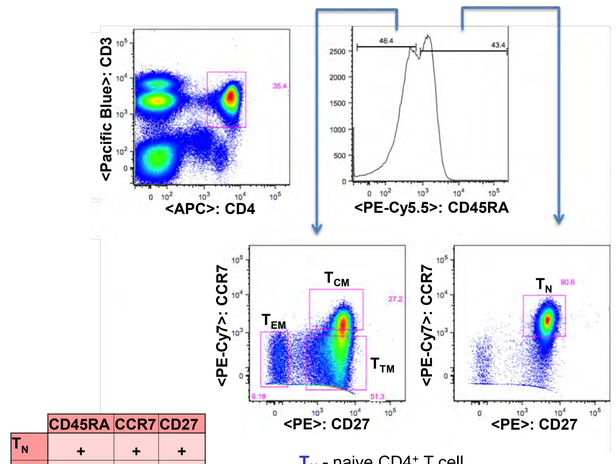


Fig 5. Primary CD4⁺ cells were activated with PMA+PHA for 16H and then infected with HIV. 16H post-infection cells were transduced with sh-Ctrl and sh-Blimp-1. 72H post-knock down cells were activated with anti-CD3 and anti-CD28 antibodies for 24H. Expression of Blimp-1**(C** & **E)** and HIV **(A, B** & **D)** was measured by qRT-PCR using β-actin as a housekeeping gene, immunoblots and HIV p24 ELISA.
*p < 0.05, **p < 0.01 and ***p < 0.001 (Student's t test).

Gating Strategy for Sorting Memory CD4⁺ T Cell Populations



	CD45RA	CCR7	CD27
T_N	+	+	+
T_{CM}	-	+	+
T_{TM}	-	-	+
T_{EM}	-	-	-

T_N - naive CD4⁺ T cell
T_{CM} - central-memory CD4⁺ T cell
T_{TM} - transitional-memory CD4⁺ T cell
T_{EM} - effector-memory CD4⁺ T cell

Blimp-1 is Upregulated in Memory CD4⁺ T Cells

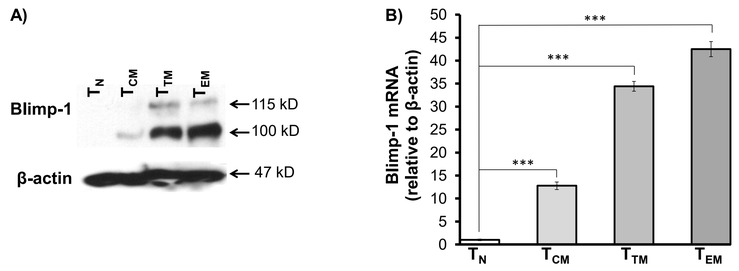


Fig 6. CD4⁺ T cells were isolated from whole blood by negative selection. To sort T cell subsets cells were stained with Pacific Blue–labeled antibody to CD3, APC–labeled antibody to CD4, PE-Cy7–labeled antibody to CCR7, PE-Cy5.5-labeled antibody to CD45RA and PE-labeled antibody to CD27. T cell subsets were separated by FACSAria. Expression of Blimp-1 in sorted memory CD4⁺ T cell populations was measured by immunoblots **(A)** and qRT-PCR using β-actin as a housekeeping gene **(B)**. The two bands detected by immunoblots correspond as reported by Dr. Tirosh, *et al.* (FEBS Lett, 2011) to the unmodified (~100 kD) and sumoylated (~115kD) form of Blimp-1. ***p < 0.001 (Student's t test).

Blimp-1 Represses HIV Transcription in Memory CD4⁺ T Cells

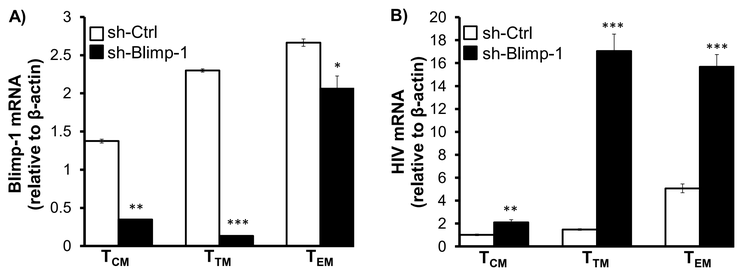
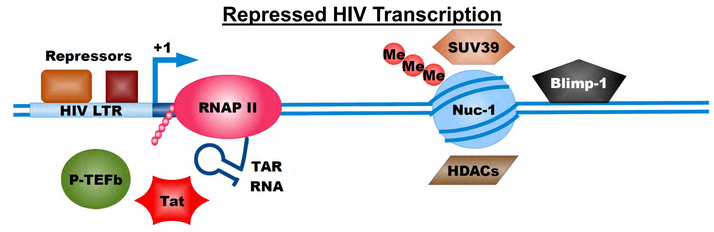


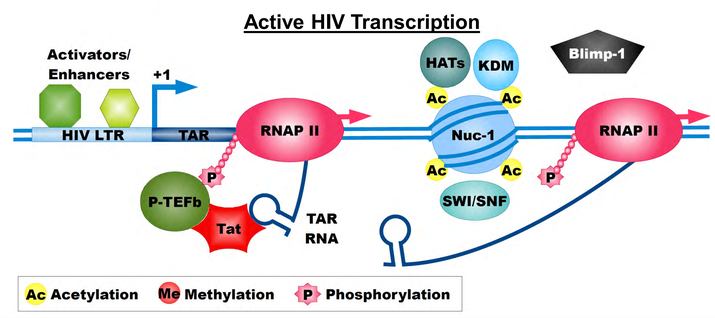
Fig 7. T_{CM}, T_{TM} and T_{EM} were infected with NL4-3 HIV via spinoculation. 16H post infection cells were transduced with sh-Ctrl and sh-Blimp-1. 72H post-knockdown mRNA was collected. Expression of Blimp-1 **(A)** and HIV **(B)** was measured by qRT-PCR using β-actin as a housekeeping gene. *p < 0.05, **p < 0.01 and ***p < 0.001 (Student's t test).

ROLE OF BLIMP-1 IN HIV LATENCY

Repressed HIV Transcription



Active HIV Transcription



CONCLUSIONS

- Blimp-1 is expressed in primary CD4⁺ T cells and induced upon T cell activation.
- HIV infection inhibits Blimp-1 induction.
- Blimp-1 inhibits Tat-dependent HIV transcription.
- Blimp-1 represses HIV transcription in primary CD4⁺ cells.
- Blimp-1 binds to the HIV LTR in primary resting CD4⁺ cells.
- Upon T cell activation with anti-CD3 and anti-CD28 antibodies Blimp-1 is released from the HIV LTR and this correlates with significant increase in HIV transcription.
- Blimp-1 is highly expressed in memory CD4⁺ populations.
- Decreasing Blimp-1 expression in memory CD4⁺ T cells results in induction of HIV transcription.

FUTURE DIRECTIONS

- Examine how HIV modulates Blimp-1.
- Investigate if Blimp-1 recruits chromatin modifying complexes to repress HIV transcription.
- Determine if Blimp-1 is post-translationally modified during HIV infection.
- Test if Blimp-1 downregulates the expression of HIV host restriction factors and cytokines.

ACKNOWLEDGEMENTS

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