Recent developments in human immunodeficiency virus-1 latency research

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Almost 30 years after its initial discovery, infection with the human immunodeficiency virus-1 (HIV-1) remains incurable and the virus persists due to reservoirs of latently infected CD4⁺ memory T-cells and sanctuary sites within the infected individual where drug penetration is poor. Reactivating latent viruses has been a key strategy to completely eliminate the virus from the host, but many difficulties and unanswered questions remain. In this review, the latest developments in HIV-persistence and latency research are presented.

Introduction

Before the introduction of highly active anti-retroviral therapy (HAART), a diagnosis of HIV/AIDS would have been a death sentence for most patients. However, modern anti-retroviral regimes are able to preserve the health of the patient and routinely reduce the plasma viral load to less than 50 copies of HIV-1 RNA ml⁻¹ (Volberding & Deeks, 2010). Although HAART is very effective at blocking HIV-1 spread within the body, it is not a cure, as viral loads readily rebound when treatment is interrupted (Chun et al., 1999; Davey et al., 1999). Furthermore, ultrasensitive detection assays have shown that in most HAART-treated patients, a low-level viraemia of less than 5 copies ml⁻¹ persists even after years of therapy (Chun et al., 2005; Palmer et al., 2008; Tobin et al., 2005). This low-level persistent viraemia is a major obstacle to the complete elimination of HIV-1 from the body.

HAART cannot fully restore the health of an infected individual. Long-term-treated HIV-1 patients have reduced lifespans and increased susceptibilities to non-AIDS related conditions such as cardiovascular disease, cancer, liver and kidney dysfunctions as well as neurological decline, which may be a consequence of the toxicity of the drugs or the chronic inflammation caused by HIV-1 infection (Deeks, 2011; d’Arminio et al., 2004). The financial cost of life-long treatment, especially in resource-poor settings, is prohibitive (Hecht et al., 2010). Until the discovery of an effective vaccine, or other interventions that can halt the continuing spread of HIV-1, it will become increasingly difficult for high disease burden countries in the developing world to control the epidemic using only current anti-retroviral regimes (Lewin et al., 2011). Thus, an effective cure of HIV/AIDS would not only alleviate the suffering of the millions of infected persons, it may be the only way to check the progress of the HIV-1 epidemic. In this article, we provide an overview of HIV-1 latency and address some of the major gaps in our understanding of the phenomenon. We examine recent advances in translational research aiming to find a sterilizing (complete eradication of the virus) or a functional (virus replication is on-going but does not lead to clinical problems) cure.

