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NF- κ B/Rel: agonist and antagonist roles in HIV-1 latency

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Abstract

Purpose of review—To discuss recent advances in our understanding of the diverse roles of NF- κ B/Rel family members in HIV-1 latency.

Recent findings—Various NF- κ B/Rel family members can reinforce maintenance of HIV-1 latency. For example, p50 recruits histone deacetylase 1 to the HIV-1 long terminal repeat promoting chromatin condensation and reduced RNA Pol II recruitment. Low-level NF- κ B activation during homeostatic proliferation of memory CD4 T cells induced by IL-7 and TCR signaling or OX40 action promotes expression of antiapoptotic gene targets like *BCL2* and *BCLXL*. Additionally, the I κ B kinase phosphorylates FOXO3a transcription factor, blocking its induction of proapoptotic genes. These combined effects promote memory CD4 T-cell survival, thus maintaining the latent reservoir. Conversely, when the nontumorigenic phorbol ester, prostratin, is combined with histone deacetylase inhibitors, potent synergistic activation of latent HIV-1 occurs involving nuclear expression of NF- κ B.

Summary—These recent findings highlight both the antagonistic and agonistic effects of the NF- κ B signaling pathway on HIV-1 latency. Synergistic inducers acting might be useful for flushing of latent virus from reservoirs in infected patients. The ultimate, albeit lofty, goal is to achieve full viral eradication. However, a more reasonable goal might be a functional cure where patients experience a drug-free remission.

Keywords

drug-free remission; HIV-1 latency; homeostatic proliferation; NF- κ B; viral eradication

Introduction

Combination antiviral therapies effectively reduce viral loads in HIV-1-infected patients but are unable to achieve full eradication of the virus. HIV-1 persists in a latent form at least in part within a small pool of CD4 memory T cells (1,000,000 cells - 10,000,000 cells/patient). As all antiviral drugs require viral replication in order to act, this reservoir of latently infected cells ensures survival of the virus despite administration of highly active antiretroviral therapy (HAART). New strategies to attack this latent reservoir are urgently needed. Studies in this area have been challenging, however, principally because latent HIV-1-positive cells are rare and lack markers that distinguish them from other CD4 T cells, thwarting attempts at enrichment.

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NF- κ B/RelA is an important host factor that promotes HIV-1 replication during acute infection. However, the role of this transcription factor family in postintegration latency is more complicated. In this review, we describe recent advances focusing on how the NF- κ B signaling pathway influences the establishment, maintenance, and loss of HIV-1 latency through diverse effects on HIV-1 transcription and the survival of memory CD4 T cells.

Rounding up the suspects

As noted, memory CD4 T cells form the major recognized reservoir for latent HIV-1 proviruses. These cells are ideally suited to harbor latent HIV-1 because of their long survival in a relatively quiescent state. HIV-1 latency may be initially established within these cells as they retreat from antigen activation to a resting state. The rarity of latently infected cells could reflect the narrow temporal window that allows integration to occur in the absence of subsequent replicative steps in the viral life cycle.

The pool of memory CD4 T cells includes three subsets:

1. Central memory T cells (TCM cells; CD45RO⁺CCR7⁺CD27⁺): these cells display receptors that enable trafficking to lymphoid organs. They proliferate rapidly in response to interleukin (IL)-2, but produce only very small amounts of cytokines.
2. Effector memory T cells (TEM cells; CD45RO⁺CCR7⁻CD27⁻): these cells are derived from TCM cells through T-cell-receptor (TCR) ligation and cytokine stimulation. They target sites of inflammatory tissues, have a low proliferative capacity, and rapidly produce effector cytokines.
3. Transitional memory T cells (TTM cells; CD45RO⁺CCR7⁻CD27⁺): these recently described cells are a cellular intermediate generated by TCM cells transiting to become TEM cells. Their functions and gene expression profiles are intermediate between those of their cellular precursors and successors [1].

TCM cells have been implicated as the predominant cellular reservoir of latent HIV-1. However, recent studies suggest latent HIV-1 is also present in TTM cells, particularly in patients whose CD4 T-cell counts remain low despite administration of HAART. It seems likely that the persistence of latent HIV-1 in these cells is linked to their homeostatic proliferation driven by IL-7 [2^{**}]. TEM cells are a poor cellular reservoir for latent HIV-1 because they are more prone to activation-induced cell death than TCM or TTM cells due to upregulation of FOXO3a-driven proapoptotic genes, such as *FASL*, *BIM*, and *GADD45*. Because of their rapid turnover, they do not contribute significantly to the pool of latently infected memory CD4 T cells [1,2^{**}].

