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HIV persistence in the setting of antiretroviral therapy: when, where and how does HIV hide?

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Abstract

Advances in the treatment of HIV infection have dramatically reduced the death rate from AIDS and improved the quality of life of many HIV-infected individuals. However, the possible long-term toxicity associated with antiretroviral therapy (ART), stigma and cost, all contribute to the necessity of finding a cure for HIV infection. In infected individuals taking ART, HIV persists in a small number of cells that can survive for the lifetime of the infected person. These persistently infected cells, usually referred as the 'reservoirs for HIV infection', are the main barriers to a cure. The diversity of the tissues and cellular types in which HIV persists, as well as the multiplicity of the molecular mechanisms contributing to HIV persistence, complicate the efforts to develop a safe, effective, and globally accessible cure for HIV. In this review, we summarise recent data that contribute to our understanding of HIV persistence during ART by addressing three questions pertaining to the HIV reservoir: (1) when is the reservoir established; (2) where is the reservoir maintained; and (3) how does the reservoir persist?

Keywords

HIV reservoir; HIV latency; HIV cure; acute infection; CD4+ T cells; tissues; molecular mechanisms

Introduction

Despite its unquestionable success at reducing HIV replication and improving the quality of life of many people living with HIV/AIDS, combination antiretroviral therapy (ART) does not eradicate the virus [1,2], due to the early establishment of a long-lived viral reservoir [3–5]. The best clinical evidence for the existence of a reservoir for HIV is provided by the rapid viral rebound observed in the vast majority of individuals who interrupt ART. HIV persistence during ART is the reason why current therapies are not curative and has been the subject of intense research during the past 15 years since ART was implemented.

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There are multiple reasons underlying HIV persistence during ART, which include the following:

- residual levels of viral replication that may not be fully suppressed in drug-privileged anatomical compartments;
- the persistence of a small pool of cells carrying silent integrated genomes that can be reactivated and reignite infection;
- persistent immune dysfunctions that fail at controlling residual replication and reactivation from latently infected cells.

The use of the word ‘reservoir’ to define the pool of cells in which replication-competent HIV persists during ART is still a matter of debate, as there is no consensus on the main mechanism by which HIV persists during ART. None the less, viral reservoirs can be defined as cell types or anatomical sites in association with which replication-competent forms of the virus persist with more stable kinetic properties than the main pool of actively replicating virus [6,7]. Identifying these cells and tissues and characterising the mechanisms by which HIV persists in these sites is a prerequisite to the design of therapeutic strategies aimed at eradicating HIV.

When is the reservoir established?

Soon after the implementation of ART in 1996, the possibility that ART initiated very early in infection could prevent the establishment of the long-lived HIV reservoir and shorten the duration of HIV persistence after prolonged therapy was proposed [8]. The rationale for this intervention originates from the fact that the latent reservoir is not created but rather revealed by ART, as latently infected CD4+ T cells are generated during untreated HIV infection (Figure 1). Therefore, a reasonable hypothesis is that by reducing the duration of exposure to the virus through early ART initiation, one would limit the overall number of infected cells, thereby reducing the possibility for some of them to revert to a resting state or to directly establish latency.

By 1998, Chun *et al*. demonstrated that initiation of ART in infected individuals as early as 10 days after the onset of symptoms of primary infection does not prevent the generation of latently infected CD4+ T cells carrying infectious virus [8]. This is in line with recent data generated in the non-human primate model of SIV infection, in which institution of ART as early as 3 days post infection could not prevent the establishment of a viral reservoir, evidenced by viral rebound after ART interruption [9]. Importantly, the time to viral rebound correlates with total viraemia during acute infection and with proviral DNA at the time of ART discontinuation [10], suggesting that the size of the reservoir is a critical parameter that can predict a clinical readout such as the time to viral rebound. Although these two studies suggest that even very early ART intervention may not be able to prevent the establishment of a reservoir for HIV, the capacity of early ART to reduce the size of this persistent reservoir has been demonstrated in several independent studies, using a variety of virological readouts [11–15]. The precise timing at which the reservoir is established is difficult to determine, as latency is likely to occur primarily in tissues that are difficult to access in recently infected individuals. The ‘when’ question may be easier to address if

