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Blimp-1, an Intrinsic Factor that Represses HIV-1 Proviral Transcription in Memory CD4+ T Cells

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CD4+ T cell subsets differentially support HIV-1 replication. For example, quiescent CD4+ memory T cells are susceptible to HIV-1 infection but do not support robust HIV-1 transcription and have been implicated as the primary reservoir of latent HIV-1. T cell transcription factors that regulate maturation potentially limit HIV-1 transcription and mediate the establishment and maintenance of HIV-1 latency. We report that B lymphocyte–induced maturation protein-1 (Blimp-1), a critical regulator of B and T cell differentiation, is highly expressed in memory CD4+ T cells compared with naïve CD4+ T cells and represses basal and Tat-mediated HIV-1 transcription. Blimp-1 binds an IFN-stimulated response element within HIV-1 provirus, and it is displaced following T cell activation. Reduction of Blimp-1 in infected primary T cells including CD4+ memory T cells increases RNA polymerase II processivity, histone acetylation, and baseline HIV-1 transcription. Therefore, the transcriptional repressor, Blimp-1, is an intrinsic factor that predisposes CD4+ memory T cells to latent HIV-1 infection. The Journal of Immunology, 2015, 194: 3267–3274.

A remaining challenge in efforts to cure HIV-1 infection is targeting the latent reservoir that is resistant to current antiretroviral therapies. Upon cessation of antiretroviral therapy, HIV-1 rapidly reemerges from latently infected cells to pretreatment viral loads (1, 2). Strategies to target this reservoir require characterizing the cell populations that harbor latent HIV-1 and understanding the biochemical mechanisms that regulate provirus expression in these cells. Quiescent memory CD4+ T cells have been implicated as the primary HIV-1 reservoir because they are susceptible to HIV-1 infection, are long-lived, and with their ability to self-renew, potentially maintain pools of latently infected cells. Numerous T cell transcription factors, such as NFAT, GATA-3, c-Maf, and RORyt have been suggested to rapidly reactivate latent HIV-1 (3), but whether there are T cell–specific factors that predispose memory cells to latent HIV-1 infection has not been demonstrated.

The Prdm1 gene encodes B lymphocyte–induced maturation protein-1 (Blimp-1), a Kruppel-like zinc-finger factor that is critical for the differentiation of mature B cells into plasma cells, and recently has been demonstrated to be expressed in dendritic cells, macrophages, keratinocytes, and T cells (4–14). In T cells, Blimp-1 regulates the activation and generation of CD4 and CD8 T cell effector populations (15–18). Blimp-1 represses the transcription of several regulatory factors, including Bcl-6, T-bet, IL-2, IFN-γ, and IFN-β, while enhancing the transcription of IL-10 (19-22). In the context of HIV-1, Blimp-1 expression is increased in chronically infected patients and correlates with enhanced expression of negative regulators of T cell activation, including PD-1, LAG3, and CTLA-4, and with T cell exhaustion and apoptosis (23–26). The HIV-1 long-terminal repeat (LTR) includes binding sites for Blimp-1, suggesting that this factor directly binds provirus and regulates HIV-1 transcription (3).

We demonstrate regulated expression of Blimp-1 in human CD4+ T cells including memory CD4+ T cell subsets. Furthermore, we show that Blimp-1 binds sequences downstream of the HIV-1 LTR limiting HIV-1 transcription in memory T cells. These results support a model in which Blimp-1 is a memory T cell–specific factor that directly contributes to the establishment of HIV-1 latency.