Other cell types have also been suggested as potential latent reservoirs, but their prevalence and kinetics of persistence are not yet well characterized. These include resting CD4 T cells in gut-associated lymphoid tissue [3^{**},4], hematopoietic stem cells in bone marrow [5^{**}], and a unidentified cellular source in the blood that is not CD4 T cells or monocytes [6^{**}].

NF- κ B and establishment of the latent reservoir

During the development of memory T cells, NF- κ B helps to ensure T-cell survival during the initial differentiation of effector cells into memory cells. Co-stimulatory receptors in the tumor necrosis factor (TNF) receptor superfamily, including OX40 (CD134) and CD30, induce NF- κ B-dependent expression of antiapoptotic genes, such as *BCL2* and *BCLXL* (Fig. 1) [7,8,9^{**},10^{*},11^{*},12,13^{**},14–16]. However, prolonged and stronger NF- κ B signaling can thwart establishment of a latent cell reservoir because of its activating effects on the HIV-1 long terminal repeat (LTR). Thus, for latency to be established, competing activities must be carefully balanced. T-cell activation must be suboptimal and NF- κ B activity must

be sufficient to promote cell survival but not so great as to encourage proviral gene expression.

Indeed, the overall status of nuclear NF- κ B expression in the cell at the time of HIV-1 infection may be the chief factor governing the likelihood of latent infection [17^{**}]. In Jurkat cells, prestimulation with NF- κ B inducers like phorbol myristate acetate (PMA) supports productive rather than latent infection; as a result, these cells harbor very few silent proviral integrations. Similarly, basal levels of NF- κ B activity characteristic of distinct CD4 T-cell lines negatively correlate with the propensity of the cells to support latent HIV-1 infection [17^{**}].

How is nuclear NF- κ B activity fine-tuned? One mechanism involves dynamic posttranslational modifications of RelA, the transcriptionally active subunit of the prototypical NF- κ B heterodimer (p50/RelA). The activity of NF- κ B, like that of other proteins, enzymes, and transcription factors, can be modulated by the addition or removal of chemical groups. For example, addition of a methyl group to lysine 37 of RelA in response to stimulation with TNF- α causes RelA to activate a subset of genes [18]. Conversely, with stronger or longer acting agonists, RelA undergoes additional methylations at lysines 314 and 315, which targets it for a very different fate, specifically degradation by the 26S proteasome [19].

Thus, during latency establishment and suboptimal T-cell activation, specific posttranslational modifications of RelA might direct its activity to favor expression of antiapoptotic genes rather than HIV-1 transcription. A similar scenario occurs with p53. Depending on the strength of the genotoxic stimulus, p53 is differentially acetylated, leading to the expression of genes involved in DNA excision and repair versus genes that trigger apoptosis [20].

A higher activation threshold for NF- κ B action at the HIV-1 LTR might favor the establishment of latency through the pro-survival effects of NF- κ B. In this regard, the HIV-1 LTR harbors two tandem κ B sites that are highly conserved among different HIV-1 subtypes. These two sites can simultaneously bind two p50/RelA heterodimers. However, because these sites are separated by only four base pairs, the formation of such a dimeric heterodimer–DNA complex requires considerable free energy to bend the spacer DNA to make room for both heterodimers [9^{**}].

Of note, this arrangement of the κ B sites is observed in most HIV-1 subtypes [21^{**}]. The resulting ternary complex is reinforced by interactions between the bound heterodimers and is thermodynamically quite stable. Mutating either or both κ B sites significantly compromises latent proviral reactivation by TNF- α . Interestingly, the major defect occurs at the level of transcriptional elongation rather than transcriptional initiation at the HIV-1 LTR [15,21^{**},22^{**}] (see also below). Similarly, we observed that activation of latent proviruses in J-Lat cells requires prolonged stimulation with TNF- α , which leads to repeated rounds of nuclear NF- κ B activation; briefer stimulation with TNF- α induces transcriptional initiation, but the elongation step is impaired and HIV-1 gene expression is aborted [14].

NF- κ B and preservation of HIV-1 latency

After CD4 T cells differentiate into the various memory subsets, the cells must survive to mount an anamnestic response during future antigen exposures. To execute this critical and characteristic function of the adaptive immune response, these cells are replenished by homeostatic proliferation involving a convergence of TCR and IL-7 signaling. The survival of TCM involves phosphorylation of STAT5a and FOXO3a, the latter involving Akt-activation of IKK α / β and modification of FOXO3a. Phosphorylated FOXO3a is rendered

inactive and thus unable to induce its proapoptotic gene targets including *FASL*, *BIM*, and *GADD45* (Fig. 1).