The question over the source of the persistent viraemia

The half-life of the HIV-1 virion in the plasma is very short (Ho et al., 1995; Ramratnam et al., 2000) and it is generally believed that the persistent viraemia is either the result of the reactivation of latently infected resting T-cells or on-going virus replication in ‘sanctuary’ sites within the body (Lewin et al., 2011; Palmer et al., 2011). The existence of a latently infected population of CD4⁺ T-cells was first indicated by the discovery that the number of cells expressing HIV-1 mRNA in vivo was lower than the number of cells carrying proviral DNA (Schnittman et al., 1989). Subsequent studies have demonstrated that a small number (approx. one million cells) of resting CD4⁺ T-cells in HAART-treated individuals harboured replication-competent latent viruses that could be reactivated by stimulation of the cells with mitogens (Chun et al., 1995, 1997; Finzi et al., 1997). While dormant, the virus is hidden from the host immune response and it has been shown that the decay rate of these latently infected resting CD4⁺ T-cells is very low, requiring an estimated period of 73.4 years for complete eradication using the current anti-retroviral regime (Siliciano et al., 2003). Alternatively, on-going low-level virus replication may be responsible for the persistent viraemia. Persistent virus production has been found within sanctuary sites such as the central nervous system (Canestri et al., 2010; Churchill et al., 2006; González-Scarano & Martín-García, 2005), the gastrointestinal tract (Chun et al., 2008) and the male and female genital tract (Halfon et al., 2010; Launay et al., 2011). Recent studies have indicated that anti-retroviral drug penetration is site- and compound-specific, and drugs that penetrate poorly may allow virus replication at that site even when plasma viral load is below 50 copies ml⁻¹ (Best et al., 2012; Di Mascio et al., 2009; Else et al., 2011; Halfon et al.,...
2010; Kwara et al., 2008; Launay et al., 2011). Since the rate of reactivation of latent viruses in resting T-cells is unknown in vivo (Siliciano & Siliciano, 2010), it is unclear whether it occurs frequently enough to maintain the low-level viraemia that is detected in patients. Thus, the most probable origin of the low-level viraemia may be the sanctuary sites where productive infection is expected to be occurring constantly. In order to determine the contribution of each of these factors to the low-level viraemia in the body, phylogenetic studies were performed on the viral sequences isolated from the residual viraemia. The results were contradictory: while some studies showed a lack of evolution among the sequences found, suggesting that the progeny virions came from one stable reservoir among CD4+ T-cells (Bailey et al., 2006; Joos et al., 2008; Ruff et al., 2002), others found viral sequences that were not detected among the resting T-cell population, indicating another cellular source for the residual viraemia (Bailey et al., 2006; Brennan et al., 2009; Sahu et al., 2009). Another indication of on-going productive infection would be if treatment intensification (the addition of a fourth anti-retroviral to the standard three-drug regime) reduced the basal level of viraemia further. The majority of treatment intensification studies using the HIV-integrase inhibitor Raltegravir (RGV) showed no significant reduction of the residual plasma viraemia (Dinoso et al., 2009b; Gandhi et al., 2010; Hatano et al., 2011; McMahon et al., 2010). However, in one study RGV increased the number of '2-LTR (long terminal repeat) circles' found in the PBMCs of 29% of the treated subjects (Buzón et al., 2010). Since RGV blocks the integration of linear viral DNA from a productive infection and encourages the formation of 2-LTR circles (Middleton et al., 2004; Svarovskaia et al., 2004), these data indicate the presence of an on-going infection. In a separate study, intensification with RGV reduced unspliced HIV-1 RNA within the ileum, but caused no significant reduction in plasma viraemia (Yukl et al., 2010), illustrating the possibility of on-going infection occurring in a compartment other than the blood (Table 1).

Most of the CD4+ T-cells in the body reside within the gastrointestinal tract and the lymphatic tissues rather than within peripheral blood (Mowat & Viney, 1997). In contrast, the majority of studies on HIV-1 replication dynamics and CD4+ T-cell depletion have been performed in peripheral blood because it is the easiest compartment to access. It has been shown that the gastrointestinal tract is the major site of HIV-1 replication and CD4+ T-cell depletion during all stages of HIV/AIDS (Brenchley et al., 2004a; Chun et al., 2008), and that the destruction of the CD4+ T-cell population within the gastrointestinal tract leads to the translocation of microbial products to the circulatory system and contributes to the chronic inflammation and immune exhaustion that are associated with HIV/AIDS (Douek et al., 2009). Thus, we may be overlooking vital pieces of the jigsaw if we focus solely on the peripheral blood compartment.

Apart from CD4+ T-cells, HIV-1 can also infect cells of the monocytic lineage (Coleman & Wu, 2009; Gartner et al., 1986; Le Douce et al., 2010). HIV-1 infection of macrophages tends to be less cytopathic than infection of activated T-cells (Ho et al., 1986, 1995; Nicholson et al., 1986). Also, infected monocytes can migrate to the central nervous system and the gastrointestinal tract before maturing into macrophages, potentially sheltering the virus from the full potency of HAART (Le Douce et al., 2010). The contribution of infected macrophages to HIV-related neurological decline is well documented (González-Scarano & Martín-García, 2005). It is also well known that dendritic cells can transport whole virions to lymph nodes where susceptible activated CD4+ T-cells reside. Moreover, dendritic cells themselves can become infected under certain circumstances (Coleman & Wu, 2009). However, it is not clear whether proviral clones or individual infected cells within the monocytic population can survive for long enough to function as long-lived latency reservoirs (Eisele & Siliciano, 2012). It is possible that within the safety of sanctuary sites and with continuous replenishment of susceptible cells, continuous productive infection may be maintained by macrophages and dendritic cells. In addition it has been proposed that infection of immature CD4+ /CD8+ 'double positive' thymocytes during thymopoiesis may generate a

