

The latent HIV-1 reservoir in patients undergoing HAART: an archive of pre-HAART drug resistance

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Recent studies on patients with a history of pre-HAART drug resistance, but currently on a successful regimen, provided new insights into the dynamics of the latent cellular viral reservoir. Results indicated that the latent reservoir is an archive, composed of a mixture of wild-type and drug-resistant strains. The studies showed that, even after years of successful HAART, the wild-type viral strains that circulated before the initiation of the therapy as well as all the different drug-resistant viral strains that evolved over time during eventual periods of non-suppressive treatment, remain detectable in the proviral reservoir. These findings support the hypothesis that during active viral replication, new variants, including drug-resistant ones, continuously enter the latent viral reservoir. It can be concluded that, as a consequence of the lifelong conservation of this latent reservoir, the potency of drugs for which resistance once developed will remain reduced, even after years of withdrawal of the drug.

Keywords: reservoirs of resistance, mechanisms of resistance, mutations

Introduction

Since the availability of sensitive assays for viral load quantification in plasma, it has become clear that HIV-1 infection is characterized by a continuous massive virus replication, even during the asymptomatic phase of the disease. Although the immune system exerts some control, it generally fails to completely arrest the replication or reduce it to a form of true latency as is seen for other viral infections. The process of continuous virus replication can be interrupted or at least significantly reduced by HAART. But, although the introduction of HAART results in undetectable levels of plasma virus in the majority of patients, HAART fails to completely eradicate the virus *in vivo*, even after years of uninterrupted therapy. Viral persistence is thought to be the result of the long-term survival of a pool of infected, resting CD4 cells. Recent studies in patients on successful HAART but with a history of pre-HAART drug resistance, provided new evidence for the dynamic nature of the latent reservoir and showed that any viral variant, including any drug-resistant variant that has been allowed to replicate for a certain time during the infection, will enter the reservoir and remain conserved.

Long-term latent reservoir

Finzi *et al.*¹ showed the establishment of a latently infected CD4 cell compartment already very early in infection. The cellular reservoir is found predominantly in resting DR⁻CD4 cells with a memory phenotype.^{2,3} The half-life of these cells is long (44

months) and this long lifespan, combined with the possibility of self-renewal by proliferation, ensures their lifelong presence. How these cells originate is still a matter of controversy, but it has been postulated that the reservoir of latently infected cells is generated when lymphoblasts that are in the process of reverting to a resting state, become infected.^{4,5} Whether the long half-life of the infected cells is the only reason for the persistence of these cells during HAART treatment or whether the latent pool is fully or partly maintained by ongoing low-level viral replication despite treatment, has long been unclear. But the lack of detectable evolution in the envelope and polymerase sequences of viral strains in the cellular reservoir during HAART, argues against entry of new genotypes in the latent pool during successful treatment, and supports the belief that persistence depends primarily on the intrinsic stability of the infected cells.⁶

The HIV reservoir during HAART treatment

In patients on successful HAART, the presence of resting CD4 cells harbouring replication-competent virus has been demonstrated by several groups.^{1,7–9} In line with these observations is the consistent clinical finding of a quick rebound of plasma virus in all patients who stop treatment, indicating the presence of a latent reservoir that enables quick reinitiation of replication whenever the drug pressure is removed.^{10,11}

But perhaps the most convincing argument for long-term conservation of viral strains comes from the observation that a cessation of treatment or a switch of antiretroviral drugs in patients treated for more than 2 years with suboptimal drug regimens,

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resulted in the replacement of the resistant virus in the plasma by wild-type variants.^{12–14} In the majority of the patients studied, the replacement of the mutant by the wild-type virus was abrupt and fast, indicating that it was the result of the reappearance of archived wild-type virus and not of the reversal of mutations in the resistant variants. This finding was remarkable since drug-resistant virus predominated in the plasma of these patients for several years and at least an important reduction in the population of wild-type virus through a natural process of cell death could be presumed. If wild-type virus persists in the latent reservoir for such a long time, then it could be postulated that drug-resistant strains too will be conserved.

Ruff *et al.*¹⁵ were the first to demonstrate that drug-resistant viruses, selected by non-suppressive regimens in infected children, entered the reservoir and persisted during HAART. Subsequently, Lambotte *et al.*¹⁶ compared a polymorphic region of the *env* gene and part of the reverse transcriptase gene in pre-HAART plasma and in the reservoir lymphocytes, in nine treated patients with long-term undetectable plasma viral load. They observed archiving of pre-HAART plasma clones in six patients and confirmed the co-existence of wild-type and drug-resistant virus in reservoir T cells in two. We studied the variability of the RT and protease gene in the provirus of 11 patients on successful HAART for years, but with a period of suboptimal regimens before.¹⁷ Not only could we confirm the co-existence of wild-type and drug-resistant virus in the proviral reservoir after 5 years or more of HAART, but we were also able to show that, with a few exceptions, all mutant virus variants that were detected in the plasma before HAART initiation, during the process of gradually building up resistance, were still present in the latent reservoir. Moreover, we observed a correlation between the time period on suboptimal therapy before HAART and the proportion of mutant, drug-resistant, proviral sequences in the cells, indicating that the quantity that a certain variant occupies within the reservoir depends in part on the period that this variant has been able to replicate. These data prove that the reservoir of latently HIV-1-infected cells is dynamic, and that newly infected cells continuously turn into latency to enter the reservoir. These data also indicate the extreme long-term conservation of all variants that have ever entered the reservoir. The latent reservoir can be considered as the life-long archive of whatever viral strain that ever evolved and replicated.

Clinical implications of the persistence of drug resistance

Once it has arisen, long-term persistence of any drug-resistant virus jeopardizes, in a permanent manner, the use of drugs to which resistance has developed. This finding again emphasizes the importance of considering the whole treatment history of a patient whenever a new combination therapy is initiated. Recycling of any drug that was part of a non-suppressive treatment regimen, even if the drug was taken years ago, might result in the reactivation of archived resistant strains and must be avoided unless there are no valid alternatives.

However, the observation that the viral reservoir contains a heterogeneous mix of wild-type variants and mutant variants with different degrees of drug resistance, also indicates that even drugs to which resistance has developed may still ‘add’ to the activity of a combination therapy by preventing the replication

of the drug-sensitive virus pool in the reservoir. Since the wild-type virus is believed to be the fittest variant, suppressing the replication of these wild-type strains can be important, especially in patients with limited treatment options, and might contribute to the reduced viral-load set-point as is observed in many treated patients with drug-resistant viraemia.^{18,19} On the other hand, continuation of a failing regimen risks the further accumulation of drug-resistance mutations and an expansion of the reservoir of cells infected with drug-resistant variants, and is not advisable. Whether the proportion of cells infected with drug-resistant strains in the latent reservoir has any impact on the success or failure of subsequent salvage regimens still remains to be examined.

Currently, plasma is the only compartment used routinely for drug resistance testing. However, the observation that the proviral compartment contains an archive of the different strains, wild-type and drug-resistant, that have evolved during the infection, makes this proviral reservoir the ideal substrate for analysis of the ‘resistance-potential’ in a patient. This can be of special importance in those patients from whom no samples have been conserved and no historical data are available.

Conclusion

An important number of data indicate that the long-lived cellular reservoirs of HIV in patients reflect a heterogeneous population of replication-competent viral strains. The diversity of the reservoir is dynamic and results from successive archiving of circulating plasma viruses during the course of HIV infection, including the drug-resistant variants. Archived variants are assumed to remain life-long, thereby precluding the successful recycling of any drug towards which resistance has arisen.

Our knowledge of the latent HIV-1 latent reservoir is rapidly increasing. Only a thorough understanding of the development and maintenance of the latent reservoir will allow the development of new therapeutic strategies, aimed at a combined effect of arresting viral replication and eliminating the latent reservoir.

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