The preservation of viral latency likely involves multiple processes, including repressive chromatin remodeling [8,11*,21**,22**,23], transcriptional interference [24,25], transcription factor or co-regulator insufficiency [10*], nuclear retention of multisplliced HIV-1 RNA [26], host microRNA antiviral defense [27], and perhaps the noted physical constraint within the LTR- κ B sites, which imposes a relatively high threshold for p50/RelA transactivation [9**]. Of note, most latent HIV-1 proviruses are located within the introns of active genes, strengthening a role for transcriptional interference; however, latent proviruses are also found in 'gene deserts', such as alphoid centromeric repeats that are rich in repressive heterochromatin suggesting a very different mechanism for latency establishment [28–30].

One member of the NF- κ B/Rel family of transcription factors, p50, appears to play an active role in maintaining HIV-1 latency. We have demonstrated that p50, which lacks a functional transactivation domain, recruits histone deacetylase (HDAC) 1 to the HIV-1 LTR that promotes a hypoacetylated state in the surrounding chromatin [8] (Fig. 1). This repressive chromatin environment appears to occlude the binding site for RNAPII, as addition of HDAC inhibitors allows for effective recruitment of RNAPII to the HIV-1 LTR. Of note, these studies were performed in J-Lat cells [28], which may not completely model latency occurring *in vivo*. HDAC1 recruitment to the HIV-1 LTR has also been detected in a primary CD4 T-cell model of HIV-1 latency [10*]. Of note, HDAC inhibitors alone often fail to antagonize latency in J-Lat containing full-length replication-competent HIV-1 viruses [11*,17**]. This finding could reflect the fact that HDAC inhibitors stimulate LTR-histone acetylation and RNAPII-dependent transcription initiation but cannot render the polymerase fully processive. Effective polymerase processivity requires parallel induction of nuclear p50/RelA expression, which recruits P-TEFb to phosphorylate serine 2 within carboxyl heptad repeats of RNAPII [8,10*,14, 15]. In most J-Lat clones containing full-length infectious virus, stimulation by an HDAC inhibitor alone leads only to short, ineffective HIV-1 transcripts [8].

Analysis of HIV-1 integration sites in resting CD4 T cells from patients on HAART has revealed that the provirus strongly prefers to insert within introns of actively transcribing genes [29]. This tendency is consistently observed in many J-Lat clones. Within these clones, transcription interference from nearby active promoters may be responsible for repressing latent HIV-1 transcription [17**,24,25]. Indeed, this action might supersede other mechanism. However, a very low level of residual viremia and multisplliced HIV-1 mRNA transcripts can be detected within resting CD4 cells in aviremic patients on HAART [6**,26]. Therefore, a repressive mechanism that relies solely on interfering with latent HIV-1 transcription might be rather inefficient and might require positive reinforcement by HDAC1 recruited by p50 or by other transcription factors, such as Sp1, LSF, or YY1 [31,32].

Of note, in several non-J-Lat, Jurkat-based latency models, HDAC inhibitors alone can induce latent HIV-1 transcription [21**,22**,33**]. The reason for this discrepancy between J-Lat and non-J-Lat clones is not entirely clear. J-Lat clones are derived with a full-length- Δ env- Δ nef HIV-1 virus, whereas the HDAC inhibitor-inducible cell models employ 'mini-viruses', which express only Tat and a GFP reporter [21**,22**,33**]. It is possible that these mini-viruses may more frequently integrate into heterochromatin [28], where transcriptional interference is unlikely to occur. This might explain why HDAC inhibitors alone cannot induce proviral reactivation in the absence of p50/RelA induction, and potentially highlight the importance of using full-length HIV-1 to model latency. It is also possible that activation of NF- κ B leads to binding of p50/RelA heterodimers to the HIV-1 LTR and in turn this

binding blocks transcriptional interference by inhibiting elongation of the competing RNAP II (B.M. Peterlin, unpublished data).

Will NF- κ B inducers antagonize HIV-1 latency?

In all Jurkat-based models of HIV-1 latency, inducers of prototypical NF- κ B heterodimer (p50/RelA) such as phorbol esters and TNF- α consistently activate latent HIV-1 proviruses [8,11^{*},12,14,21^{**},22^{**},23,33^{**}]. However, in some recently described primary CD4 T-cell models, these inducers do not appear to effectively antagonize HIV-1 latency. In general, two strategies have been used to generate primary CD4 T cells latently infected with HIV-1. First, resting CD4 T cells are infected with HIV-1 by spinoculation, which overwhelms the postentry blocks that oppose HIV-1 infection in these cells. Secondly cells are activated with various agents, infected with HIV-1, and then the cultures are allowed to rest to encourage establishment of viral latency. Some studies have employed TCM-like cells [34^{**}]. Additionally, activated CD4 T cells have been infected with HIV-1 and engineered to express antiapoptotic gene products to improve cell survival as they are allowed to rest [10^{*}, 13^{**},21^{**}].

