HIV-1 latency in proliferating T cells
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Chapter Nine

microscope

General discussion
GENERAL DISCUSSION

The search for an HIV cure continues. HIV-1 replication can be successfully repressed by combined anti-retroviral therapy (cART) in drug-adherent patients, delaying, and sometimes preventing progression towards AIDS. If therapy is interrupted the virus will re-emerge, therefore lifelong administration of anti-retroviral drugs is required. Long-term treatment with cART can induce life-threatening toxicities including disorders of the pancreas, kidney and liver. Additionally, HIV can develop resistance against the anti-retroviral therapy, triggering re-emergence of the virus. For these reasons the search for an HIV cure continues.

When one thinks of an HIV cure, the ‘Berlin’ patient immediately comes to mind. This patient acquired acute myeloid leukemia after being infected with HIV for 10 years and receiving cART for the last 4 years. To treat the leukemia, the patient underwent two hematopoietic stem cell transplantations because the leukemia relapsed after the first transplant. After the second transplant there was no relapse of leukemia. To this day, there is also no re-emergence of HIV, even though the patient has been off anti-retroviral therapy since the transplantations. Both transplants came from the same donor, who was homozygous for the CCR5 delta 32 allele (CCR5Δ32/Δ32). This 32 base pair deletion in the CCR5 gene prevents expression of the CCR5 protein on the T cell surface. Although HIV-1 can use different co-receptors for entry and fusion with a target cell, CCR5 is predominantly used. By replacing the patient’s immune system with cells lacking CCR5 expression, the new immune system should be resistant to HIV-1 infections and able to clear any remaining virus. However, another important co-receptor for HIV-1 is CXCR4 and by replacing the immune system with CCR5-minus donor cells, one could set the stage for selection of CXCR4 using HIV-1 variants. Even though ultra-deep sequence analysis suggested the presence of a small population of such variants prior to the transplant, no X4 viruses emerged after the procedure. To investigate if persistent HIV was present in non-circulating immune cells such as tissue macrophages that can resist chemotherapy, a rectal biopsy was performed 159 days after the second transplant. The intestinal lamina propria is an important reservoir for HIV in which viral DNA can be detected even when no viral load is detectable in plasma. Nevertheless, residual virus could not be detected in the intestinal lamina propria. Interestingly, the biopsy did reveal original CCR5-expressing macrophages that were not yet replaced by the new immune system. Even though macrophages represent a long-lived reservoir for HIV-1, no viral DNA could be detected. During the time of immune reconstitution these CCR5-expressing macrophages disappeared and in samples from 24 and 29 months after transplantation the mucosal macrophages were negative for CCR5. Although it is not feasible to analyze every single cell in the patient for the presence of viral DNA, it seems that the ‘Berlin’ patient is ‘cured’ from HIV.
Bone marrow transplantations require elimination of the immune cells and replacement of the immune system with bone marrow cells from an appropriate donor. This intervention remains risky and a fair percentage of patients die as a result of the procedure. Therefore, bone marrow transplants will not be the solution to treat an HIV-1 infection unless the patient is also diagnosed with leukemia. Nonetheless, it does provide encouraging evidence that it is possible to cure HIV. The CCR5-expressing macrophages did not contribute to re-emergence of HIV-1, possibly because there were no CCR5-expressing T cells. Since these cells are present when the immune system is replaced with cells from a “normal” donor, viral eradication does not occur and the virus rebounds when anti-retroviral therapy is interrupted. Therefore, most patients receiving a bone marrow transplantation must continue cART.

A different approach towards long-term HIV suppression concerns gene therapy protocols, including the development of small interfering RNAs (siRNAs) targeting mRNAs and decreasing protein expression via RNA interference. The siRNAs can affect viral proteins as well as host cell proteins, like CCR5, that are essential for viral replication. Different successful siRNAs have been developed, but the problem remains how to deliver these small molecules to the right cells. Vectors carrying gene cassettes that encode these siRNAs could be injected in the blood, but these vectors have to target the appropriate cell type. Alternatively, T cells or hematopoietic stem cells from an HIV-infected individual could be treated ex vivo with the vector. These cells expressing siRNAs are transplanted back into the patient and the T cell progeny will be protected against HIV. Several international research projects are gearing up for clinical tests.