Materials and Methods

Cell culture

Discarded deidentified tissues from otolaryngology surgeries performed at Boston Medical Center were mechanically separated and cultured on plastic plates for 2–3 d to eliminate adherent cells. Cells in suspension were then positively selected for CD4+ T cells using the Dynabeads CD4-Positive Isolation Kit (Invitrogen). Whole blood from healthy, anonymous donors was purchased from NY Biologicals. The Boston University School of Medicine Institutional Review Board reviewed the use of tonsils and blood for these studies and assigned it as nonhuman subject research. PBMCs were isolated from whole blood by centrifuging through Histopaque gradient (Sigma-Aldrich). CD4+ T cells were positively selected using the Dynabeads CD4 Positive Isolation Kit. Jurkat clone E6-1 was originally purchased from American Type Culture Collection (Manassas, VA). Primary CD4+ T cells and Jurkat cells were propagated in RPMI 1640 medium supplemented with 10% FBS, 100 U/ml penicillin, 100 μg/ml penicillin/streptomycin, and 0.2 M L-glutamine. Human embryonic kidney 293T (HEK293T) cells were purchased from the American Type Culture Collection and cultured in DMEM containing 10% FBS and penicillin/streptomycin. Cells were incubated in a 37°C humidified incubator with 5% CO2. Cells were left either untreated or activated with 0.1 μg/ml anti-human CD3 (BD Biosciences) and 1 μg/ml anti-human CD28 (BD Biosciences) for 30 min. Goat anti-mouse Ab (5 μg/ml; Sigma-Aldrich) was added to cross-link the receptors. T cells were harvested

Abbreviations used in this article: AcH3, histone H3 acetylation; Blimp-1, B lymphocyte–induced maturation protein-1; FIP, foamy virus internal promoter; HEK293T, human embryonic kidney 293T; ISRE, IFN-stimulated response element; LTR, long-terminal repeat; RNAP II, RNA polymerase II; RSV, Rous sarcoma virus; sh, short hairpin; Tcm, central memory T cell; Tcm, transitional memory T cell; Tn, naive T cell.

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24 h post-stimulation. All experiments with primary cells were performed with tissues from at least three different donors.

Polychromat flow cytometry

CD4+ T cells were isolated from whole blood by negative selection using RosetteSep Human CD4+ T Cell Enrichment Cocktail (StemCell Technologies). To sort T cell subsets, cells were stained with CD3-PacificBlue (BD Biosciences), CCR7-PE-Cy7 (BioLegend), CD45RA-PE-Cy5.5 (Invitrogen), CD27-PE (BD Biosciences), and CD4-allophycocyanin (BioLegend). T cell subsets were separated with FACSAria.

Transfections, virus generation, and infections

HIV-1-1 LTR/GALS-luciferase reporter constructs with an intact or mutated (5′-GAAAGCGAGAG-3′ mutated to 5′-GGCCGCGCGC-3′) ISRE or NL4-3 Tat-luciferase, Blimp-1, Tat, Rous sarcoma virus (RSV) LTR-LUC (27) and foamy virus internal promoter (FIP)-LUC (28) expression constructs were transiently transfected into HEK293T cells via calcium phosphate transfection as described previously (29). The RSV-LUC and FIP-LUC were provided by Dr. S. Gummula, Boston University School of Medicine. Luciferase assays were performed 48 h posttransfection using Lucifere Assay System (Promega).

Lentiviral vectors pNL4-3-LUC-eNev(−)Nef(−) (30) obtained from National Institutes of Health AIDS Research and Reference Reagent Program, Blimp-1 short hairpin (shRNA) (Dr. K. Calame, Columbia University, New York, NY) were packaged by cotransfecting Tat, RSV-Rev, Gag/Pol, and vesicular stomatitis virus-G into HEK293T cells using calcium phosphate transfection as described previously (29). HIV-1 titers were determined using a p24 ELISA (PerkinElmer).

 Luciferase assays were performed 48 h posttransfection using Luciferase Assay System (Promega).

Quantitative RT-PCR

RNA was prepared by resuspending cells in TRIzol (Life Technologies), and cDNA was generated using SuperScript II Reverse Transcriptase (Invitrogen). Primers and random primers (Promega) GoTaq qPCR Master Mix (Promega) was used for quantitative real-time PCR reaction. Blimp-1 transcripts (+2074 to +2372) were amplified using 5′-CCCTGGAAGCA-3′ and 5′-GTTAGA-3′ primers. Initiated HIV-1 transcripts (+1 to +40) were amplified using 5′-GGCTTCTCTGTGTTAGA-3′ and 5′-AGAGCTTCAGCTA-3′ primers as described previously (29). B-Actin mRNA was amplified using a QuantiTect primer assay (Qiagen). PCR was carried out for 45 cycles, and the relative quantification was calculated using the ∆∆CT method (32), normalizing specific amplification of the transcripts of interest to the β-actin control for each specific sample. The product detected in the sh-Control was a calibrator, and the transcript levels in samples were calculated as fold changes in comparison with sh-Control.