considered together with the ‘how’ question: the well-accepted model of the generation of latently infected cells proposes that they originate from activated cells, most likely specific for HIV antigens [16,17], that are infected and differentiate into long-lived resting memory cells [18]. As a consequence, the latent reservoir may not be established before the generation of memory CD4+ T cells. In contrast, if HIV latency can be directly established in resting CD4+ T cells without the need for these cells to go through an activation state [19,20], the reservoir may be seeded in the first days following infection. Identifying the precise timing during which the latent reservoir is established is technically challenging as at the early stages the bulk of infected cells are likely to be productively infected, which complicates the effort to identify the minute fraction of latently infected CD4+ T cells.

In addition to the quantitative restriction by early ART, the reservoir is less genetically diverse in subjects who start ART early in infection [21]. More importantly, it may be more easily targeted by autologous HIV-specific CD8+ T cell responses, as viruses archived at a later stage of the disease are more likely to present escape mutations [22]. Therefore, the benefits of early ART intervention in the context of curative strategies are both qualitative and quantitative.

Of note, early ART is the only currently available intervention that limits the size of the latent HIV reservoir and leads to clinical benefits in HIV-infected individuals. A prime example is the case of the ‘Mississippi child’ who started ART by 2 days of life for 18 months and was able to remain virally suppressed for 27 months in the absence of ART [23]. Similarly, ‘post-treatment controllers’ from the VISCONTI cohort of adults started ART within the first 2 months of infection and were able to control viraemia without ART for more than 5 years [24,25]. The mechanisms by which early ART can lead to natural viral control in a subset of individuals are still under investigation. Although the mechanisms are likely to differ between individuals who are able to control replication-competent virus for different periods of time (‘Boston patients’ [26], Mississippi child [23], post-treatment controllers [25], elite controllers [27]), a reduced frequency of infected cells is common to all. This reinforces the clinical relevance of early ART interventions, which greatly limit the size of the reservoir, in curative strategies.

Where is the reservoir maintained?

Cellular reservoirs

Although alternative reservoirs may contribute to HIV persistence (detailed below), CD4+ T cells represent the best-characterised reservoir for HIV in virally suppressed subjects on long-term ART [3,4,28,29]. CD4+ T cells can be further subdivided into subsets according to their memory status or their effector functions upon stimulation (Figure 2).

Within the CD4 compartment, naïve cells and recent thymic emigrants can carry HIV DNA and replication-competent HIV [30–32], but their frequency of infection is usually much lower than that of memory CD4+ T cells [21,29,33,34]. Within the memory compartment, central (T_{CM}), transitional (T_{TM}) and effector memory (T_{EM}) cells are the three major reservoirs for HIV in individuals on suppressive therapy and harbour replication-competent virus [29], although their contribution to the functional reservoir may vary [35]. In addition,

two groups have recently shown that the recently identified CD4+ T memory stem cells (T_{SCM}) harbour high per-cell levels of HIV-1 DNA and make increasing contributions to the total reservoir measured by HIV DNA over time [36,37].

As an alternative to the CD4+ T cell subsets that distinguish different stages of T cell differentiation (T_{CM}, T_{TM} and T_{EM}), the CD4 compartment can be divided into subsets that demarcate different functional programs and homing capacities including Th1, Th2, Th17, regulatory T cells (Tregs) and follicular T helper cells (Tfh). It is well established that the effector function of CD4+ T cells, which is based on the expression of particular transcription factors, chemokine receptors and cytokine secretion upon stimulation, influence the capacity of this particular cell to serve as a long-term reservoir for HIV during ART [38,39]. In particular, regulatory Tregs [40] and Tfh cells [41] may represent preferential cellular reservoirs for HIV in virally suppressed individuals. CD4+ T cells expressing CCR6, a marker of Th17 cells with homing capacity to the gut, are highly sensitive to HIV infection [42–44]. This could be attributed to the intrinsic nature of Th17 cells rather than to their homing potential, since CD4+ T cells expressing the integrin beta 7, another marker for homing potential, do not display this increased sensitivity to HIV infection [38]. Indeed, the majority of HIV type 1 DNA in circulating CD4+ T lymphocytes is present in non-gut-homing resting memory CD4+ T cells [45].