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<th>Persistent viraemia originated from one source (resting T-cells)</th>
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<td>● Viral genomes recovered from persistent viraemia show little variation</td>
<td>Bailey et al. (2006); Joos et al. (2008); Ruff et al. (2002)</td>
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<td>● HAART intensification does not reduce residual viraemia</td>
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<th>Multiple sources contribute to persistent viraemia</th>
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<td>● Persistent HIV-1 infection has been found within different parts of the body</td>
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<td>● Viral sequences distinct from those isolated from resting T-cells are found</td>
<td>Bailey et al. (2006); Brennan et al. (2009); Sahu et al. (2009)</td>
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<td>● HAART intensification increased 2-LTR circles from PBMCs</td>
<td>Buzón et al. (2010)</td>
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<td>● HAART intensification reduced HIV-1 RNA within the ileum</td>
<td>Yukl et al. (2010)</td>
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population of latently infected naïve T-cells (Brooks et al., 2001). Despite the continuing debate over the true origin of the low-level viraemia, it can be agreed that a viable therapeutic intervention to cure HIV/AIDS should involve the elimination of all these proven and potential reservoirs.

**Haematopoietic stem cells (HSCs) are a viral reservoir?**

HSCs are a population of primitive, self-renewing precursor cells that reside in the bone marrow (Cabrita et al., 2003). HSCs can proliferate and differentiate into all the cell types found in peripheral blood. HSCs and other more mature precursor cell types such as the multipotent progenitor cells (MPPs) can express the HIV-1 receptors CD4, CCR5 and CXCR4; thus in theory these cells can be infected by HIV-1 (Alexaki & Wigdahl, 2008; McNamara & Collins, 2011). However, whether this is the case in vivo is controversial, with contradictory evidence emerging from different studies (Davis et al., 1991; Folks et al., 1988; Neal et al., 1995; Stanley et al., 1992).

If HSCs and other progenitor cells are proven to be latent reservoirs of HIV-1, it would make the difficult task of curing HIV/AIDS even more challenging as these cells are very long-lived, can self-propagate and the provirus in these cells may not be affected by HAART or any novel therapies that target latently infected CD4+ T-cells. Recently, it has been shown that CD34+ progenitor cells (that include HSCs and MPPs) are susceptible to latent infection ex vivo, and that integrated provirus was detected among CD34+ cells from HAART-treated patients (Carter et al., 2010). A follow-up study showed that only X4 or dual R5/X4-tropic viruses could efficiently infect these CD34+ progenitor cells (Carter et al., 2011). Furthermore, this study also showed that human HSCs infected with a GFP-reporter virus could be successfully engrafted into irradiated non-obese diabetic (NOD)/SCID IL-2R-GFP-reporter virus could be successfully engrafted into this study also showed that human HSCs infected with a provirus and a highly artificial small animal model, relevant to the situation in the human body? The existence of an HSCs reservoir remains a controversy and requires further study.

**Latent HIV-1 infection of resting CD4+ T-cells**

Although HIV-1 can persistently replicate within sanctuary sites, improvements in drug penetration or HAART intensification may overcome this barrier to eradication in the future. However, enhancing the effectiveness of HAART will not affect the latent viruses hiding within the resting CD4+ T-cell populations of the body. Thus, the latent infection within resting T-cells remains the biggest proven obstacle to a sterilizing cure of HIV-1 infection. The majority of the circulating CD4+ T-cells in the body at any given time are in a resting state (Berard & Tough, 2002). These cells are typically defined by the lack of activation marker expression (CD25, CD69 and HLA-DR), as well as the maintenance of the cells in the G0 phase (Chun et al., 1997). They can be broadly divided into those that have not undergone antigen-stimulated expansion (naïve T-cells) and those that have remained behind after the end of an immune response (memory T-cells) (Berard & Tough, 2002). Among infected resting T-cells, HIV-1 gene expression is largely suppressed (Hermankova et al., 2003). However, some transcription of HIV mRNA can be detected within the resting T-cells of HAART-treated patients, although full virus production is inhibited by inefficiencies at various stages of the viral life cycle (Lassen et al., 2004, 2006; Vatakis et al., 2010) (Fig. 1). Since most of these latently infected resting CD4+ T-cells are CD45RO+ memory cells (Brenchley et al., 2004b; Chomont et al., 2009; Chun et al., 1997; Pierson et al., 2000), it is hypothesized that the majority of the latently infected T-cells come from activated CD4+ T-cells that were infected and then reverted back to a resting memory state before the start of virus replication (Han et al., 2007). Latent provirus can be maintained within the memory T-cell population by the homeostatic proliferation of the infected host cells, driven by IL-7 (Bosque et al., 2011; Chomont et al., 2009).