Immunoblot analysis and Abs

Whole-cell lysates were prepared by washing cells with cold PBS and lysing them with buffer containing 10 mM Tris-Cl (pH 7.4), 150 mM NaCl, 1.0 mM EDTA (pH 8), 2 mM sodium vanadate, 10 mM sodium fluoride, 10 mM sodium pyrophosphate, 1% Triton X-100, 1 mM PMSF, and protease inhibitor mixture III (Calbiochem). Protein was measured using the BSA assay (Pierce). Samples were heated for 5 min at 100°C before loading onto a 10% SDS-PAGE gel. Proteins were transferred to a polyvinylidene difluoride membrane (Millipore) by electroblotting. Abs used were as follows: anti-Blimp-1 serum (provided by Dr. K. Calame), anti-β-actin (Sigma-Alrich), anti-Tat (4138; National Institutes of Health AIDS Research and Reference Reagent Program), anti-Sp1 (Upstate Biotechnology), CD3 (BioLegend), anti-IRF-8 (Santa Cruz Biotechnology), anti-histone H3 acetylation (AcH3; Upstate Biotechnology), and rabbit IgG (Upstate Biotechnology).

Chromatin immunoprecipitation–real-time PCR

Chromatin immunoprecipitations (ChIPs) were performed as described previously (29). Quantitative real-time PCR analysis was carried out using Sybyr green reagents and the primers 5′-GACCTTCCGGTGGAGCTTT-3′ and 5′-CTAACCAGAAG ACCGAGATC-3′, which amplify the +102 to +46 region of the HIV-1 LTR. 5′-CTGGGAGCTCTCTGCTAACTA-3′ and 5′- TTACGAGCTACACAAACAGG-3′, which amplify the +30 to +134 region of HIV-1, 5′-TCCCTAGACCCTTTTATGCAG-3′ and 5′- GTGCGAGAGCTCTCAGGTT-3′, which amplify the +142 to +237 region of HIV-1, and 5′-ACAGTACCTGTAGTG GAGTAT-3′ and 5′-AATCCCTGGTGGTCTCAGTTT-3′, which amplify the +2415 to +2522 region of provirus.

Statistical analysis

Statistical analysis was carried out using Student t test. A two-tailed distribution was performed on paired samples. A p value < 0.01 was considered significant.

Results

Blimp-1 is expressed in primary human CD4+ cells including memory CD4+ T cells

Although Blimp-1 expression has been characterized in murine T cells, we wanted to confirm that it shared a similar expression pattern in human primary CD4+ T cells. CD4+ T cells were isolated from tonsils, a rich source of follicular Th cells, and from peripheral blood. Blimp-1 mRNA was detected by qRT-PCR and protein with immunoblots. Consistent with previous reports (33) tonsillar CD4+ T cells expressed low levels of Blimp-1 compared with the CD4+ T cells isolated from whole blood (Fig. 1A). Activation with anti-CD3 and anti-CD28 Abs resulted in 3-fold increase in Blimp-1 mRNA and protein levels (Fig. 1B, 1C).

We also examined Blimp-1 expression in different memory CD4+ T cell populations obtained from peripheral blood. Flow cytometry based on CD45RA, CD27, and CCR7 expression was used to enrich for naive T cells (TNA, CD4+CD3−CD45RA+), central memory T cells (T CM, CD4+CD3−CD45RA−CCR7+CD27+), transitional memory T cells (T TM, CD4+CD3−CD45RA−CCR7−CD27+), and effector memory T cells (TEM, CD4+CD3−CD45RA−CCR7−CD27+). Although TEM have been implicated as the primary cell type that is latently infected, recent reports suggest that TEM and TCM contribute to the latent HIV-1 reservoir (34–36). Blimp-1 mRNA was measured in memory cells by qRT-PCR, and protein levels were determined by immunoblots. Blimp-1 expression was 10-fold higher in long-lived Tem compared with naive T cells, whereas even higher levels of Blimp-1 were observed in TCM and TEM (Fig. 1E, 1F). Overall, Blimp-1 is present in multiple T cell populations but is expressed at significantly higher levels in CD4+ T memory cells found within the latent reservoir.

