HIV-1 latency in proliferating T cells
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Chapter One

General introduction
HIV-1 latency and Outline of this thesis.
GENERAL INTRODUCTION

HIV origin and diversity. In 1983 human immunodeficiency virus (HIV) was discovered as the causative agent of acquired immunodeficiency syndrome (AIDS). Since the beginning of the epidemic in the 1970’s an increasing number of people died of AIDS-related causes and in 2011 it was estimated that more than 34 million people, including 3.3 million children, were living with an HIV infection worldwide (www.unaids.org). The first infections in humans occurred long before the AIDS epidemic became apparent. The oldest HIV-positive blood sample dates back to 1956 and, based on HIV sequence analysis, it was estimated that the first HIV infections in humans occurred around 1920-1930.

HIV originated from zoonotic transmissions of simian immunodeficiency virus (SIV) from non-human primates to humans in West and Central Africa (reviewed in Hemelaar). The cross-species transmission probably occurred during the hunting of primates for meat or the capture, trade and housing of monkeys as pets. Several independent transmission events have occurred, resulting in 2 different lineages of HIV, known as HIV type 1 (HIV-1) and type 2 (HIV-2), each of which can be further subdivided into several groups and subtypes. The contribution of each virus type and subgroup to the pandemic differs significantly.

HIV-1 comprises 4 groups, each originating from an independent zoonotic transmission: M (for major/main), N (for new or non-M/non-O), O (for outlier) and P. Group M and N originate from SIV found in chimpanzees in West and Central Africa. Group N has only infected a small number of individuals in Cameroon. Group M viruses most likely spread from Cameroon via the Congo River to Kinshasa in the Democratic Republic of Congo to initiate the epidemic and is now responsible for the HIV-1 pandemic. Group O and P probably originate from SIV in gorillas living in Cameroon. Group O has infected about ten thousand individuals in Cameroon and surrounding countries. Group P has thus far been detected in only 2 individuals from Cameroon.

HIV-2 can be subdivided into 7 groups termed A through G. It appears that these strains resulted from 7 individual transmission events from the sooty mangabey to humans. HIV-2 infections occur primarily in Western Africa, but a significant number of infected individuals have also been reported in India, Brazil and Portugal (reviewed in Reeves and Doms).

The pandemic. The pandemic is caused predominantly by virus variants of HIV-1 group M, which can be divided into subtypes A through K. In addition, recombination between the different subtypes occurs frequently and this resulted in more than 51 circulating recombinant forms (CRFs, http://www.hiv.lanl.gov/content/sequence/HIV/CRFs/CRFs.html). The different subtypes and CRFs are unequally distributed over the world (Fig. 1). The subtype B variants are mainly found in Western Europe and
Northern America, whereas in Thailand the recombinant AE (CRF_01) subtype is dominant. All HIV-1 subtypes and many different CRFs circulate in Africa, but subtype C viruses dominate the Sub-Saharan area and recombinant AG (CRF_02) variants are prevalent in West and West-Central Africa. However, these geographical boundaries are slowly disappearing as the number of non-B infections in Europe and Northern America is increasing due to immigration and tourism\textsuperscript{14}. In addition, the prevalence of recombinant forms is still increasing\textsuperscript{15,16}.

In countries where infected individuals have access to antiretroviral therapy, the HIV-1 infection has become a chronic disease. Unfortunately, there are still many regions worldwide where infected individuals have limited or no access to therapy; for these people HIV-1 remains a life-threatening infection.

**The HIV-1 genome and proteins.** The HIV genome, as present in the virion particle, consists of 2 copies of a single stranded RNA molecule of 9.7 kb encoding 9 open reading frames (ORFs) (Fig. 2). The three major ORFs gag, pol and env are translated into the Gag, Pol and Env polyproteins that are processed into functional proteins. Gag is cleaved into 4 proteins that are important for the structure of the virion and packaging of the viral RNA genome. Pol is cleaved into 3 enzymes: protease (PR), reverse transcriptase (RT) and integrase (IN). PR cleaves the polyproteins Gag and Pol into the functional units, RT reverse transcribes the viral RNA into DNA, and IN facilitates integration of the viral DNA into the host cell genome. Env encodes the precursor gp160 glycoprotein that is cleaved into a gp41 transmembrane domain.

