HIV-1 latency in proliferating T cells

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SUMMARY

In this thesis we focus on the ability of HIV-1 to establish a latent provirus in proliferating T cells and on how the silent provirus can be activated from latency. HIV-1 latency is a major barrier towards virus eradication from the infected individual. Knowledge on how the latent reservoir is established and understanding the molecular mechanisms that control latency may contribute to the development of therapeutics aiming at prevention and/or eradication proviral latency and describes why the current anti-retroviral therapy cannot cure an HIV-infected individual.

HIV-1 isolates can be classified in four different groups: Major (M), New (N), Outlier (O) and P, with group M viruses being mainly responsible for the current pandemic. The M group is divided into subtypes A through K and contains more than 51 circulating recombinant forms (CRFs). The increasing diversity of HIV-1 isolates makes virus quantitation challenging, especially when diverse isolates co-circulate in a geographical area. Measuring the HIV-1 DNA levels in cells has become a valuable tool for fundamental and clinical research. Chapter 2 describes the development of a quantitative HIV-1 DNA assay based on TaqMan technology. Primers that target the highly conserved Long Terminal Repeat (LTR) region were designed to detect a broad array of HIV-1 variants and subtypes with high sensitivity. Introduction of a pre-amplification step prior to the TaqMan reaction allowed us to quantitate fully reverse transcribed viral DNA and 2-LTR circles.

HIV-1 transcription initiation depends on cellular transcription factors that bind to promoter sequences in the LTR. Each HIV-1 subtype has a specific LTR promoter configuration and even minor sequence changes in the transcription factor binding sites (TFBS) or their arrangement can impact transcriptional activity. Such subtype-specific promoter characteristics correlate with significant differences in terms of viral replication kinetics and the response to environmental changes. Such latency differences may influence the size of viral reservoirs and the possibility to purge these reservoirs by therapeutic intervention. Chapter 3 describes the degree to which LTR promoter variation contributes to differences in proviral latency. We constructed recombinant viral genomes with the subtype-specific promoter inserted in the common backbone of the subtype B LAI isolate. The recombinant viruses are isogenic except for the core promoter region that encodes all major TFBS, including NF-κB and Sp1 sites. We developed and optimized an assay to investigate HIV-1 proviral latency in T cell lines. Our results indicate that the majority of HIV-1 infected T cells only start viral gene expression upon TNFα activation. There were no gross differences among the subtypes, both in the initial latency level and the activation response, except for subtype AE that combines an increased level of basal transcription with a reduced TNFα response. This subtype AE property is related to the presence of a GABP instead of NF-κB binding site in the LTR.
There are different cell types that harbor latent HIV-1 provirus but the majority of the reservoir consists of long-lived resting CD4+ T lymphocytes. How this reservoir is established is not yet fully understood and there are currently two ideas. The first possibility is direct infection of resting CD4+ T lymphocytes. This is an inefficient process in these resting cells due to several blockades in the virus replication cycle. The second possibility is infection of proliferating T lymphocytes, which are the primary target cells for HIV-1. If infection results in the establishment of a latent provirus, these T lymphocytes will not be recognized by the immune system because no viral antigens are expressed and presented. If the proliferating cells subsequently return to the resting state, they become part of the long-lived reservoir. It has generally been assumed that proliferating T lymphocytes do not harbor a latent HIV-1 provirus because these activated cells express many transcription factors that induce proviral gene expression. In Chapter 4 we present the surprising finding that HIV-1 does in fact frequently establishes a latent provirus in proliferating primary T lymphocytes. Latency can only be recognized upon activation of the silent provirus. However, conventional anti-latency drugs that are used to activate the latent provirus in resting cells had no effect on the latent provirus in proliferating lymphocytes. We describe that co-culturing of the HIV-1 infected proliferating T lymphocytes with monocytes-derived dendritic cells (DCs) is able to activate the provirus from latency. Activation did not involve the DC-SIGN signaling or TCR stimulation, but was mediated by DC-secreted component(s) and cell-cell interaction between the DC and T lymphocyte. The cell-cell contact mediated activation could be blocked by ICAM-1 antibodies, implicating the ICAM-1 LFA-1 interaction. These results imply that circulating DCs could purge HIV-1 from latency in activated T cells and re-initiate virus replication. Moreover, our data show that viral latency can be established early after infection and support the idea that actively proliferating T lymphocytes with an effector phenotype contribute to the latent viral reservoir.

Chapter 5 combines the insights obtained in the two previous chapters to study the effect of the subtype-specific LTR promoter on viral latency and replication in primary T lymphocytes. No clear differences in latency and replication among the HIV-1 subtypes were observed. Additionally, the subtype B and AE LTRs were studied in more detail regarding a putative AP1 binding site in transfection experiments with luciferase reporter constructs. The results from these assays indicate that c-Jun, a member of the AP1 transcription factors family, can bind to both the subtype B and AE LTR, but the latter showed a stronger response. This is in line with the fact that the subtype AE LTR matches the AP1 consensus sequence more closely than the subtype B LTR. The AP1 binding site was recently proposed to be important for viral latency as part of the latency establishment element (LEE)\(^1\). We measured that c-Jun suppresses LTR activity in the absence of Tat, reflecting the establishment of a latent provirus that will likely occur before Tat is synthesized. However, c-Jun instead boosts LTR promoter activity in the presence of Tat. In other words, c-Jun provides the setting for Tat to act as a dominant molecular switch to control HIV-1 LTR activity.
Chapter 6 describes whether different myeloid cell types can induce gene expression from the latent HIV-1 provirus in proliferating T lymphocytes. Knowledge on ‘natural’ purging mechanisms may contribute to the design of novel eradication strategies that target the long-lived viral reservoir. Monocyte-derived macrophages type I and II can moderately activate the latent provirus, but not as efficiently as monocyte-derived DCs. Monocytes and plasmacytoid DCs isolated from blood cannot activate the latent provirus. Conventional CD1c⁺ and cross-presenting CD141⁺ myeloid DCs efficiently purged the provirus out of latency. In our in vitro experiments the T lymphocytes are cultured outside their natural habitat. The observation that viral latency in T lymphocytes is influenced differently by various DC subsets suggest that viral latency in vivo might depend on the specific location of the T lymphocytes. For instance, production of virus particles from the latent reservoir may occur in the lymph node or other lymphoid organs due to the presence of DCs. Interestingly, maturation of primary myeloid DCs with several TLR ligands indicated that these mature myeloid DCs activate latent provirus as efficiently as immature myeloid DCs, except for poly(I:C) maturated cells that lost almost all purging capability.

In Chapter 7 we describe the first steps in characterizing the molecular mechanisms of DC-mediated activation of latent HIV-1 provirus in proliferating T lymphocytes. The activation appears to be multi-factorial, as both cell-cell interaction and DC-secreted factor(s) mediate activation of the latent provirus, indicating that multiple signaling routes are involved. The DC-secreted factor is a surprisingly large protein or protein complex (>100 kDa) and the cell-cell interaction may involve tetraspanins. Understanding the molecular mechanisms that purge HIV-1 out of latency may be useful to improve intervention therapies designed to overcome latency.

Chapter 8 presents a general overview of the molecular mechanisms that contribute to viral latency and the role of latently infected proliferating T lymphocytes in the establishment of the long-lived viral reservoir in resting T lymphocytes.

Chapter 9 covers the prospects of finding a cure for HIV-1, the effect of current ‘shock and kill’ approaches on the viral reservoir in proliferating T lymphocytes and the influence of this newly described reservoir on the ongoing debate regarding HIV-1 latency versus continued low-level replication.

REFERENCES