Interestingly, the primary models have yielded surprising results about the involvement of the NF- κ B pathway as an antagonist of HIV-1 latency. Despite unanimous results obtained with all Jurkat-based latency models [8,11^{*},12,14,21^{**},22^{**},23,33^{**}], three of four primary models found that TNF- α is a very weak inducer of latent HIV-1 (TNF- α was not tested in the fourth model). Results in the Bosque latency cell model were even more surprising, as common inducers of NF- κ B like phorbol esters or other agents that activate the PKC-IKK α / β -NF- κ B pathway did not effectively induce latent virus in the TCM-like cells. Conversely, these ‘nonpolarized, TCM-like’ CD4 cells readily responded to TCR stimulation or addition of phytohemagglutinin. Further, reactivation of virus by these agents was blocked by cyclosporin A, arguing for a potential role for NFAT transcription factors in the response. Indeed the authors concluded that reactivation of latent HIV-1 in these cells principally proceeds through a Lck–calcineurin–NFAT pathway.

The crystal structure of NFATc2 indicates that this transcription factor associates with the double LTR- κ B sites in a unique dimer–monomer configuration [35]. The dissociation constant of the engagement of NFATc2 to the first LTR- κ B site is 20 nmol/l [36]. The second monomer interacts with the second site, presumably with a lower affinity because of fewer DNA contacts. In contrast, despite a free-energy requirement to simultaneously incorporate two p50/RelA heterodimers at the two LTR- κ B sites, the first p50/RelA heterodimer binds exceptionally tightly to the HIV-1 LTR with a K_d of 0.1 nmol/l to 0.1 pmol/l [9^{**},37]. Assuming equal cellular concentrations of NFATc2 and p50/RelA *in vivo*, NFATc2 would appear to be an extremely poor competitor of p50/RelA binding to the LTR. Of note, the calcineurin signaling pathway also augments NF- κ B activation in T cells, although the precise mechanism remains unclear [38–40]. Although PHA activates NFAT, this mitogen also induces NF- κ B [34^{**},40]. Thus, although NFAT appears to occupy center stage as an activator of latent virus in the Bosque cell model, additional study is required to discern the relative roles of NFAT versus NF- κ B in the activation of latent HIV-1 *in vivo*.

Why might TNF- α fail to reactivate latent HIV-1 in primary models? Notably, in resting CD4⁺ T cells, the P-TEFb complex is predominantly sequestered in an inactive 7SK-ribonucleoprotein complex, whose release requires calcium–calcineurin signaling [41]. The Tyagi primary model elegantly reveals that TNF- α stimulates p50/RelA nuclear translocation and possibly LTR occupancy. However, unlike TCR signaling, which induces cytosolic calcium flux leading to the release of P-TEFb, TNF- α signaling does not trigger calcium mobilization in the cell. Thus, the lack of free P-TEFb may explain the failure of these primary T cells to respond to TNF- α .

Clinical implications

The ‘flushing’ or ‘purging’ strategy involving activation of latent HIV-1 in the presence of HAART is commonly regarded as the most promising approach for eradicating the virus from the latent reservoir. However, as noted, prior attempts to stimulate latent provirus expression *in vivo* with anti-CD3 or IL-2 were not successful [42,43]. More recently, valproic acid was tested as an inducer of latent HIV-1 but with limited success [44]. However, valproic acid is a weak HDAC inhibitor and, when used in the absence of NF- κ B agonists, cannot promote NF- κ B/RelA persistence in the nucleus [8,11*,45]. Conversely, in combination with an NF- κ B inducer like prostratin, valproic acid or more potent HDAC inhibitors like trichostatin A synergistically activate HIV-1 proviruses in JLat cells (Williams and Greene, unpublished data) and promote viral reactivation in cells from patients who are receiving HAART and have undetectable viral loads [11*].