![Global distribution of HIV-1 subtypes](image)

**Fig. 1. Global distribution of different HIV-1 subtypes and CRFs.** Letter sizes indicate the relative frequency of that particular subtype in a region. URFs are unique recombinant forms. The figure is adapted from the review by Hemelaar\textsuperscript{5}. 

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and a gp120 outer surface domain. Trimeric gp41 together with trimeric gp120 forms
the envelope on the outer surface of the virus particle that is required for binding to
the target cell. Two smaller ORFs encode the essential regulatory proteins Tat and
Rev. The Tat protein is important for transcription of the viral genome and Rev is
essential in regulation of viral mRNA biosynthesis. For a more extended overview of
the HIV-1 genome and the encoded proteins see the review by Frankel and Young\textsuperscript{17}. Additionally, there are 4 ORFs encoding the “accessory” proteins Nef, Vpu, Vif and Vpr. The term accessory was applied because these proteins are dispensable for viral
replication in T cell lines. However, in the search for new viral functions that can be
therapeutically targeted, these accessory proteins have gained renewed interest
because they are well conserved among virus isolates, are important for viral
pathogenesis and mediate evasion from host restriction factors. For a recent update
on the function of the accessory proteins, see the reviews by Vermeire \textit{et al.}\textsuperscript{18} and
Malim and Bieniasz\textsuperscript{19}.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{Fig2.png}
\caption{HIV-1 genome and virus particle. A: Schematic representation of the HIV-1 DNA. Indicated are the open reading frames (ORFs) encoding the structural proteins Gag, Pol, Env, the regulatory proteins Tat, Rev and the accessory proteins, Vif Vpr, Vpu and Nef. B: Mature HIV-1 particle. Depicted are the Env spikes on the outer surface and the typical cone shaped structure containing the two single stranded RNA molecules.}
\end{figure}

\textbf{The HIV-1 replication cycle.} HIV-1 is a lentivirus belonging to the \textit{Retroviridae}
family. The viral replication cycle is initiated by binding of the viral envelope protein
(Env) to the CD4 receptor on the target cell (Fig. 3, step 1). This interaction induces a
conformational change in Env that triggers binding to a co-receptor. HIV-1 generally
uses the chemokine receptors CCR5 or CXCR4 as co-receptor, but others such as
CCR3 have been described (reviewed by Pollakis and Paxton\textsuperscript{20}). Binding to the
co-receptor triggers fusion of the viral and cellular membrane, such that the viral
core containing the viral RNA genome is released into the host cell cytoplasm. The
viral RNA genome is reverse transcribed into double stranded DNA by the viral RT enzyme, one of the unique characteristics of the Retroviridae family. The viral IN protein inserts the viral DNA into the host cell genome, which is the second hallmark of this virus family. The integrated form is termed the provirus, which uses the cellular gene expression machinery (transcription, splicing and translation) for the production of new viral mRNAs, proteins and viral RNA genomes. The structural proteins, together with two single stranded RNA genomes, assemble at the plasma membrane to form new virus particles that bud from the cell. Within the newly formed virion the viral PR induces proteolytic cleavage of the Gag polyprotein into the different structural proteins. A mature infectious virion is formed upon Gag cleavage and formation of the typical cone-like structure with the viral genome. For a more detailed description of the HIV-1 replication cycle see the review by Freed\textsuperscript{21}.