Blimp-1 inhibits HIV-1 transcription and binds HIV-1 provirus

On the basis of the transcriptional repressor function of Blimp-1 and our data showing its high expression in CD4+ memory T cells, we hypothesized that Blimp-1 limits HIV-1 expression. To determine whether Blimp-1 regulates HIV-1 LTR activity, we performed cotransfection experiments with Blimp-1 and an HIV-1 LTR reporter. Blimp-1 inhibited the HIV-1 LTR–driven luciferase reporter gene (HIV-1 LTR–LUC) by 67% when overexpressed in HEK293T cells (Fig. 2A). Overexpression of the HIV-1 transcriptional activator, Tat, was unable to rescue HIV-1 LTR-mediated transcription in the presence of Blimp-1 (Fig. 2A). In addition, Blimp-1 inhibited an HIV-1 clone that lacked Tat (ΔTat-HIV-1-LUC) by only 30% but repressed transcription by greater than 70% when Tat was added back by cotransfection (Fig. 2B). Blimp-1 is not acting as a general repressor in these experiments because it failed to repress expression driven by RSV LTR (27) and FIP (Fig. 2C) (28). These data indicate that Blimp-1 inhibits basal HIV-1 transcription as well as transcription in the presence of Tat.
We explored whether Blimp-1 regulates HIV-1 expression in CD4+ T cells. We established a Jurkat T cell line that stably overexpressed Blimp-1 and infected it with a single cycle env-minus HIV-1-luciferase virus (HIV-1-LUC). We do see a modest decrease in the doubling time in Jurkat cells overexpressing Blimp-1 (<10%); however, for all experiments, cell numbers were adjusted so that assays were performed on comparable numbers of cells. HIV-1 transcription, as measured by the luciferase assay, was decreased by 55% in cells overexpressing Blimp-1 (Fig. 2D). In addition, primary human CD4+ T cells enriched from whole blood were infected with a HIV-1-LUC virus (30), and Blimp-1 was overexpressed using a lentiviral vector. Transduction of cells with Blimp-1 lentivirus resulted in >13-fold increase in Blimp-1 mRNA (Fig. 2E). Cells overexpressing Blimp-1 had 65% decrease in HIV-1 transcription measured by HIV-1 mRNA levels (Fig. 2E) and >90% inhibition of HIV-1 replication measured by HIV-1 p24 ELISA (Fig. 2E). The data from Jurkat T cells and primary human CD4+ T cells confirmed that Blimp-1 is a repressor of HIV-1 transcription.

HIV-1 provirus has four putative Blimp-1 binding sites, GAAAAG, with two sites upstream of the transcription start site that overlap the NF-kB sites (−104 to −80 bp) and a second set of sites located downstream of the transcriptional start site in an ISRE (+200 to +218 bp) (Fig. 3A) (37). Both regions have been reported to modulate HIV-1 transcription (37, 38). Mutating the HIV-1 ISRE ameliorated Blimp-1–mediated repression of HIV-1 following cotransfection into HEK293T cells (Fig. 3B). Furthermore, ChIPs demonstrate that Blimp-1 directly binds HIV-1 provirus. Chromatin was prepared from CD4+ T cells enriched from peripheral blood, infected with HIV-1, and protein–DNA complexes were enriched with Blimp-1–specific Ab. ChIPs show modest Blimp-1 binding at the −104/−80 bp site and 9-fold higher Blimp-1 binding at the +200/+218-bp ISRE site (Fig. 3C). Following T cell activation, Blimp-1 binding was not detected at the HIV-1 ISRE element. This inability to bind the HIV-1 LTR following activation explains in part why, despite increases in Blimp-1 mRNA and protein (Figs. 1B, 1C, 3C), repression of HIV-1 is not observed in activated CD4+ T cells. Blimp-1 binding does not exclude the recruitment of other factors to the HIV-1 ISRE. ChIPs show that IRFs 1 and 8 bind to the HIV-1 ISRE in the presence of Blimp-1 (Fig. 3D), suggesting that Blimp-1 is not simply competing with other key transcription factors for binding to the HIV-1 LTR. These results suggest that Blimp-1 represses HIV-1 transcription by directly binding the proviral ISRE element.