The molecular basis for this synergy involves changes in chromatin structure around the LTR as well as acetylation of RelA, which promotes improved DNA binding and resistance to I κ B- α inactivation [45,46]. Additionally, HDAC inhibitors promote RNAPII recruitment to the LTR, while prostratin enhances processivity of the bound polymerase through the actions of NF- κ B and P-TEFb. Acetylation of RelA at lysine 310 recruits the bromodomain protein BRD4, which, in turn, recruits P-TEFb to phosphorylate serine 2 within the carboxyl heptad repeats of RNAPII, thereby enhancing RNAPII-transcriptional elongation [16]. However, the potential toxic effects of prostratin and HDAC inhibitors like SAHA, including their ability to cause T-cell activation and secretion of proinflammatory cytokines, could make their clinical use problematic [17**,33**]. Nevertheless, if the synergy observed in J-Lat cells can be translated into the *in vivo*-situation, it may be possible to achieve the desired effects at lower and thus less toxic doses of these agents. Recently identified antagonists of HIV-1 latency that activate NF- κ B in the absence of generalized T-cell activation might form the basis for a more attractive treatment modality [13**,47*] (Fig. 1). However, because HIV-1 latency is probably multifactorial in the cells of a single patient, it seems likely that combinations of agents attacking the different mechanisms in play will be required. It is also possible that full eradication will not be required. Rather, appropriate inducers might both shrink the reservoir and enable the immune response against the virus such that the host can effectively control the virus in the absence of HAART – a so-called drug-free remission. Such a functional cure might be more likely to achieve than complete viral eradication.

Conclusion

The important role of NF- κ B in memory T-cell survival and homeostatic proliferation could be problematic when NF- κ B inducers are used as components of a ‘flushing strategy.’ These agents could inadvertently promote the survival of latently infected cells. However, it is now clear that NF- κ B is fine-tuned after its translation to manifest pro-survival versus HIV-activating effects. Discerning the molecular basis for these different NF- κ B activities could lead to a novel and effective approach for flushing latent HIV-1 without affecting the survival of memory T cells. Finally, it will be important to assess the role of NF- κ B in HIV-1 latency in cells other than memory T lymphocytes.

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Additional references related to this topic can also be found in the Current World Literature section in this issue (pp. 000–000).

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Figure 1. A model depicting the agonistic (left) and antagonistic (right) roles of NF- κ B/Rel family members in HIV-1 latency

Homodimers of p50 reinforce HIV-1 latency by associating with and recruiting HDAC1 to HIV-1 LTR. HDAC1-mediated deacetylation of surrounding histones leads to chromatin condensation and decreased binding of RNAPII [8] (step 1). Stimulation through the OX40 and CD30 cellular co-receptors activates the I κ B kinase complex (IKK) resulting in the phosphorylation, polyubiquitylation, and proteasome-mediated degradation of I κ B- α . These less than full stimulatory events liberate moderate amounts of the prototypical NF- κ B heterodimer (p50/RelA) allowing its translocation into the nucleus where it produces pro-latency effects by activating the expression of antiapoptotic genes like *BCL2* and *BCLXL* [7] (step 2). Furthermore, after weak TCR and IL-7-dependent stimulation associated with homeostatic proliferation of memory CD4 T cells [2^{**}], IKK phosphorylates the FOXO3a transcription factor, decreasing its ability to induce expression of various proapoptotic genes, like *FASL*, *BIM*, and *GADD45* [1] (step 3). These combined effects promote survival of memory T cells thus preserving the latent reservoir. Of note, NF- κ B induction during homeostatic proliferation is not sufficient to drive latent HIV-1 transcription, which has a high activation threshold [9^{**}]. However, HIV-1 latency is antagonized during subsequent anamnestic immune responses, where robust TCR signaling induces high-level expression of nuclear p50/RelA [10^{*}]. Likewise, pharmacological agents that stimulate protein kinase C (PKC) and activate accumulation of reactive oxygen species (ROS) induce nuclear NF- κ B expression [11^{*},12,13^{**}] (step 4). ROS activators that stimulate NF- κ B in the absence of global T-cell activation have been identified and could represent a novel approach for flushing latent proviruses [13^{**}] without adverse side effects. LTR-targeted p50/RelA complexes effectively displace the bound p50-HDAC1 repressor complexes and lead to effective recruitment of P-TEFb [8,14] (step 5). This recruitment involves either the direct binding of P-TEFb to RelA [15] (LTR-bound RelA on the right) or the acetylation of RelA at lysine 310 followed by binding of the bromodomain-containing protein BRD4 that in turn interacts with P-TEFb [16] (LTR-bound RelA on the left). In both cases, P-TEFb phosphorylates the carboxyl terminal domain of RNAPII, resulting in robust activation of RNAPII elongation [14] (step 6). The initial NF- κ B-induced expression of HIV-1 mRNAs in turn triggers the production of Tat, which powerfully amplifies HIV-1 expression by binding to TAR and recruiting P-TEFb leading to high level, sustained phosphorylation of RNAPII.