**Fig. 3. The HIV-1 replication cycle.** Replication starts by binding of the viral Env protein to CD4 and a co-receptor (1), mediating fusion of viral and cellular membranes (2). The viral core is released into the host cell cytoplasm (3) and the viral RNA genome is reverse transcribed by the viral RT (4) into dsDNA that migrates into the nucleus (5). The viral IN protein integrates the dsDNA into the host cell genome (6), the integrated viral DNA is termed the provirus. Production of viral mRNAs (7) and proteins (8) depends on the host cell transcription and translation machinery. Viral proteins and the viral RNA genome assemble at the plasma membrane (9) to form new virus particles that subsequently bud from the cell surface (10). Proteolytic cleavage of the Gag polyprotein into the different structural proteins, forming the typical cone structure with the viral genome, induces maturation of the virion (11) to become an infectious particle.
**Disease course.** HIV-1 infects cells of the immune system and causes chronic immune activation, which can over time result in the collapse of this defense system, leading to opportunistic infections, various forms of cancer and eventually death of the infected individual. The natural course of an untreated HIV-1 infection can be divided into four stages, (reviewed in Sierra et al.)\(^2\). The first stage is the incubation period, also known as the primary phase, lasting 2 to 4 weeks, in which the viral RNA load in plasma increases, concomitant with a decrease in the number of CD4\(^+\) T lymphocytes. The second stage is known as the acute phase, lasting for 1 to 3 months, which can include fever-like symptoms, rash and sores. During this period the immune system becomes fully activated in response to the HIV-1 infection. As a consequence, the viral RNA load declines, HIV-1-specific cytotoxic CD8\(^+\) T lymphocytes emerge, HIV-1-specific antibodies are produced and CD4\(^+\) T lymphocyte counts are partially restored. When the viral load stabilizes at a relative low level, known as the viral setpoint, replication is controlled by the immune system, which can last for 2 to 15 years. This third stage of disease represents clinical latency, which should not be confused with viral latency. During this asymptomatic period the virus continues to replicate but the infected cells are cleared by the immune system and replaced by new cells, creating an equilibrium between cell death and cell replacement. This process results in exhaustion of the immune system over time. The CD4\(^+\) T cell numbers gradually decline when the immune system can no longer produce a sufficient number of new lymphocytes, which leads to the fourth phase. This is the stage in which the patient will be diagnosed with AIDS. Cell-mediated immunity is lost and the immune system can no longer protect the patient from opportunistic infections or tumors, which will eventually lead to death of the infected individual.

The rate of disease progression leading to AIDS differs significantly between individuals. There are patients who will develop AIDS within 5 years after infection, the so-called rapid progressors, and there are patients that effectively control viral replication, such that the asymptomatic phase can last up to 25 years. These patients are known as long term non-progressors. A very select group of HIV-1 infected individuals are known as elite controllers, their immune system controls virus replication so efficiently that they do not progress towards AIDS.

**Anti-retroviral therapy.** Therapy for HIV-1 became available in 1987 and consisted of a single RT inhibitor (AZT). Therapy success was limited due to the rapid emergency of drug-resistance by selection for viruses with point mutations in the RT encoding gene\(^2\). The discovery in 1996 that HIV-1 replication can be durably inhibited by a combination of minimal 3 anti-retroviral drugs has led to a significant decrease in HIV-related mortality. The triple therapy or combined anti-retroviral therapy (cART) usually consists of two RT inhibitors and one PR inhibitor. In theory many steps of the viral replication cycle can be inhibited and several drug classes have been developed; fusion and entry inhibitors prevent viral infection of the target
cell, RT inhibitors prevent the formation of viral DNA, IN inhibitors prevent integration of the viral DNA, PR inhibitors prevent maturation of the newly formed virus particles. Triple therapy is very efficient in suppressing HIV-1 replication to levels below the detection limit, but will not cure the infected individual and medication has to be administered on a daily basis for a lifetime.

**HIV-1 evolution.** Because the viral RT enzyme lacks a proofreading mechanism the process of reverse transcription has a high error rate of about one mutation per round of viral genome amplification\(^{24}\). In combination with the estimated production of \(10^{10}\) viral particles per day in infected individuals and the high rate of genomic recombination, HIV-1 has a high overall mutation rate\(^{25,26}\). Due to this high mutation rate many closely related, yet genetically distinct virus variants will emerge over time in an infected individual. This pool of closely related viruses is termed the viral quasispecies. The introduced mutations will be random in nature and can either be neutral or affect the viral replication capacity in a positive or negative manner. It is also possible that mutations are introduced that will cause the formation of a defective provirus: a dead end product. The high diversity also enables HIV-1 to escape from the pressure imposed by the patient’s immune system and to become resistant to anti-retroviral drugs.

**HIV-1 LATENCY**

**Proviral latency is the primary obstacle to a cure for HIV-1.** Combined anti-retroviral therapy (cART) is very successful in suppressing the plasma viral RNA load below the limit of detection (50 copies HIV RNA / ml plasma), providing lifelong protection in drug-adherent patients. Unfortunately, even after many years of continuous treatment, the virus persists and the plasma viral load will rebound rapidly when cART is interrupted\(^{27-29}\). The reason for this rapid virus repopulation is the presence of a long-lived reservoir of latent HIV-1 proviruses in resting memory T lymphocytes that was first described in 1995\(^{30}\). Viral latency is defined as a silent or non-productive state of infection that can be reversed. Such latent viruses are not sensitive to cART and will persist in infected individuals. Consequently, when cART is interrupted, these viruses can re-establish systemic infection. Cells carrying a latent provirus are difficult to detect in the infected individual because they differ only from uninfected cells in having a transcriptionally inactive HIV-1 genome. Attempts to eliminate these proviruses have thus far not been successful\(^{31-35}\). The long-lived latent reservoir is therefore considered a major obstacle towards a cure for HIV-1.