Blimp-1 limits HIV-1 transcription in CD4+ memory T cell subsets

The above gain of function experiments demonstrated that Blimp-1 represses HIV-1 expression. To determine whether Blimp-1 limits HIV-1 expression in the context of primary cells, we reduced Blimp-1 expression in primary CD4+ T cells using shRNA. We infected CD4+ T cells purified from peripheral blood with HIV-1-LUC, transduced the infected cells with sh-Blimp-1 lentivirus, and monitored HIV-1 expression. The efficacy of Blimp-1 knockdown was confirmed 96 h posttransduction by qRT-PCR (Fig. 4A) and immunoblots (Fig. 4B). Diminishing Blimp-1 increased basal HIV-1 transcription and replication as measured by qRT-PCR (Fig. 4C), luciferase assay (Fig. 4D), and p24 ELISA (Fig. 4E), indicating that Blimp-1 limits HIV-1 transcription in primary CD4+ T cells. Activating T cells through CD3 + CD28 following Blimp-1 knockdown did not further induce HIV-1 transcription and actually decreased HIV-1 transcription by 80%. This apparent paradoxical result suggests that Blimp-1 is required for optimal induction of HIV-1 transcription (Fig. 4C) and indicates that
Blimp-1 acts as both a repressor and transcriptional activator in the context of HIV-1–infected primary T cells. However, the ability of Blimp-1 to activate HIV-1 transcription is independent of Blimp-1 binding to provirus because ChIPs show it is displaced following T cell activation (Fig. 3C).

To gain insight into how Blimp-1 limits HIV-1 transcription, we examined with ChIPs the distribution of RNAP II on the HIV-1 genome. In cells expressing Blimp-1, there was an accumulation of RNAP II at the transcriptional start site and modest amounts of RNAP II downstream, suggesting that Blimp-1 leads to RNAP II promoter proximal pausing (Fig. 4F). Diminishing Blimp-1 reduced RNAP II at the promoter and increased RNAP II downstream in provirus by 3-fold consistent with RNAP II release and greater processivity (Fig. 4F). We also used qRT-PCR to measure initiated versus elongated transcripts. In control cells, there was an accumulation of initiated short HIV-1 mRNA but low expression of full length mRNA (Fig. 4G) as would be expected with RNAP II pausing (39). Decreasing Blimp-1 with shRNA altered the ratio of initiated to elongated HIV-1 mRNA so that the ratio was approximately one indicating processive transcriptional elongation (Fig. 4G). Furthermore, knocking down Blimp-1 led to an increase in AcH3 at the positioned nucleosome (nuc-1) (Fig. 4H). These data suggest that Blimp-1 targets multiple steps of transcription regulation to limit HIV-1 transcriptional elongation.
Expression of Blimp-1 was measure by qRT-PCR (A) were transduced with sh-Ctrl and sh-Blimp-1. Seventy-two hours postknockdown, cells were activated with anti-CD3 and anti-CD28 Abs for 24 h. Blimp-1 expression was assayed by qRT-PCR using primers for elongated HIV-1 mRNA (C) and initiated short transcripts (G), luciferase assay (D), and HIV-1 p24 ELISA (E). ChiP analysis used anti-rabbit and anti-RNAP II (F) or anti-AcH3 (H) Ab and +30F/+134R and +2415F/+2522R HIV-1 primer sets. These experiments were performed in triplicate, and the data are representative of at least three independent experiments. * sh-control; ** sh-Blimp-1. Bars show average values ± SD; n = 3, *p < 0.05, **p < 0.01, ***p < 0.001 (Student t test).