In addition to CD4+ T cells, non-conventional cellular HIV reservoirs have been described. They include CD8+ T cells [46,47], the double negative CD3+CD4−CD8-subset [48,49] and cells from the myeloid lineage including circulating monocytes [50–53]. In the NHP model of SIV infection, myeloid cells containing viral DNA show evidence of T cell phagocytosis *in vivo*, suggesting that their viral DNA may be attributed to phagocytosis of infected T cells [54], questioning the role of myeloid cells as a major source of SIV *in vivo*. The contribution of these potential alternative reservoirs to HIV persistence in humans is difficult to assess, as the majority of these cells may reside in tissues that are not easily accessible. Furthermore, obtaining sorted myeloid cells with sufficient yield and purity from these tissues is very challenging. Nevertheless, in HIV-infected humans, a few lines of evidence suggest that cells from the myeloid lineage could serve as a long-term reservoir for the virus in mucosal tissues such as the lung [55,56] and duodenum [57]. Of note, these studies usually only demonstrate the presence of viral nucleic acids or viral proteins in myeloid cells isolated from blood and tissues. Whether replication-competent virus can persist in these cells after prolonged ART is largely unknown.

Anatomical reservoirs

As mentioned above, HIV primarily infects CD4+ T cells and cells from the myeloid lineage. Although these cells are found in the circulating blood, the bulk of the cellular targets for HIV primarily reside in lymphoid tissues [58]. Most of the studies aimed at measuring viral persistence (both residual viral replication and latency) have been conducted in the peripheral blood, although it is clear that tissue reservoirs such as the gut and lymph nodes are important sites for HIV persistence [59,60]. Studies in mice have revealed the existence of tissue-resident memory T cells that play an important role in protective immunity to site-specific pathogens and mucosal sites, such as lung and intestine, contain

tissue-retained memory populations that do not recirculate [61,62]. In humans, a recent study conducted in blood, lymphoid and mucosal tissues obtained from organ donors revealed that the subset composition and phenotype of peripheral blood T cells does not reflect that of spleen, lymph nodes or mucosal tissues, suggesting that blood is a distinct compartment [63]. This reinforces the importance of conducting studies aimed at understanding the mechanism of HIV persistence and quantifying persistent HIV directly in tissue samples because these reservoirs may not be reflected in the circulating blood. Most of the studies conducted in tissue reservoirs for HIV have used PCR-based assays to assess the levels of HIV persistence, as recovering replication-competent HIV from these sites is technically challenging.

As mentioned above, T cell homeostasis driven by cytokine or T cell receptor-mediated signals in T cell subsets varies with their differentiation stage and their tissue localisation, and cannot be inferred from blood [64]. T_{EM} CD4+ T cells producing IL-2 predominate in mucosal tissues and accumulate as T_{CM} cells in lymphoid tissue [63]. In the gut, high frequencies of activated cells [63] as well as persistent elevated levels of inflammation may favour residual viral replication even after prolonged ART. Conversely, the high frequency of the relatively quiescent T_{CM} cells in lymph nodes [63,65], which are in constant interactions with stromal cells and antigen-presenting cells to ensure the homeostasis of the CD4 compartment, may promote the survival of latently infected cells in this site. Most HIV DNA and RNA in the blood is found in T_{CM} cells, whereas in ileum and rectum, most HIV DNA and RNA was found in T_{EM} cells [66]. The characterisation of the mechanisms of HIV persistence in these anatomical reservoirs will require the use of assays that can distinguish residual viral replication from latency and sorting procedures that will ensure high levels of purity of the cell population examined in different anatomical sites.

