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Meaning of DNA detection during the follow-up of HIV-1 infected patients: a brief review

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SUMMARY

A growing body of evidence indicates that proviral DNA load quantitation is an important parameter in establishing the dynamics of HIV infection. Proviral DNA load can be determined during the follow-up of infected individuals to evaluate reservoir status in addition to viral replication. Hence, the study of viral reservoirs, represented by HIV-1 latently infected cells, including resting memory CD4+ T cells, monocytes and macrophages, by which HIV-1 can be reactivated, opens new perspectives in the assessment and the comprehension of HIV-1 infection. However, the identification of viral reservoirs, that can store both wild and drug resistance viruses, is one of the most important steps in developing treatment strategies because it is now clear that viral reservoirs not only prevent sterilizing immunity but also represent a major obstacle to curing the infection with the potent antiretroviral drugs currently in use.

Even if only careful evaluation of virological and immunological markers is necessary to fully characterize the course of HIV-1 infection and to provide a more complete laboratory-based assessment of disease progression, the availability of a new standardized assay such as DNA proviral load will be important to assess the true extent of virological suppression in treated patients and to verify the efficacy of new immune-based therapies aimed at purging HIV-1 DNA reservoirs.

Several studies demonstrate, in fact, that HIV-1 cellular DNA load may be an indicator of spread of infection whereas the plasma RNA load is indicates active infection.

This article will review the importance of monitoring HIV-1 proviral load DNA during the follow-up of HIV-1 infected subjects, suggesting that additional information complementing HIV RNA load could provide crucial information to monitor viral replication and the effectiveness of HAART therapy.

HEY WORDS: HIV-1, DNA proviral load, PCR, viral reservoirs

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The importance of DNA during the follow-up of HIV-1 infected subjects

Recent research has focused on the importance of HIV-1 DNA quantification as a useful marker in long-term longitudinal studies of patients under therapy. Even though HIV-1 RNA plasma viral load is a pivotal parameter to monitor viral replication, a growing number of observations showed that the measurement of HIV-DNA proviral load could provide crucial information on the reservoir and dynamics of HIV infection.

It is well known that highly active antiretroviral therapy (HAART) can control viral replication and decrease plasma HIV-1 to undetectable levels, but early optimism has been dampened by evidence that latent replication-competent virus can persist in resting memory CD4+ cells despite triple drug combinations (Finzi *et al.,* 1997; Siliciano *et al.,* 2003a; Lambotte *et al.,* 2004; Garbuglia *et al.*, 2004; Haggerty *et al.,* 2006). Besides the most worrisome reservoir represented by latently infected resting memory $CD4^+$ T cells

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carrying integrated HIV-1 DNA, there are several potential cellular and anatomical reservoirs, such as central nervous system and the male urogenital tract, that may contribute to long-term persistence of HIV-1 (Krieger *et al.,* 1991; Hamed *et al.,* 1993; Liuzzi *et al.,* 1996; Gupta *et al.,* 1997; Vernazza *et al.,* 1997; Pierson *et al.,* 2000; Clements *et al.,* 2005; Harrington *et al.,* 2005). It is now clear that long lived reservoirs of HIV can persist for years and the extremely long half-life of these cells, combined with a tight control of HIV-1 expression, make this reservoir ideally suited to maintain hidden copies of the virus, which are in turn able to trigger a novel systemic infection upon discontinuation of therapy (Marcello A., 2006). However, the eradication of infection has been hampered by the presence of viral reservoirs established early in infection (Chun *et al.,* 1998; Dianzani *et al.,* 2000; Pierson *et al.,* 2000; Hermankova *et al.,* 2003; Riva *et al.,* 2003; Sharkey *et al.,* 2005, Palmisano *et al.,* 2005) and not reachable by currently used drugs.

Latent reservoirs might be defined as cell types or anatomic sites in which replication competent virus persists stably over time (Haggerty *et al.,* 2006) despite prolonged antiviral treatment. Moreover, the virus is protected from biochemical decay, the immune system and antiviral therapy and the archivial nature of a reservoir guarantees lifetime persistence of infection. Both wild type and drug resistant virus (Re *et al.,* 2003; Marcello A., 2006) can coexist in the same cell reservoir, which can replenish and revive viral infection. However proviral DNA could be an informative marker to explore viral reservoirs and assess long-term impact of treatment, offering significant information complementing the input provided by plasma viremia (Ramratnam *et al.,* 2000).