Blimp-1 was highly expressed in CD4+ TCM, TEM, and TM (Fig 1) memory cells that do not to support efficient HIV-1 transcription and contribute to the latent reservoir (34–36, 40). To examine whether Blimp-1 was limiting HIV-1 transcription in these cells, we isolated different memory CD4+ T subsets from peripheral blood as described in Fig 1, infected the cells with HIV-1 by spinoculation (31), and then decreased Blimp-1 with sh-Blimp-1 lentivirus. Spinoculation enhances viral binding, which increases infection of cells without activating stimuli and results in higher integration rates than infection of cells in the absence of spinoculation. Decreasing Blimp-1 levels in all three CD4+ T cell memory populations resulted in significant increases, 2- to 12-fold, in HIV-1 transcription (Fig. 5B). The levels of induction correlated with the efficiency of Blimp-1 knockdowns observed with more modest induction in TCM and TEM cells in which Blimp-1 knockdowns were inefficient compared with robust induction in TM where Blimp-1 expression was decreased by >80% (Fig. 5A). These findings support a model in which Blimp-1 expression correlates with limited basal HIV-1 transcription in T memory cells (Fig. 6).

Discussion
Memory CD4+ T cells are a major reservoir of latent HIV-1 and their longevity and homeostatic proliferation prevents virus clearance and supports HIV-1 persistence. Although several mechanisms potentially inhibit HIV-1 transcription, T cell–specific transcription factors that intrinsically program the maturation of CD4+ memory T cells could contribute to the propensity of HIV-1 to become repressed in these cells. We present evidence that the lymphoid differentiation factor Blimp-1 limits HIV-1 transcription in CD4+ memory T cell subsets.

Blimp-1 is expressed in a range of immune cells, such as B cells, macrophages, dendritic cells, and T cells. We confirmed that Blimp-1 is differentially expressed in CD4+ T cells, with low levels in CD4+ TCM and CD4+ tonsillar cells but elevated expression in cells that have been shown to contribute to the latent reservoir, CD4+ TCM, TEM, and TM. Blimp-1 expression is primarily made up of CD4+ TCM and to a lesser degree CD4+ TEM with CD4+ TEM representing the minority of latently infected cells (36, 40). The contribution of these cell populations to the reservoir inversely correlates with the expression levels of Blimp-1 with Blimp-1 being most highly expressed in TEM and more modestly expressed in TCM (Fig. 1). Although it is tempting to speculate that Blimp-1 may dictate the levels of HIV-1 found in each of these memory compartments, we have no evidence that Blimp-1 inhibits HIV-1 entry or the establishment of infection. Overexpression of Blimp-1 or knocking down Blimp-1 prior to HIV-1 infection has no effect on the number of proviruses as measure by Alu-HIV-LTR quantitative real-time PCR (data not shown). Therefore, we conclude that Blimp-1 is primarily limiting transcription in these different T memory subsets.

We also showed that Blimp-1 is induced upon T cell activation, suggesting that Blimp-1 may have a role in limiting HIV-1 expression in nonmemory T cell subsets. HIV-1 has been reported to be expressed at higher levels in activated T cells, which may be relevant to the findings described here.
Blimp-1 is expressed in memory CD4+ T cells, binds the HIV-1 ISRE, and inhibits HIV-1 transcription in the presence of Tat. Following T cell activation, Blimp-1 is released from HIV-1 provirus, which correlates with increased RNAPII processivity, AcH3, and enhanced HIV-1 transcription.


References

Acknowledgments

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Disclosures

The authors have no financial conflicts of interest.

FIGURE 6. Model for the role of Blimp-1 in HIV-1 transcription. Blimp-1 is highly expressed in memory CD4+ T cells, binds the HIV-1 ISRE, and inhibits HIV-1 transcription in the presence of Tat. Following T cell activation, Blimp-1 is released from HIV-1 provirus, which correlates with increased RNAPII processivity, AcH3, and enhanced HIV-1 transcription.

preferentially replicate in different T effector cells such as T(H2) relative to T(H1) cells (41), and although we did not specifically examine Blimp-1 expression in T effector subsets, Blimp-1 may influence HIV-1 expression as well as T cell maturation and function by regulating the expression of key lineage restricting transcription factors including PAX5, STAT6, Bcl-6, and T-bet (6, 7, 21, 42) and cytokines such as IL-10 (15, 43).