**Cellular reservoirs for HIV-1.** The cART drugs prevent new rounds of viral replication and as the half-life of a viral particle is estimated to be only 6 hours, the reduction of HIV-1 RNA levels in plasma is determined by the half-life of virus-producing cells\(^{25}\). Therefore, effective cART allowed measurement of decay rates of different populations of virus producing cells that were infected prior to the start of therapy.
(Fig. 4A). A very fast initial decline in viral load reflects the loss of virions produced by activated CD4$^+$ T lymphocytes that have a half-life of approximately 1 to 2 days when infected (Fig. 4B)$^{36,37}$. The second phase decline reflects virus production by infected cells with half-lives of several weeks to months that steadily produce small amounts of virus$^{38,39}$. It is not yet fully clear which cell types belong to this population, but macrophages are most likely part of it (Fig. 4C)$^{33}$. Based on these two phases of viral decay it was predicted that cART could cure HIV-1 infected individuals within 2 to 3 years$^{25}$. Unfortunately this proved not to be the case, as there is a third phase that shows no further (or hardly detectable) viral decay, reflecting virus production by long-lived memory T lymphocytes (Fig. 4D). Because of their long half-life and their ability for self-renewal this population allows for HIV-1 persistence. Other cell types have been suggested to contribute to the viral reservoir as well, including dendritic cells$^{40}$, monocytes$^{41,42}$, astrocytes$^{43}$ and different subsets of hematopoietic stem cells$^{44}$.

**Establishment of the long-lived HIV-1 reservoir.** Latently infected resting T cells are present in all infected patients$^{45,46}$. Despite ongoing therapy, the frequency of these HIV-1 infected memory cells remains fairly constant over time$^{47,48}$. This is probably due to the homeostatic proliferation of the resting T lymphocyte$^{49,50}$. The latent reservoir is established early after infection of the individual, but exactly how this reservoir in resting T lymphocytes is established is not known$^{51}$. Two possible scenarios have been proposed: direct infection of resting memory T lymphocytes or infection of activated T lymphocytes that subsequently return to the resting state.

Direct infection of memory T cells can occur, but this is not efficient since reverse transcription and integration processes often remain incomplete$^{52,53}$. Even if reverse transcription is completed, the linear non-integrated dsDNA is rapidly degraded with a half-life of approximately 1 day$^{53}$. Immediate cellular activation can rescue viral integration, but subsequent replication is not always efficient$^{54,55}$. Cytokine stimulation of quiescent T lymphocytes can increase HIV-1 infection efficiency by boosting the reverse transcription and integration processes without inducing cell proliferation or up-regulation of cellular activation markers$^{56-58}$. *De novo* infection of resting T lymphocytes is not likely to play a major role in the formation of the long-lived reservoir, but it does represent a very stable component of the reservoir. The resting cells do not support virus production and the proviruses remain in a latent state. This can be alleviated via cellular activation, resulting in the production of new viral particles$^{59}$. The alternative, more indirect route towards the long-lived reservoir is via infection of activated T lymphocytes. However, due to their short half-life, productively infected T cells can hardly revert back to the resting state$^{37,60}$. Therefore, it seems more likely that the proliferating T lymphocyte returns to the resting state after infection, but before the production of new virus is initiated.
Regulation of HIV-1 gene expression