Integrated and non integrated DNAs

Following HIV-1 infection, the viral RNA genome is reverse transcribed into a collinear DNA duplex. After DNA synthesis, the linear viral DNA is incorporated into a preintegration complex that enters the host cell nucleus where unintegrated viral DNA is found in two different forms: circular and linear. Circular DNAs can contain either one (1-LTR) or two (2-LTR) long terminal repeats and are found only in the nucleus. Linear DNA represents the precursor of proviral DNA, a stable structure that serves as a template for viral transcription (Furtado *et al.,* 1999; Butler *et al.,* 2002).

This peculiar replication cycle with the concomitant presence of integrated and unintegrated DNAs, which can be extracted and evaluated by molecular biology methods (Desire *et al.,* 2001; Gibellini *et al.,* 2004; Vitone *et al.,* 2005; Re *et al.,* 2005) might account for some contrasting results, which depend on the type of DNA analyzed as well as the typology of patients enrolled in the different studies and the timing of the start of therapy. Several studies have focused on the different form of DNAs and most concur that 2-LTR circles a) are quickly degraded in HIV-1 infected cells and b) might be considered a marker for ongoing de novo infection and c) might identify HIV patients at risk for immunologic and clinical decline (Sharkey *et al.,* 2000; Panther *et al.,* 1998). To further support the idea that 2LTR circles are labile and do not persist over time, a recent study demonstrated that unintegrated 2LTR DNA was undetectable after several years of uninterrupted HAART (McDermott *et al.,* 2005) in the majority of patients. Moreover, the demonstration that episomal cDNAs acquires drug resistance mutations (Sharkey *et al.,* 2005), while proviruses remain wild type, continue to provide evidence of its instability in vivo. In particular, the dynamics of episomal cDNA turnover in vivo, studied by following the emergence of an M184V polymorphism in plasma viral RNA, episomal cDNA and proviral DNA, demonstrated that wild-type episomal cDNAs are replaced by M184V-harboring episomes during acquisition of drug resistance. Importantly, this complete replacement indicates that episomal cDNAs are turned over by degradation rather than through death or tissue redistribution of the infected cell itself. However, evolution of episomal viral cDNAs is a valid surrogate of ongoing viral replication in HIV-1-infected individuals.

Hatzakis and coworkers (2004), using a pool of unintegrated and integrated linear double-stranded HIV-1 DNA structures as a marker of cellular HIV-1 DNA load, showed that the rate of decline was clearly associated with the long-term success of therapy. Despite decreasing proviral load, replication-competent viruses can be isolated from several cell types, including resting CD4 lymphocytes, macrophages, and natural

killer cells many years after initiation of HAART (Finzi *et al.,* 1997; Wong *et al.,* 1997; Zhang *et al.,* 1999; Valentin *et al.,* 2002). These findings emphasize the virus's ability to escape and reproduce itself by new rounds of infection.

Is it possible to define a DNA threshold?

It is extremely difficult to define a DNA threshold for use as an informative marker in clinical practice. McDermott *et al.,* (1999) reported high DNA levels in sequential samples from individuals who did not respond to therapy, resulting in an elevated viremia due to de novo infection of naïve cells and an increase in the number of cells containing viral DNA. For a better knowledge of DNA levels, Kostrikis *et al.,* (2002) measured the concentration of HIV-1 DNA forms which underwent the second template switch (STS DNA) and 2-long-terminal-repeat DNA circles in peripheral blood mononuclear cell samples. Results obtained showed that among the patients who progressed to AIDS, the median levels of STS HIV-1 DNA, but not 2LTRC, were significantly higher than those of patients who remained AIDS-free for a long period of time. In agreement with these results, Pellegrin *et al.,* (2003) found that patients with proviral DNA levels ≥ 2.7 $\log_{10}$ copies /10^6 PBMCs are more likely to experience virological failure, even if individual parameters involved in the constitution of the pool of HIV-1 infected cells such as host genetic factors and individual response to treatment (Berger *et al.,* 1999; O'Brien *et al.,* 2000) should not be underestimated.

More recently (Rouzoiux *et al.,* 2005) it was demonstrated that HIV DNA level was a major predictor of progression to AIDS independently of HIV RNA level and CD4 also during the acute phase of infection. Even if DNA and RNA have different merits in clinical practice, it is plausible that the stock of HIV infected cells plays a specific role in determining risk progression even though the rate of virus replication has a direct influence on the preservation of this stock.

DNA load in patients with undetectable RNA load

It is well established that an accurate and reproducible measurement of HIV-1 RNA can help in the evaluation of new antiviral therapies (Guidelines for the Use of Antiretroviral Agents in HIV-1-Infected Adults and Adolescents, 2005). On the other hand, despite a powerful long-term inhibition of viral production, the persistence of detectable amounts of proviral DNA even after prolonged treatment suggests that HIV-1 DNA quantification could be a helpful marker in the follow-up of patients on therapy (Wong *et al.,* 1997; Brostrom *et al.,* 1999, Shiramizu *et al.,* 2005). Although the unquestionable importance of RNA quantification to better understand the meaning of proviral DNA load in patients with undetectable RNA load has received much attention (Perelson *et al.,* 1996; Re *et al.,* 2005), several researchers studied the course of DNA in patients whose plasma viremia was well suppressed by antiretroviral therapy. It clearly emerged that the inability of current drugs to suppress viral replication completely allows the replenishment of the pool of latently infected cells.

Even if some studies suggest that the latent HIV reservoir in the resting CD4 T cell compartment is virologically quiescent in the absence of activating stimuli, a strong line of evidence suggests that low levels of ongoing viral replication persist and prolong the overall half-life of HIV in patients receiving antiretroviral therapy. The concept that low levels of proviral DNA are present in the PBMC of patients with undetectable viral load supports the idea that the latent reservoir decays faster in patients who consistently maintain plasma HIV-1 RNA levels of fewer than 50 copies/ml (Vitone *et al.,* 2005) By contrast, some reports showed that a lack of response in plasma HIV-1 RNA load by antiretroviral therapy might be independent of any response in the viral DNA copies/cell (Ramratnam B *et al.,* 2000; Shiramizu *et al.,* 2005).

Even if DNA amount was not correlated to other immunovirological markers (CD4 and RNA levels), we tended to find a higher but not significant level of proviral DNA in patients with lower levels of $CD4^+$ cells and detectable RNA value (Vitone *et al.,* 2005). This result may be explained by the high rate of both defective or functional integrated/unintegrated HIV-1 proviral DNA synthesis and/or antiretroviral therapy effects in patients with a low $CD4^+$ cell count. Concerning DNA and CD4 levels, Tierney *et al.,* (2003) demonstrated that the HIV DNA level in peripheral blood mononuclear cells is not strongly associated with

the $CD4^+$ cell count, even if the relatively narrow range of the $CD4^+$ cell count used in this study made it difficult to observe any true association between $CD4^+$ T cell counts and HIV DNA levels. On the contrary, Riva *et al.,* (2003) showed a significant inverse association between the $CD4^+$ cell count and HIV DNA level. However, several papers reported that DNA levels gradually decrease in time (Wong *et al.,* 1997; Lafeuillade *et al.,* 2001, Ngo-Giang-Huong *et al.,* 2001) even when the level of viral RNA was persistently undetectable. In particular, McDermott *et al*. (2005) demonstrated that HIV-1 DNA levels in PBMCs were correlated with therapeutic efficacy, suggesting that DNA quantification might be a useful tool to monitor the DNA decay of HIV-1 reservoirs. In particular, low levels of DNA proviral load were constantly found in most individuals with low levels of viral replication whereas non responders had the opposite results. However, the strong evidence that HIV DNA levels in PBMCs correlate with the therapeutic efficacy is undeniable (Tierney *et al.,* 2003).

HIV-1 DNA persistence with time

The continuous persistence of detectable proviral DNA levels in PBMC is a major problem in the management of HIV-1 infection. One of the problems widely tackled by several researchers is how long DNA levels persist in PBMC. The establishment and the persistence of a reservoir is a consequence of T cell physiology, where the latently infected cells are protected from viral cytopathic effects and host immune mechanisms. This situation is characterized by stably integrated virus in definite cell compartments persisting over time. Since HAART is not able to eradicate the stably integrated forms of HIV-1, chronic infection creates an archive of virus ready to be reactivated (Riva *et al.,* 2001; Haggerty *et al.,* 2006).

One of the most important questions is whether early treatment can prevent the establishment of a reservoir. Currently available data (Finzi *et al.,* 1997; Chun *et al.,* 1998; Zhang *et al.,* 1999; Strain *et al.,* 2005) suggest that early treatment might limit the size of the reservoir, even if theoretically a single infected cell can rekindle infection. In this connection, Strain (2005) showed rapid rates of decay in patients treated early after seroconversion.

Moreover some studies performed on resting DR- CD4 lymphocytes from chronically infected individuals demonstrated that the half-life of these cells is extremely long (up to 44 months) (Finzi *et al.,* 1999; Siliciano *et al.,* 2003b) This long lifespan, combined with the possibility of self-renewal by proliferation, ensures their lifelong presence due not only to the slow turnover of residually infected memory lymphocytes, but also to the inability of current regimens to suppress viral replication completely, allowing replacement of latently infected cells. It has been suggested that the stability of the reservoir may be associated with the intermittent episodes of low level viremia (the so-called "blips") common in patients on HAART (Havlir *et al.,* 2001; Hermankova *et al.,* 2001; Ramratnam *et al.,* 2000; Ruff *et al.,* 2002; Garbuglia *et al.,* 2004).

This long viral persistence creates several problems when therapy is interrupted. Viral rebound is typical when HAART is stopped and the viral sequence found in the rebounding plasma virus is genetically identical to those present in the reservoir. This suggests that the rapid resurgence of plasma viremia observed after discontinuation of therapy and the viruses cocultured from PBMC are derived from a relatively stable pool of the replicating form of virus rather than from activation of a previously latent pool (Sharkey *et al.,* 2000; Imamichi *et al.,* 2001).

Apart from the patients enrolled in the severe protocols of therapy interruption, a recent study on a small cohort of patients who refused to continue any antiviral regimen showed an increase or a rebound in viral DNA in most patients despite high levels of plasma viremia. This confirms that the absence of therapy reflects an increase and/or a persistence of cells containing viral DNA, but also that the DNA rebound occurs from two to five months after therapy suspension without any significant correlation with plasma HIV-1 RNA levels (Re *et al.,* 2005).

CONCLUSION

HIV-1 infection is characterized by ongoing massive viral replication throughout the disease. This

process of continuous virus replication has been significantly reduced but not completely eradicated by therapeutic protocols. HIV persistence might result from the long-term survival of a pool of infected resting CD4 cells. Due to the extremely long half-life of latent reservoir, its eradication would require several years of treatment (Pierson *et al.,* 2000). Thus, latently infected resting $CD4^+$ T cells provide a mechanism for life-long persistence of replication-competent forms of HIV-1, rendering unlikely hopes of virus eradication with current antiretroviral regimens.

Whether the long half life of the infected cells is the only reason for the persistence of these cells during HAART or whether the latent pool is fully or partially maintained by ongoing low-level viral replication despite treatment is not yet completely understood (Noe *et al.,* 2005).

However the presence of archived viruses able to remain life-long in cells and tissues despite highly active antiretroviral therapy (HAART) explains viral rebound during therapy interruptions. This rebounding virus derives from latent reservoirs characterized by low drug penetration and the ability to maintain viral particles for a long period of time.

Since PBMCs harbour archival proviral DNA, their utility in drug resistance testing has recently received much attention (Chew *et al.,* 2005) even if it is not clear whether higher levels of plasma viremia during HAART always correlate with the appearance of drug resistance mutations in plasma and/or PBMCs. An additional advantage of comparing cell-free and cell-associated virus in drug resistance testing will be to predict the emergence of future drug resistance and subsequent treatment failure, especially when plasma viral load remains subdued or below detection in the face of HAART.

Even though plasma HIV RNA levels remain the basic parameter to monitor the intensity of viral replication, we are firmly convinced that DNA levels could represent an adjunct prognostic marker in monitoring HIV-1 infected subjects. Moreover understanding the diverse mechanisms of residual HIV-1 disease is critical for the possible development of clinical research protocols targeting long-term viral remissions and determining the long-term durability of treatment strategies.

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