**Popular ‘shock and kill’ approaches.** Another scenario to cure an ongoing HIV-1 infection is eradication of the latent reservoir with a so-called ‘shock and kill’ approach. By the addition of anti-latency drug(s) to normal cART cocktails, induction of viral gene expression in the latently infected cells occurs (‘shock’). The anti-retroviral drugs will prevent new infections by the released virus and the virus-producing cells will die due to cytopathic effects or via host CD8+ T lymphocyte-induced apoptosis (‘kill’). The main challenge to this approach is to identify the right drug (or drug combination) that reaches and activates all latent proviruses. Purging the latent provirus from resting CD4+ T lymphocytes may be accomplished by cellular activation, but this is likely to be toxic as was demonstrated by global T cell activation trials with anti-CD3 antibodies and recombinant IL-2. An alternative approach uses IL-7, a cytokine essential for maintenance of T cell homeostasis, which induces HIV-1 gene expression via JAK/STAT5 signaling without T cell activation. However, IL-7 treatment may induce homeostatic proliferation of latently infected resting cells, thereby possibly expanding the latent reservoir. Anti-latency agents used to purge HIV-1 should be highly efficient in activating latent provirus without
inducing T cell activation. To accomplish this, we first need an accurate understanding of the molecular mechanisms of HIV-1 latency.

Promising anti-latency drugs are the histone deacetylase (HDAC) inhibitors. HDAC inhibitors do not induce T cell activation or the secretion of cytokines. The advantage of HDACs is that they have been extensively investigated, especially in anti-cancer therapy, and much is known about the toxic effects. At least 12 HDAC inhibitors have been tested for anti-latency properties, mostly in cell lines but some also in resting cells isolated from patients, and a few are currently being evaluated in clinical trials (reviewed by Xing and Silicano)\(^{23}\). The best results thus far have been obtained with valproic acid (VPA) and vorinostat, also known as suberoylanilide hydroxamic acid (SAHA). VPA can induce virus production from resting CD4\(^+\) T lymphocytes isolated from HIV-1 infected individuals on cART\(^{24}\). In another study, VPA administration in combination with cART induced a decline in the level of latently infected resting CD4\(^+\) T lymphocytes in three out of four patients\(^{25}\). However, other studies indicated that VPA administration has no effect on diminishing the latent reservoir\(^{26-28}\). Vorinostat can induce virus production in resting CD4\(^+\) T lymphocytes isolated from patients on cART\(^{29}\) and a single dose of vorinostat increased HIV-1 gene expression in resting T lymphocytes in HIV-1 infected patients receiving cART\(^{30}\). Clinical trials of vorinostat in patients are ongoing\(^{23}\). A major concern is that these agents do not selectively target HIV-1, but also affect many other genes that are regulated by histone modification. Additionally, HAT/HDACs are also able to modify numerous non-histone targets, such as proteins involved in cellular proliferation, migration, cell death, DNA repair, angiogenesis, inflammation, and the immune response\(^{31}\).

Although conflicting results have been published regarding the impact of HIV-1 LTR DNA methylation on viral latency, there is an interest in DNA methyltransferase (DNMT) inhibitors as anti-latency agents. DNMT 5-aza-2’deoxycytidine (5-aza or aza-Cdr) alone is a weak inducer of HIV-1 gene expression but has been described to synergize with TNF\(\alpha\) and prostratin in two different T cell line models of HIV-1 latency\(^{32-34}\). This encourages further research on DNMTs in combination with other anti-latency compounds but, as is the case for HDACs, concerns remain that DNMTs may affect the expression of many genes.

Transcriptional activity of the HIV-1 LTR promoter can be induced by protein kinase C (PKC) activators that mediate the activation of the transcription factors NF-\(\kappa\)B and AP-1\(^{35}\). The best described PKC activators are PMA and 12-deoxyphorbol-13-acetate (prostratin). PMA activates the latent provirus by cellular activation, but also induces mitogenesis, is tumor promoting and therefore not suitable for clinical use\(^{23}\). Prostratin does not promote tumor development or cell proliferation, but does induce enhanced expression of the cellular activation markers CD69, CD25 and the IL-2 receptor, while downregulating the expression of CD4, CCR5 and CXCR4\(^{36}\). Another transcription activator that does not upregulate T cell activation is
hexamethylene bisacetamide (HMBA), which induces activation of the positive transcription elongation factor-b (P-TEFb)\textsuperscript{37-40}. P-TEFb recruitment to the HIV-1 LTR is dependent on SP-1 and independent of Tat. Agents that activate NF-κB via PKC or activate pTEFb are likely to affect the transcription of many host genes (reviewed by Margolis)\textsuperscript{41}. For a more extensive overview of anti-latency compounds and the HIV-1 latency activation strategies see the reviews by Xing and Siliciano\textsuperscript{23}, Barton \textit{et al.}\textsuperscript{42} and Margolis\textsuperscript{41}.

The current purging strategies are aimed at targeting the long-lived reservoir in resting T lymphocytes via transcriptional activation of the HIV-1 LTR. However, most of these compounds, such as TNFα, prostratin, PMA, TSA and vorinostat, appear to be ineffective with respect to the latent provirus in proliferating T lymphocytes\textsuperscript{43}. This implies that alternative pathways may also contribute to viral latency and that transcriptional activation alone may not be sufficient to target all reservoirs.