The importance of the gastrointestinal tract as a privileged site for HIV persistence after prolonged ART is still debated. In the blood, the majority of HIV DNA is present in non-gut-homing resting memory CD4+ T cells [45]. Several studies have directly examined the frequency of infection in biopsies obtained from different regions of the gastrointestinal tract. In some studies, infection frequencies measured in the gastrointestinal tract are higher than in the matched blood samples [59,60], whereas other investigators found no significant differences in the frequency of infection of blood and rectal CD4+ T cells [67]. Similar to peripheral blood mononuclear cells (PBMCs), the decay in the frequency of infected cells in the gastrointestinal tract appears to be minimal [68,69] and the frequencies found in the two compartments are well correlated [29,70]. This questions the existence of a compartmentalisation between the blood and gut reservoirs. Compartmentalisation is suggested by the existence of gut-residing CD4+ T cells [63] and the fact that different HIV-1 quasispecies populate different parts of the gut [71]. In addition, several studies suggest that HIV DNA resides in cells other than CD4+ T cells, including myeloid cells [72] and more specifically tissue macrophages [57] in the gastrointestinal tract, although this may partially be attributed to contamination by T cells [21], at least in some of the samples tested. The contributions of the gut reservoir to viral rebound upon ART interruption is still unclear [73,74]. Of note, the relative contribution of viral latency and residual replication to viral persistence during suppressive ART in the gut are largely unknown, for the technical reasons mentioned above.

Replication-competent virus has been recovered in cells isolated from lymph nodes in virally suppressed individuals [28,41] and non-human primates [75]. The recent identification of follicular T helper cells, which reside in the germinal centres of lymph nodes, as a major compartment for HIV production, and perhaps persistence [41], will most probably refocus attention around these lymphoid organs. Interestingly, a recent study indicates that B cell follicles constitute 'sanctuaries' for persistent SIV replication in the presence of potent antiviral CD8 responses, as a result of the relative exclusion of cytotoxic T lymphocytes (CTL) from this site [76]. Moreover, concentrations of antiretroviral drugs are much lower in lymph nodes than in peripheral blood, which correlates with continued virus replication measured by detection of viral RNA in productively infected cells [77]. Therefore, lymph nodes may play a major role in HIV persistence through residual levels of viral replication in Tfh cells. In addition, T_{CM} cells, which represent a preferential cellular subset for latent HIV, are enriched in lymph nodes. Therefore, lymph nodes could contribute to HIV persistence both through residual levels of viral replication and latency.

In addition to the gut and lymph nodes, several other tissue compartments could serve as a long-term reservoir for HIV. While it is still unclear if the brain could serve as a long-lived reservoir for replication-competent HIV during suppressive ART [78], it clearly contains cells with an integrated provirus in untreated HIV-infected individuals [79,80]. The male [81] and female [82,83] genital tracts could also serve as long-lived reservoirs for HIV during ART. Other previously neglected potential reservoirs such as the kidney [84] and the liver, which may play an important role in viral persistence, at least in the humanised mouse model [85], warrant additional studies. In all cases, it would be important to demonstrate that these potential reservoirs fulfil the criteria of a long-lived viral reservoir during ART, including the replication competency of the persistent viral genomes.

How is the reservoir maintained?

Residual viral replication during ART

ART achieves prolonged suppression of viral replication; however, through the use of highly sensitive PCR assays that are able to detect single copies of HIV RNA, residual plasma viraemia has been shown to exist even in patients who have been virally suppressed for prolonged periods of time [18,86,87]. This residual viraemia could originate from reactivation of virus in latently infected memory CD4+ T cells that are undergoing antigenic stimulation. Interestingly, activated CD4+ T cells in the peripheral blood have been shown to spontaneously release viral particles even in the absence of stimuli [88]. Viraemia could also come from productively infected CD4+ T cells sequestered in lymphoid tissues. Low levels of viral replication in lymphoid organs can play a role in HIV persistence via the spread of virus through cell-to-cell contact in the virological synapse [89]. Significantly, residual plasma viraemia in virally suppressed individuals was shown to correlate with the size of the CD4+ T cell reservoir, but not with markers of immune activation, suggesting that immune system activation alone is not responsible for the observed low-level viraemia [90].

Maintenance of latently infected cells

The persistence of HIV in memory CD4+ T cell subsets is ensured by T cell survival and homeostatic proliferation in response to interleukin-7 (IL-7) signalling [29]. IL-7 mediates the proliferation of latently infected CD4+ T cells without disrupting latency [91], and when administered to virally suppressed subjects, induces a modest but significant expansion of the reservoir [92]. In addition to homeostatic proliferation, latently infected CD4+ T cells undergo proliferation in response to antigenic stimulation, as suggested by phylogenetic analyses showing high numbers of identical sequence expansions in virally suppressed individuals [93,94]. Recently, several groups sequenced the integration sites of the HIV genome within the host DNA and found that specific HIV-integration sites are linked to clonal expansion [95–97]. These studies also indicate that integration of the viral DNA into cancer genes contributes to persistent infection [96]. While the majority of these expanded integrated viral genomes is likely to be incompetent for HIV replication [97,98], it is possible that these defective integrants retain the capacity to generate viral RNA and perhaps viral proteins that could contribute to abnormally elevated levels of immune activation in virally suppressed individuals.