HIV-1 transcriptional latency. Once the viral DNA is integrated into the host cell genome, regulation of HIV-1 gene expression depends on the cellular transcription machinery. Transcription initiation is controlled by the viral promoter that is located in the U3 region of the Long Terminal Repeat (LTR). The promoter encodes numerous transcription factor binding sites (TFBSs) that bind cellular transcription factors (TFs), which can either induce or repress transcription. The core promoter (-78 to -1, relative to the transcription start site at +1) plays a crucial role in HIV-1 transcription as deletion of the three SP1 sites or the two NF-κB binding sites severely impairs or even abolishes viral replication. A low level of basal transcription is triggered by host TFs, but high levels of HIV-1 gene expression and virus production require the viral Tat protein (Fig. 5). Tat interacts with the trans-activation-response region (TAR) element, an RNA motif located at the 5' end of every HIV transcript. Through this interaction transcription is induced by the positive transcription elongation complex-b (P-TEFb), composed of cyclin-dependent kinase 9 (CDK9) and cyclin T1. P-TEFb mediates hyperphosphorylation of the carboxy-terminal domain of RNA polymerase II, thereby strongly enhancing its elongation efficiency.

Fig. 4. Cellular reservoirs for HIV-1. A: Schematic of the decay rates of different populations of virus producing cells as reflected by the drop in viral RNA load in plasma upon initiation of cART. B: Start of cART reveals the first fast phase of decline representing the virus produced by activated CD4+ T lymphocytes, which have a half-life of 1 to 2 days. C: The second phase reflects the loss of virus production by cells with a half-life of weeks to months, which includes macrophages and possibly other cell types. D: The third phase shows no or very little viral decay, reflecting a low level of virus production from long-lived memory T lymphocytes. If cART is interrupted, the virus will rebound from this cellular reservoir and re-establish systemic infection.
If the HIV-1 promoter is not sufficiently active to support virus production, this results in transcriptional latency. Latently infected CD4+ T cells are enriched for HIV-1 Tat variants with impaired activity and cellular models for HIV-1 latency frequently carry inactivating Tat or TAR mutations. Fluctuations in Tat levels will result in stochastic gene expression. Low levels of P-TEFb kinase activity can also prevent full HIV-1 gene expression. Additionally, host TF availability can be a limiting factor in viral transcription, especially in resting T lymphocytes that lack active NF-κB and NFAT. HIV-1 gene expression can subsequently be induced by cellular stimulation with compounds that (in)directly activate these host TFs.

**Transcriptional interference.** It has been noticed that transcriptional interference mediated by flanking genes can contribute to proviral latency. HIV-1 preferably integrates in introns of actively transcribed genes. Transcription of that host gene can interfere with transcription initiation at the viral promoter when host gene transcription runs into the HIV-1 genome. This block can be alleviated by silencing of the cellular promoter, e.g. during cell division, or by massively activating HIV-1 transcription by supplementing Tat protein.

**Chromatin restrictions.** The cellular DNA genome is packaged by histone proteins in so-called nucleosomes (Fig. 6). A nucleosome consists of a histone octamer and double-stranded DNA. A DNA strand of approximately 146 bp is wrapped 1.7 times around the histone protein complex. Two nucleosomes are linked via a stretch of DNA that can be up to 80 bp long. A chain of nucleosomes is referred to as chromatin. Different signaling pathways can trigger enzymatic modification of specific amino acids in the histone tail (acetylation, methylation and phosphorylation). This may lead to a more open chromatin conformation, making the DNA more accessible, or a more closed conformation to suppress TF binding to the viral promoter. The “histone code” hypothesis states that combinations of distinct
modifications at specific sites direct which proteins can interact with histone-DNA complexes to determine the transcriptional activity\(^75\).

HIV-1 transcription can be restricted by a nucleosome that is positioned close to the transcription start site (referred to as Nuc-1, +10 to +155)\(^76\). Transcriptional activation requires remodeling of Nuc-1 and increased accessibility of chromatin has been associated with transcriptional activation in models of chronic HIV infection (reviewed in Demonte \textit{et al.})\(^77\). Methylation of histone tails can either induce an open or closed chromatin conformation, depending on the amino acid residue that is modified. Histone acetylation correlates with a more open chromatin structure. Histone acetyl transferases (HATs) modify the histone tail and histone deacetylases (HDACs) remove the modification. HDACs are unable to bind to DNA directly, but can be recruited through cellular TFs that bind to the viral LTR, including YY1, NF-κB p50 and AP-4\(^78,79\). Alternatively, Tat-induced transcriptional activation recruits the transcriptional co-activator p300 and cAMP-responsive transcription factor binding protein (CBP)\(^80,81\). These large HAT proteins can induce an open chromatin conformation, thus favoring transcription. Additionally, Tat can be modified by p300/CBP to increase the transcription efficiency\(^82,83\). Consistent with a role for histone acetylation in HIV-1 latency, many drugs that inhibit HDAC activity are effective inducers of HIV-1 transcription in latently infected cells\(^84\) and some have