Furthermore, Blimp-1 is required for robust CD4 and CD8 T cells antiviral responses against influenza and lymphocytic choriomeningitis virus and mediates murine gammaherpesvirus latency in splenocytes (16, 17, 44–46). Blimp-1 is associated with increased expression of inhibitory receptors and decreased polyfunctionality of exhausted CD4+ T cells during lymphocytic choriomeningitis virus infection (47). In chronically infected HIV-1 patients Blimp-1 is elevated and correlates with an increase in T cell exhaustion markers (25, 26). This increase in Blimp-1 may be a direct result of infection because HIV-1–pulsed dendritic cells enhance Blimp-1 expression (23, 24). It would be interesting to explore whether Blimp-1 limits HIV-1 transcription in different CD4+ T cell subsets similar to what is observed in different T memory subsets.

Blimp-1 binds a GAAAG consensus sequence, which is also found in ISREs present in Blimp-1–regulated genes CIITA, IDO1, and IFN-λ1 (48-50). Although HIV-1 provirus contains four putative Blimp-1–binding sequences (3), we detected Blimp-1 binding only at the HIV-1 ISRE element. This cis-element has been implicated in regulating HIV-1 transcription by recruiting IRF family proteins IRF-1 and IRF-8 (51). Blimp-1 binds this element in resting cells and is displaced following T cell activation. It is possible that Blimp-1 antagonizes or competes with the transcriptional activator IRF-1, although we do not observe significant changes in IRF-1 or IRF-8 binding in the absence or presence of Blimp-1 or in response to T cell activation. Changes in the ability of Blimp-1 to bind the HIV-1 ISRE may reflect post-translational modifications of Blimp-1, which is regulated by sumoylation and ubiquitination (52, 53).

Blimp-1 mediates repression of genes by recruiting epigenetic factors such as methyltransferases G9a, Prmt5 and LDD1, Groucho-proteins, and histone deacetylases to promoters (54–58). LSD-1 and G9a limit HIV-1 transcription and are candidates for the repression observed in CD4+ memory T cells (59, 60). We observed that decreasing Blimp-1 increases acetylation at nuc-1 and releases RNAPII pausing. Blimp-1 limiting transcription elongation is also consistent with the observation that Tat cannot rescue HIV-1 expression in the presence of Blimp-1 and suggests that it is targeting a step prior to transcription elongation and recruitment of P-TEFb, which is mediated by Tat.

Blimp-1 can act as a transcriptional activator and is necessary for the induction of IL-10 and XBP-1 (6, 22, 61). We also observe that Blimp-1 can act as a repressor and trans-activator in primary CD4+ T cells. Intriguingly, Blimp-1 is required for efficient induction of HIV-1 transcription upon T cell activation (Fig. 4C). However, Blimp-1 does not occupy either set of binding sites following T cell activation despite increased Blimp-1 expression following CD3 + CD28 activation, suggesting that Blimp-1 is not directly inducing HIV-1 transcription. The mechanism by which Blimp-1 induces HIV-1 transcription following activation is not clear and may reflect Blimp-1 interacting with other transcription factors, posttranslational modifications of Blimp-1, or its ability to influence the expression of other host genes, cytokines, and restriction factors that regulate HIV-1 transcription. Many transcription factors have been shown to act as negative and positive regulators. For example, in the context of RNAPII pausing, hypophosphorylated DSIF limits RNAPII processiveness, whereas, following recruitment of P-TEFb and hyperphosphorylation, DSIF becomes a transcriptional activator and facilitates RNAPII transcriptional elongation (62, 63).

We propose a model in which Blimp-1 is highly expressed in memory CD4+ T cells, which do not support robust HIV-1 replication. In resting cells, Blimp-1 binds the HIV-1 ISRE and represses HIV-1 transcription elongation, whereas upon T cell activation, Blimp-1 is released from HIV-1 provirus derepressing proviral transcription (Fig. 6). We show that Blimp-1 is a transcriptional repressor of HIV-1, and its expression in memory CD4+ T cells makes them prone to HIV-1 latency. Understanding how Blimp-1 is regulated and the transcriptional processes it coordinates to silence HIV-1 expression will provide insights into the establishment and maintenance of the HIV-1 reservoir.


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