Molecular mechanisms of HIV latency

In maintaining the HIV reservoir, latently infected resting memory CD4+ T cells potentially have the greatest clinical significance. Their long life-span ensures that the virus can be maintained in quiescent cells for years. Resting memory CD4+ T cells support a favourable environment for the maintenance of HIV latency, and multiple molecular mechanisms have been proposed to mediate its induction, including the site of viral integration, transcriptional interference, chromatin remodelling, restriction of transcription host factors and requirements for the HIV accessory protein Tat (transactivator of transcription).

In a study of viral integration sites in resting CD4+ T cells from virally suppressed individuals, a surprising 93% of proviruses were found in actively transcribed genes [99]. This site preference is most likely due to interaction of the pre-integration complex with cellular host factors associated with gene transcription. Integration into actively transcribed genes can lead to transcriptional interference caused by the elongating RNA polymerase II complex transcribing through the 5′ LTR. This leads to interference with pre-initiation complex formation and silencing of HIV transcription [100] that could only be partially reversed through cellular activation by TNF-α [101]. Studies in a primary cell-based model of HIV latency have shown that latent proviruses have an orientation bias when compared to productively infected cells within the same model system [102]. These data suggest that mechanistically transcriptional interference is a significant factor in silencing HIV transcription.

Another contribution to HIV latency is establishment of a repressive chromatin environment. Epigenetic modifications alter the physical structure of chromatin and affect transcription levels including CpG methylation and histone methylation. A Jurkat model demonstrated that initiation of HIV latency was associated with CCAAT-box binding transcription factor 1 (CBF-1)-dependent histone deacetylase (HDAC)-1 recruitment to the 5′LTR and histone H3 lysine 9 (H3K9) trimethylation [103,104]. H3K9/27 trimethylation

was also shown to be involved in the establishment of latency in primary cells [105]. DNA methylation at CpG islands is a repressive epigenetic modification that can inhibit transcription factor binding and can recruit HDAC-2 for histone deacetylation. The role of DNA methylation in HIV latency, however, is controversial. In studies using Jurkat cell lines or an *in vitro* primary CD4+ T cell model of latency, densely methylated proviral DNA was associated with a reduced capacity to reverse latency [106]. Subsequent studies using resting CD4+ T cells isolated from a cohort of virally suppressed HIV-infected individuals demonstrated very low levels of CpG dinucleotides within the 5′LTR, suggesting DNA methylation may not have a significant role in the maintenance of HIV latency [107]. In addition, the role of histone modification in HIV latency has also been of interest, and has led to the recent clinical trials using HDAC inhibitors as an eradication strategy [108–110]. Histone deacetylases are a family of enzymes functioning to remove the acetyl groups from lysine residues, one of the signals required for binding of activating transcription factors [111,112]. HDAC activity has been associated with the repression of HIV transcription [113,114]. Among the different classes of HDACs, the class I HDACs, HDAC-1, -2 and -3, are recruited to the HIV-1 LTR in cell-line models of HIV-1 latency [111,113–115]. In support of the role of HDACs in HIV latency, class I HDAC inhibitors have been shown to induce HIV expression in both *in vitro* cell models of latency and in resting CD4+ T cells from HIV-infected patients [116–120]. Although promising conceptually as an eradication strategy, therapeutically, the results have been mixed. The HDAC inhibitors vorinostat and panobinostat induced a significant increase in cell-associated HIV RNA in the clinical setting [108–110]. However, these drugs had no effect on the size the of the latent HIV reservoir. One of the assumptions made in HIV eradication strategies is that latent provirus reactivation will induce either cell death in the now productively infected cell, or allow it to be recognised by HIV-specific CTL. Of note, a recent study showed that HDAC inhibitors have a negative impact on CTL activities by impairing IFN-γ production and their ability to recognise and eliminate HIV-infected target cells *in vitro* [121]. These data highlight the importance of considering how the anti-latency compounds will influence multiple arms of the immune response to maximise clinical effectiveness.

HIV latency is also affected by the availability of host transcription factors. The specific recruitment of factors such as NF-κB, Sp1 and NFAT are required for HIV transcription. NF-κB binds to the HIV 5′ LTR and initiates pre-initiation complex formation and transcription initiation [113]. Phosphorylation of RNA polymerase II on the serine 5 position in the heptapeptide repeats of its C-terminal domain (CTD) promotes transcription initiation but not transcript elongation. In order for efficient elongation to occur, sustained activation of NF-κB expression is required to lead to the synthesis of the HIV Tat protein. Tat functions to recruit P-TEFb, which results in the phosphorylation of serine 2 of the RNA polymerase II CTD and transcription elongation.

Sequestering of NF-κB and other factors required for transcription initiation, such as NFAT, in the cytoplasm of quiescent CD4+ T cells also contributes to establishment of HIV latency [122,123]. P-TEFb becomes incorporated into an inactive complex with HEXIM and 7SK RNA, which restricts availability of P-TEFb for efficient transcription elongation [124,125].

The sequestering of P-TEFb plays a significant role in limiting Tat synthesis, a critical factor in HIV transcription.

The HIV Tat protein functions in transcriptional elongation. In the absence of Tat, the majority of the RNA polymerase II complexes prematurely terminate transcription near the promoter [126]. Tat binds to the transactivation response element (TAR), a sequence in the 5′-non-coding region of HIV mRNA that forms a stable stem-loop structure. Efficient elongation of HIV transcripts requires the recruitment of a complex of proteins comprising CycT1 and CDK9/P-TEFb [127]. CycT1 induces CDK9/P-TEFb kinase activity to facilitate phosphorylation of a number of proteins within the elongating transcription complex, including the RNA polymerase II CTD [128]. In addition to recruiting factors for transcriptional elongation, Tat also functions to counteract the activities of negative elongation factors such as the RNA-binding NELF complex associated with RNAPII pausing [129,130]. Without efficient elongation, host cell factors such as Setx, Srn2 59–39 exonuclease and microprocessor complexes function to prematurely terminate HIV transcripts [131], setting up a negative feedback loop that further downmodulates HIV transcription and induces latency [132]. Interestingly, two recent studies by the Weinberger group suggested that rather than a limiting factor that unnecessarily restricts viral replication, Tat activity functions as a regulation mechanism that mediates viral latency for HIV survival [133,134]. Using a computer modelling approach, they demonstrated that the state of HIV expression could be separated from the state of activation of the host cell, and that the two processes were in fact independent [133]. They then used a synthetic biology approach to alter either Tat expression or cellular activation and found that Tat expression was the controlling factor of virus expression irrespective of the host cell state. Mathematical modelling was further applied to explain that the function of Tat to control viral expression may have evolved to promote cell survival and, consequently, enhanced virus propagation [134]. After initial entry of the virus at mucosal surfaces, productive infection of all target cells would induce rapid cell death and therefore limit the capability of the virus to efficiently establish infection. Induction of viral latency would promote cell survival and allow the virus to be spread to other sites in the body when those cells migrated to other tissues. Their mathematical model predicted that establishment of latency benefited the virus over continued productive infection, and resulted in higher infection rates. Interestingly, these data would suggest that latency is one of the earliest steps in HIV infection, in order to limit the death of target cells. Although the implication for intervention strategies is that there may not be a clinical intervention early enough that could block the establishment of a latent reservoir, the aim of limiting the size of the reservoir to preserve immune system function and reduce inflammation is well documented.

Conclusion

The interdependency of residual replication, proviral latency and immune dysfunction complicates our understanding of the mechanisms by which HIV persists in virally suppressed individuals. Indeed, the pool of latently infected CD4+ T cells can be replenished by ongoing replication, a phenomenon that is not fully controlled by the host immune response. Recent data also suggest that viral latency (as defined by the lack of virion production) and transcriptional latency (no transcriptional activity of the LTR) may not

always overlap. This concept of ‘leaky latency’ is attracting lots of interest, as it does not only question the widely accepted model of latency, but also suggests that the so-called ‘latent reservoir’ may produce low amounts of viral products (RNA and perhaps proteins) that could contribute to the persistent immune dysfunctions seen in virally suppressed individuals.