**Ongoing replication and the latent reservoir.** In nearly all patients on cART small amounts of free virus can be detected with sensitive single-copy assays for HIV RNA in plasma\textsuperscript{44-46}. There is an ongoing debate whether this residual virus represents ongoing viral replication or virus production from cellular reservoirs that were infected prior to the onset of therapy.

An argument in favor of virus release from the latent reservoir is that drug adherent patients with plasma viral loads below the detection limit do not develop resistance to anti-retroviral drugs. Phylogenetic studies failed to show evolution of HIV-1 sequences, even during transient viral load ‘blips’ and rebounding virus when interrupting cART is similar to the virus present during acute infection\textsuperscript{47-50}.

Another argument supporting virus release from the latent reservoir is that treatment intensification does not result in a further decrease of residual virus in plasma\textsuperscript{51-54}. If residual virus is the product of ongoing viral replication one would expect the therapy to be suboptimal and therefore treatment intensification should halt low-level replication. On the other hand, if low-level replication occurs it will most likely be in tissues with suboptimal drug penetration where treatment intensification may also not work\textsuperscript{55}. Buzón and colleagues reported that treatment intensification with the integrase inhibitor Raltegravir increased unintegrated (episomal) HIV-1 DNA\textsuperscript{56}. This increase in episomal DNA indicates that new infections do indeed occur, yet are blocked at the integration step. Others have reported that such treatment intensification with Raltegravir does not result in an increase in episomal DNA\textsuperscript{57}.

Distinguishing low-level replication from virus production by the latent reservoir is not easy since virus release from the latent reservoir may also result in low-level replication in cART suboptimal environments. The main reason to continue this
discussion is that, if there is ongoing replication, further research on antiviral therapy is warranted to eliminate this. Based on our understanding of the latent reservoir dynamics, combined with the results from clinical trials with anti-latency treatment, it seems safe to conclude that the latent reservoir can be responsible for the low amounts of virus that is detectable under intensive therapy.

Another interesting question is what the impact of low-level virus replication is on the latent reservoir. Can low-level replication result in the establishment of new latent proviruses, thereby expanding the size of the reservoir? This is also important in light of the popular ‘shock and kill’ approaches. As we demonstrated in chapter 4, the anti-latency drugs used in these purging attempts have no effect on the latent provirus in proliferating T lymphocytes. Purging the latent provirus from resting T lymphocytes will release a wave of infectious particles and uninfected cells are protected by cART. However, at sites where cART levels remain suboptimal, this could result in infection of proliferating T cells, which can create latent provirus in resting cells on top of the just long-lived reservoir! Therefore, it is important to understand how exactly the long-lived reservoir is established and maintained, and perhaps an additional drug should be added to the purging compounds that specifically targets latent provirus in proliferating T cells.

**Prevention of the latent reservoir.** A different strategy to eradicate HIV from the infected individual is to prevent the establishment of the latent reservoir. As the reservoir is established early during infection this will not aid the individuals that already are infected, but it may prove useful for new patients. In 2001 it was already reported that an early start of anti-retroviral therapy during primary infection reduced progression to AIDS. In 2005 the first indications were reported that early onset of therapy (before seroconversion) reduced the size of the latent reservoir. Although these results were confirmed in later studies, not all patients start early with therapy because of the risk of drug resistance and drug toxicity, and also increased costs. Intriguing data was presented at the XIX International AIDS Conference (AIDS 2012), showing that patients who started very early with therapy and which was maintained this for 6 years, could be taken off therapy without viral rebound (www.aids2012.org/WebContent/File/AIDS2012_Media_Release_HIV_Cure_26_July_2012_EN.pdf). Another interesting study was recently presented at the 20th Conference on Retroviruses and Opportunistic Infections (CROI 2013), showing the case report of a child treated with antiretroviral therapy from 30 hours after birth. Treatment continued for 18 months until the child’s caregiver withdrew the child from therapy. When the child returned after 6 months the viral load was undetectable (http://mobile.aidsmap.com/Case-report-of-a-functional-HIV-cure-in-a-child/page/2585854/). These results suggest that it might be possible to reach a functional cure of HIV by preventing the formation of the latent reservoir, providing optimistic prospects for future clinical and fundamental research strategies.
REFERENCES

28. Archin NM, Cheema M et al. 2010. Antiretroviral intensification and valproic acid lack sustained effect on residual HIV-1 viremia or resting CD4+ cell infection. PLoS.One. 5:e9390
46. Maldarelli F, Palmer S et al. 2007. ART suppresses plasma HIV-1 RNA to a stable set point predicted by pretherapy viremia. PLoS.Pathog. 3:e46


Gandhi RT, Zheng L et al. 2010. The effect of raltegravir intensification on low-level residual viremia in HIV-infected patients on antiretroviral therapy: a randomized controlled trial. PLoS.Med. 7:


