Resistant viral variants in cellular reservoirs of human immunodeficiency virus infection

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Abstract

Since the advent of antiretroviral therapy (ART), morbidity and mortality rates in those infected with human immunodeficiency virus type 1 (HIV-1) have been significantly reduced. However, HIV-1 is known to persist in several types of cells and tissues, and will usually return to pretreatment levels when therapy is stopped, even in those individuals who have been on suppressive ART for a long time. The discovery of drug sanctuaries and viral reservoirs in the body, in which HIV may persist, has helped to explain why therapeutic eradication of HIV-1 has proved so difficult. Several studies have indicated that the latent reservoir is an archive, composed of a mixture of wild-type and drug-resistant strains. Archived variants are assumed to remain life-long, thereby precluding the successful recycling of any drug towards which resistance has arisen. Several studies have underlined the value of pro-viral DNA as an additional source of information on the total burden of resistance in an individual. The HIV mutation patterns detected in plasma do not necessarily reflect those found in the cell-associated compartment, and may not be the same as those in different anatomical compartments. Although assessment of drug resistance in plasma is of direct and immediate importance for treatment, examination of the genotypic pattern of HIV-1 in cellular compartments might also provide information allowing a more sustainable response to therapy and better disease management.

Keywords: Cellular reservoirs, drug-resistance, HIV, HIV-DNA, review

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Introduction

Although current antiretroviral therapy (ART) is effective and life-prolonging, it is not curative and does not eradicate human immunodeficiency virus type 1 (HIV-1) infection. It has been proposed that a major obstacle in HIV-1 eradication is the ability of the virus to establish a latent infection within a subpopulation of susceptible cells [1,2]. HIV-1 infection of activated CD4⁺ T-cells leads to the production of virus particles and eventually to cell death from the cytopathic effects of the virus; however, in certain cell populations, viral replication stops, and such cells represent a barrier to virus eradication, because of their long half-lives and because specifically targeting and purging such reservoirs is inherently difficult.

The pool of latently infected CD4⁺ T-cells is established early in the course of the disease, during primary infection [2–4]. There are two populations of resting CD4⁺ T-cells—the naive and the memory CD4⁺ T-cells. The exact contribution of naïve CD4⁺ T-cells to the dissemination of the virus and the establishment of latency is not clear. The relatively short lifespan of these cells is not consistent with the role of cellular reservoirs of HIV-1 pro-viral DNA. However, given that the majority of T-cells in healthy individuals are naïve ones, the fact that they can be infected with HIV-1 is of crucial importance, despite their short half-lives. Nevertheless, memory T-cells make an ideal viral reservoir, because of their long lifespans. In addition, they are quiescent cells, and their transcriptional machinery is set in a mode that greatly favours viral latency with minimal ability to support basal viral transcription. The infected memory cells can persist for decades before they receive a stimulating signal that activates the cells, concomitantly inducing virus production [4].

There is no doubt about the presence of latent pro-viral HIV-1 DNA in the memory CD4⁺ T-cell populations, and many authors support the idea that this may be the only physiologically important, long-term cellular reservoir for HIV-1, because of the long lifespan of these cells. However, other investigators have shown that latent HIV-1 pro-viral
DNA within resting CD4⁺ T-cells is not the only, and potentially not the major, source of a rebounding virus after treatment ceases. This was discovered when HIV-1 from resting CD4⁺ T-cells was compared with the virus that emerged immediately after therapy ceased, suggesting the presence of viral reservoirs in cells other than memory CD4⁺ T-cells [5]. HIV DNA has also been found to persist in cells of the monocyte–macrophage lineage, including blood-circulating monocytes [6–8]. Monocytes are known to be released into the blood from the bone marrow and to circulate in peripheral blood for 1–5 days before differentiating into immature dendritic cells and other tissue macrophages. Depending on the tissue type, the lifespan of these cells can range from a few days to several months. Cells of the monocyte–macrophage lineage are particularly important in HIV-1 persistence, because of their ability to cross the blood–brain barrier and spread HIV-1 infection in the central nervous system (CNS).

In addition to blood cells, some anatomical sites may act as reservoirs for HIV-1 replication, including the lymph nodes, brain, genital tract, semen and lungs [9].

**FIG. 1.** Main cellular reservoirs of human immunodeficiency virus type 1 (HIV-1) drug-resistant variants in the blood compartment. Following HIV-1 binding to different blood cells, the viral genome is reverse transcribed into DNA. Infection of activated CD4⁺ T-cells leads to pronounced production of virions, and eventually to cell death, because of either the cytopathic effect (CPE) of the virus or cytotoxic T lymphocytes (CTL) activity. Two populations of resting CD4⁺ T-cells exist, the naive and the memory cells. It has been hypothesized that the memory CD4⁺ T-cells, which carry the pro-virus, are infected while they are in the process of reverting to the resting state. On activation, these cells are able to release the drug-resistant archived variants. The contribution of naive cells to the dissemination of the virus is less clear. In monocytes, HIV-1 replicates at low levels, and infection has no impact on monocyte viability.
Several authors have demonstrated the presence of cellular reservoirs harbouring replication-competent viruses in patients successfully treated with ART [1,10]. The consistent clinical evidence for a quick rebound in viraemia after therapy is interrupted is in line with these findings [11]. Furthermore, it is known that, in patients failing ART, stopping treatment or switching antiretroviral drugs results in the resistant virus in plasma being replaced by wild-type variants. In most of these individuals, the replacement of the mutant virus by wild types is rapid, suggesting that it is the result of the reappearance of the archived wild-type virus [11,12].

If the wild-type virus persists in the latent reservoirs for a long time, then it could be postulated that the drug-resistant viral variant will also be conserved (Fig. 1). Several studies of patients in whom ART has been successful but who have a history of drug resistance have provided evidence of 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 length of time during infection will enter the reservoir and remain conserved [13–18]. Once this has occurred, the long-term persistence of any drug-resistant virus jeopardizes, in a stable manner, the use of those drugs to which resistance has been developed. The observation that the pro-viral compartment contains an archive of heterogeneously mixed wild-type and drug-resistant variants makes this reservoir an ideal substrate for analysis of the ‘resistance potential’ in a patient.

HIV-1 drug-resistant mutations are actually detected by analysing plasma viral RNA. However, it is possible that the HIV-1 pro-viral DNA assay could be used as an alternative marker, especially when switching therapy in patients with undetectable viraemia.

**HIV-1 Drug-Resistant Variants in Blood Compartments**

In light of the above, there is an increasing body of literature on the possible utility of assessing drug-resistant mutations in the pro-virus. Several studies in untreated or failing patients have emphasized the value of pro-viral DNA as an additional source of information on the total burden of resistance in an individual [19–24]. Using direct sequencing, Bon et al. [21] assessed the prevalence of mutations associated with drug resistance in cell-free and cell-associated viruses derived from ART-naïve patients. They reported that the key mutations conferring resistance to reverse transcriptase inhibitors were detected more frequently in peripheral blood mononuclear cells (PBMCs) than in plasma viral RNA, and that major mutations in the protease region were detectable only in the cell-associated virus. In confirmation of this, Kabamba-Mukadi et al. [24] reported that the proportion of mutations in pro-viral DNA in therapy-naïve patients was significantly higher than that detected with standard RNA genotyping, and that these mutations persisted for at least 1 year, irrespective of drug therapy. Against this, other authors have shown that there is tight concordance of resistance profiles in paired HIV-1 RNA and PBMC HIV-1 DNA in therapy-naïve patients [25,26].

Several other studies have addressed the level of agreement between mutations associated with drug resistance in both plasma and PBMCs; however, most of these studies were performed on samples derived from patients failing ART who had a history of drug resistance [20,27–29]. These studies clearly demonstrated that such drug-resistant variants can easily be found archived in PBMCs, and that the PBMC compartment does not necessarily reflect the plasma compartment. This could, indirectly, imply that PBMCs may constitute a reservoir for drug-resistant variants and might replenish plasma with drug-resistant HIV-1 variants in certain circumstances. The persistence of drug-resistant variants in the reservoir may be particularly important when the circulating virus does not reflect the archived population and when patients undergo more than one salvage treatment.

The importance of performing resistance genotyping on pro-viral DNA has also been emphasized by Delaugerre et al. [30]. HIV-1 RNA and HIV-1 DNA samples obtained perinatally from a newborn and its mother were analysed, and resistance mutations were detected early in infant lymphocytes. Clonal and longitudinal analyses showed that the primary acquisition of resistant virus was associated with long-term persistence in the infant’s cellular reservoir. This article strongly supports the use of genotype assays, not only in plasma but also in the newborn’s lymphocytes, because this would help to show whether resistance was likely to persist, which has major implications for long-term treatment.

It is recognized that HIV-1 replication in the presence of drugs is related to viral evolution and selection of resistant HIV-1 mutants. However, it is less clear whether resistance to ART can appear after a reasonable degree of viral suppression has been achieved. Some authors have reported that when the viraemia is suppressed (to below 50 copies/mL), resistance does not occur [31], whereas others have shown that resistance-related mutations can indeed emerge under such conditions [32]. Diaz et al. [33] confirmed that, even with minimal viral replication, ART resistance mutations can be selected and archived in pro-viral DNA.

Although it is plausible that, in the early stage of virological failure, detection of emerging resistance mutations is more sensitive in viral RNA than in pro-viral DNA, the latter
can be of use in detecting emerging resistance when the viral load remains at undetectable levels or at levels that preclude the recovery of HIV-1 RNA though genotyping. Palmisano et al. [34] both supported and broadened this hypothesis; they observed that, in a population of HIV-1-positive patients who were fully responsive to ART, an association existed between the presence of mutations in pro-viral DNA and the occurrence of virological failure over the subsequent 2-year period.

The authors of most studies aimed at analysing the archived drug-resistant viral variants using PBMCs, and few data are available on the resistance pattern of viral populations within individual blood cell types. Some authors compared the drug resistance pattern of HIV-1 from CD4+ T-cells with viruses harboured in blood monocytes [35,36]. Potter et al. [35] reported that, in some individuals, distinct viral populations containing specific drug-resistant mutations were present in different blood compartments. In agreement with these findings, it has been reported recently that, in patients failing ART, there is a different distribution of drug-resistant mutations in CD4+ T-cells, plasma and monocytes [36]. A number of factors may influence the compartmentalization of viral variants in different blood cell types during ART. The penetration of antiretroviral drugs into different cell types may be variable, and could alter the selective pressure responsible for the emergence of drug resistance. It should also be taken into account that some physiological factors, such as the expression of some ATP-binding cassette proteins, which can be expressed differently by the various cell types [37], might affect the uptake of drugs, thus determining what mutations and variants emerge. It is also well known that the immune system can exert a strong selective pressure on viruses, leading to the emergence of viral variants [38,39].

Although recent studies have indicated that HIV-1 replication occurs in blood monocytes in vivo [40,41], it remains unclear to what extent HIV-1 can infect and replicate in blood monocytes. Blood monocytes are able to migrate to all tissues, such as the lungs, gastrointestinal tract, kidneys, urogenital tract, primary and secondary lymphoid organs and CNS. Any viral variants harboured in monocytes could be carried into these tissues, where they could replicate, thus contributing, together with the resistant viruses harboured in CD4+ T-cells, to the persistence of the drug-resistant virus.

**HIV-1 Drug-Resistant Variants at other Anatomical Sites**

Although HIV-1 infection leads to systemic disease, several lines of evidence suggest that HIV-1 compartmentalization occurs both at the cellular level [1,9] and at anatomical sites, such as the CNS, male genital tract (MGT), kidneys and lungs [42–44]. It is recognized that these compartments act as reservoirs for HIV-1 replication and that the virus may replicate locally after suppression of blood viraemia.

The anatomical barrier may restrict the penetration of some antiretroviral drugs at some anatomical sites, thus facilitating the evolution of drug resistance [45,46]. This diminished penetration of antiviral medication could result in lower effective drug levels and in retention of replicating strains with a wild-type genotype.

A comparison of the genetic diversity and mutational resistance pattern of the plasma virus and a virus from the anatomical reservoir revealed that specific resistance mutations may vary between viruses from different body compartments. In a recent study by Canestri et al. [47], it was demonstrated that, despite successful suppression of plasma viraemia with ART, HIV-1 may replicate in the cerebrospinal fluid (CSF), where development of HIV-1 resistance results in acute or subacute neurological effects. These data confirm earlier findings that the development of drug resistance may vary between the two compartments, and that CSF can act as a viral reservoir for resistance mutations [45–48]. Furthermore, it should be noted that treatment-resistant viral strains could be exchanged between the two compartments, and this could result in treatment failure if a drug-resistant strain enters the blood from the CSF.

There are interesting data on drug-resistant strains and anatomical reservoirs from those studies in which the MGT was analysed. Several comparative studies in blood and semen have indicated that the MGT may constitute a distinct compartment in some patients, based on the isolation of phylogenetically distinct viral quasi-species from semen and blood [49]. Although ART appears to reduce viral shedding in semen [50], the rate and pattern of emergence of resistance may differ between the blood compartment and the MGT [51]. Indeed, it has been reported that, in HIV-1 infection, the MGT can act either: (i) as a viral compartment with restricted gene flow and a slower molecular clock [52]; or (ii) as a viral reservoir with viral persistence [49]; or (iii) as a drug sanctuary with variable antiretroviral penetration [53]. These characteristics allow for the possible development of drug resistance and for its long-lived persistence [54]. Smith et al. [55] demonstrated that drug-resistant HIV-1 persists in higher proportions in the MGT for longer than it does in blood. This greater persistence offers a prolonged opportunity for transmission of the drug-resistant virus.

Within the various body compartments, considerable attention has been dedicated to the rectal mucosa. The surface of the rectal mucosa represents a major route of HIV-1
infection in both homosexual men and heterosexual partners; hence, the virological aspects of this site have important implications for virus transmission. Moreover, the intestinal mucosa is an anatomical reservoir for HIV-1 [56]. Detectable levels of pro-viral DNA and HIV-1 RNA have been reported in rectal biopsy specimens of HIV-1-infected patients, both naïve and during ART. Furthermore, on comparison of gut-derived isolates with those from the blood, a discordant phenotype has been reported [57]. It has been reported that different levels of drug resistance can be detected when HIV-1 from rectal biopsy specimens is compared with the blood-derived virus.

The presence of drug-resistant viruses with different resistance profiles in body compartments other than blood might have important therapeutic consequences. It remains to be determined to what extent these anatomical sites contribute to the emergence of drug-resistant variants.

Conclusions

Current guidelines recommend the use of antiretroviral resistance testing of the plasma of drug-naïve patients and of those failing ART. However, several studies have clearly demonstrated that drug-resistant variants can easily be found archived in PBMCs, and that the PBMC compartment does not necessarily reflect the plasma compartment.

In fact, drug-resistant mutations persist at detectable levels for longer in PBMC DNA than in plasma RNA, because of the different rates of virus turnover in these two compartments. Indeed, discrepancies between drug-resistant mutations in the virus populations harboured in plasma RNA and in PBMC DNA have been reported in patients failing therapy or after treatment interruption, as well as in naïve patients.

From this point of view, routine genotypic plasma analyses underestimate the presence of resistance relative to what may be revealed by examination of the virus populations archived in PBMCs. These observations make the pro-viral reservoir the ideal substrate for analysis of the total burden of resistance in an individual. Obviously, it must be emphasized that PBMC DNA sequencing cannot be proposed as a substitute for plasma RNA sequencing.

Several studies suggest that HIV-1 compartmentalization may occur both at the cellular level and in anatomical sites, such as the CNS, genital tract, kidneys and lungs. The anatomical barrier may restrict the penetration of antiretroviral drugs at some anatomical sites, thus facilitating the evolution of drug resistance. Comparison of the genetic diversity and mutational resistance pattern of the virus in plasma and in the anatomical reservoir reveals that specific drug-resistant mutations of the virus may vary between different body compartments.

The different resistance profiles of the virus in different body compartments may have important therapeutic consequences. However, it remains to be determined whether such differences can contribute to the emergence of drug-resistant variants during treatment failure.

Although drug-resistant variants may be harboured in different anatomical sites, it is our firm opinion that PBMC DNA analysis, in conjunction with the currently recommended plasma RNA analysis, has the potential to increase the sensitivity of the detection of drug resistance in drug-naïve patients and in those with a history of ART failure. Further studies of large HIV-1 populations are warranted to define the role of DNA genotyping in both clinical and research settings.

In conclusion, plasma remains the compartment of choice for testing of drug resistance. However:

- The concomitant assessment of drug resistance mutations in both plasma and blood cell compartments might improve the choice of a new therapeutic regimen.
- In patients responding to ART, in whom plasma HIV-1 RNA cannot be sequenced by commercially available assays, pro-viral DNA may represent an alternative means of investigating the genetic evolution of the predominant viral species in an individual. Prospective studies should be conducted to verify the predictive capacity of the HIV-1 DNA genotype and its value in clinical practice.

Transparency Declaration

The authors declare no conflicts of interest.

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