While the virological mechanisms of HIV persistence have been extensively investigated in many cohorts of HIV-infected individuals receiving suppressive ART, only a few studies have evaluated the antiviral immune response (both innate and adaptive) during ART and how they influence HIV persistence. This neglected area is now getting much more attention, particularly in the context of ‘shock and kill’ strategies to reduce the size of the reservoir, which will most probably require an immune component to be successful.

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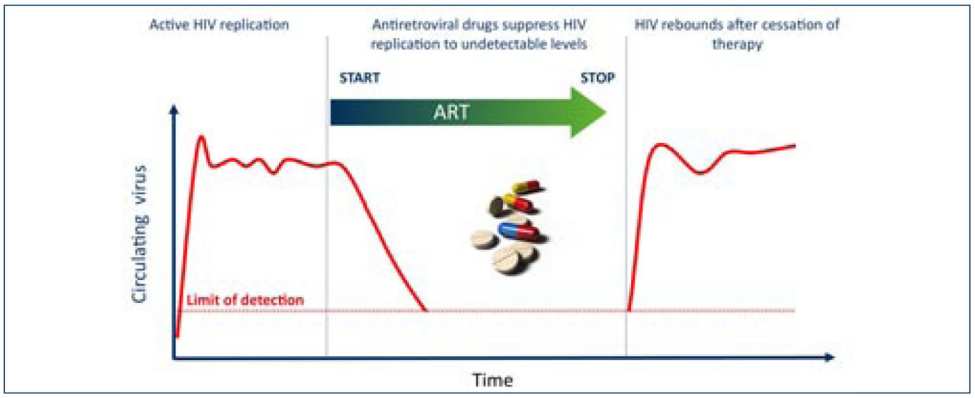

Figure 1.

Clinical definition of the HIV reservoir. Untreated HIV infection is characterised by high levels of viral replication that can be measured in the plasma of HIV-infected individuals. ART reduces viral replication to undetectable levels by standard viral load measurements. When ART is interrupted, HIV replication resumes, revealing that HIV persisted in cellular and anatomical ‘reservoirs’ during ART and that these reservoirs can re-ignite infection.

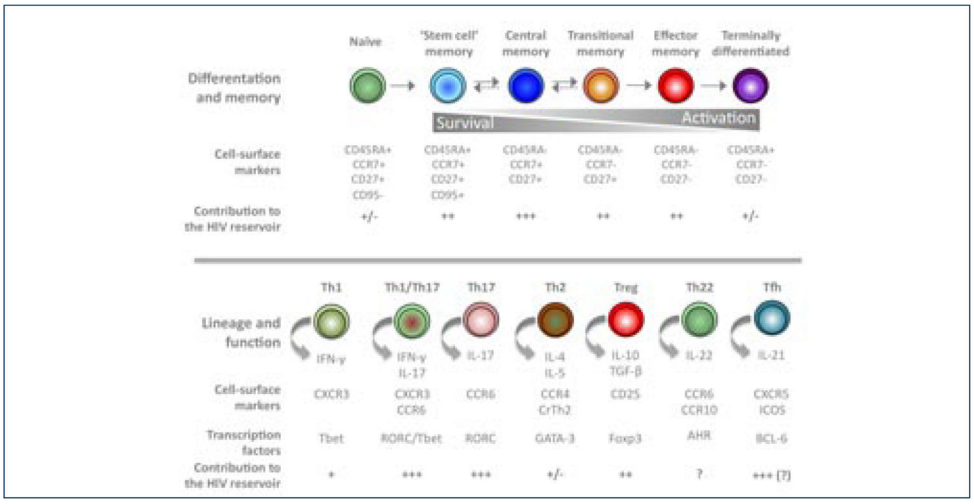

Figure 2.

Contribution of CD4+ T cell subsets to the HIV reservoir during ART. CD4+ T cell subsets can be classified according to their differentiation and memory status (top) or to their effector functions (bottom). Cell-surface markers and the production of specific cytokines can be used to identify each individual subset. The relative contribution of each subset to the HIV reservoir is indicated.

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