![Chromatin Organization](image)

**Fig. 6. Chromatin organization.** Cellular genomic DNA and the HIV-1 proviral DNA are packaged by histone proteins into nucleosomes. HIV-1 transcription can be restricted by a nucleosome (referred to as Nuc-1) that is positioned close to the transcription start site (+10 to +155). HATs can acetylate the histone tails, creating a more open chromatin conformation thereby making the HIV-1 promoter more accessible to host cell TFs. HDACs can remove the acetyl-group from the histone tails, creating a less open chromatin conformation that will prevent TF binding to the viral promoter. The figure is adapted from Verdin \textit{et al.}\(^76\).
DNA methyltransferases can maintain DNA methylation during cell division and replication of DNA and induce de novo DNA methylation. Conflicting findings have been published whether CpG methylation of the 5’ LTR acts as an additional epigenetic restriction mechanism or not. Profound de novo LTR methylation has been observed in several cellular models for HIV-1 latency that were obtained after prolonged cell culturing. It has therefore been suggested that methylation is a late event in epigenetic suppression, thus promoting the maintenance of latency.

Post-transcriptional latency mechanisms. Besides blocks at the level of transcription (initiation, elongation, Tat trans-activation and chromatin restrictions), latency can be induced by several post-transcriptional processes. The nuclear export of multiple spliced HIV-1 RNA transcripts encoding Tat, Rev and Nef can be impaired in resting T lymphocytes by the absence of the RNA-binding protein PTB. Host- or HIV-derived microRNAs can interfere with viral mRNA stability and/or translation via the RNA interference (RNAi) pathway. Alternatively, HIV-derived siRNAs can interfere with viral mRNA stability biology via RNAi mechanisms or suppress viral transcription via RNA directed transcriptional gene silencing.

OUTLINE OF THIS THESIS
In this thesis we focus on the ability of HIV-1 to establish latency in proliferating T cells and on how the silent provirus can be activated from latency. In Chapter 2 we describe the development of a TaqMan assay that quantitates viral DNA of different HIV-1 subtypes with equal efficiency. Depending on the primers used, the assay can detect different viral DNA forms, including completely reverse transcribed DNA and 2-LTR circles. Chapter 3 deals with the use of this TaqMan assay to study the influence of the LTR promoter of the different HIV-1 subtypes on proviral latency in T cell lines. We show functional differences between the HIV-1 subtypes in this respect. In Chapter 4 we describe the surprise finding that HIV-1 infection does frequently result in establishment of a latent provirus in proliferating primary T lymphocytes. Furthermore, we demonstrate that this provirus can be purged out of latency by co-culturing T lymphocytes with monocyte-derived dendritic cells (DCs). In fact, DC-mediated activation was critical in revealing this latency, as regular anti-latency drugs fail to activate HIV-1 from latency in proliferating primary cells. Chapter 5 combines the insights obtained in the two previous chapters to study the effect of the subtype-specific HIV-1 promoter on viral latency and replication in primary T lymphocytes. Additionally, subtype AE and B LTR luciferase reporter
constructs allowed us to identify an AP1 binding site in the AE promoter. C-Jun binding to the AP1 site inhibited basal LTR transcription, but in the presence of Tat, c-Jun binding enhanced transcription. In Chapter 6 we investigated whether different myeloid cell types can induce the expression of latent provirus. We observed that monocytes and plasmacytoid DCs cannot activate the latent provirus. Monocyte-derived macrophages type I and II can moderately activate the latent provirus, but not as efficiently as monocyte-derived dendritic cells. Conventional CD1c⁺ and cross-presenting CD141⁺ myeloid DCs efficiently purged the provirus out of latency. Surprisingly, poly(I:C) stimulated myeloid DCs lost almost all purging capacity. Chapter 7 describes pilot studies performed to characterize the DC-mediated activation of HIV-1 provirus from latency. The activation appears to be multi-factorial, as both cell-cell interactions and (a) DC-secreted factor(s) mediate activation of latent provirus, indicating that multiple signaling routes are involved. Chapter 8 consists of a review providing a general overview of molecular mechanisms contributing 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. The general discussion in 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 continues low-level replication.

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