Virus may infect resting T-cells directly and latent infection of naïve T-cells has been observed in patients, albeit at a lower frequency than memory T-cells (Chomont et al., 2009; Pierson et al., 2000). However, direct infection of resting T-cells is very inefficient (Pierson et al., 2000; Stevenson et al., 1990), with defects in reverse transcription and delays in integration in comparison with infection of activated CD4+ T-cells (Vatakis et al., 2007, 2009). Recently, two studies have implicated the innate restriction factor SAMHD1 in the inhibition of reverse transcription in resting CD4+ T-cells. Initially shown to be absent in transformed CD4+ T-cell lines, SAMHD1 was found to be expressed in both resting and activated primary CD4+ T-cells. In resting T-cells, SAMHD1 restricted reverse transcription by depleting the cellular pool of dNTPs (Baldauf et al., 2012; Descours et al., 2012). Nevertheless, integration of the viral genome can still occur in resting T-cells (Vatakis et al., 2009) and no method described to date has been able to distinguish between latently infected memory T-cells that were infected either during activation or during quiescence (Vatakis et al., 2010). Furthermore, a recent study showed that in patients receiving HAART treatment, the amount of HIV DNA in memory T-cells declined over time while the amount of HIV DNA in naïve cells remained constant, suggesting that direct infection of resting T-cells may be replenishing the latent viral reservoir.
as the disease progresses (Wightman et al., 2010). These observations are consistent with the finding that R5 tropic viruses [which are associated with acute infection (Choe et al., 1996; Feng et al., 1996; Zhu et al., 1993)] preferentially infect CCR5-expressing memory T-cells whereas X4 tropic viruses [which are associated with late disease progression (Connor et al., 1997)] exhibit a preference for CCR5- CXCR4+ naive T-cells (Bleul et al., 1997; Ostrowski et al., 1999; Wu et al., 1997). The stimulatory effects of HIV-1 gp120 may enable the direct infection of resting T-cells by activating calcium flux and NFAT signalling down the CCR5 signalling pathway, as well as upregulating inositol triphosphate-mediated signalling and the expression of the IL-2 receptor (Cicala et al., 2006; Kornfeld et al., 1988; Weissman et al., 1997). Stimulation of CXCR4 signalling by HIV-1 gp120 induces cytoskeleton-remodelling activity in resting T-cells, increasing the efficiency of subsequent infection with HIV-1 (Yoder et al., 2008). Thus, it is possible that the direct infection of resting T-cells plays an increasingly important role in maintaining the viral reservoir as the disease progresses.

The molecular mechanisms of latency in HIV-1 infection have been reviewed extensively (Coiras et al., 2009; Colin & Van Lint, 2009; Marcello, 2006; Marsden & Zack, 2009; Richman et al., 2009; Siliciano & Greene, 2011). In general, HIV-1 latency may be divided into pre-integration or post-integration latency. Pre-integration latency refers to the partial or complete inhibition of the viral life cycle before the integration of the virus into the host genome (see above). Most of the HIV-1 DNA found in resting T-cells is unintegrated (Chun et al., 1997; Sloan & Wainberg, 2011). Although it has been shown that linear unintegrated viral DNA within resting CD4+ T-cells is able to complete integration after the activation of the cell (Bukrinsky et al., 1991), pre-integration latency is not thought to be relevant to the establishment of the reservoir of latently infected resting T-cells due to the labile nature of viral DNA in the cytoplasm of the cell (Pierson et al., 2002). Accordingly, the unintegrated viral DNA may no longer be replication competent after a protracted period inside the host cell (Han et al., 2007; Zhou et al., 2005). Post-integration latency is the failure of expression of the viral genome after it has been integrated into the host genome. While less than 0.05%, or approximately 10^6 to 10^7 cells, carry integrated provirus, it is these integrated proviruses that are thought to constitute the latent viral reservoir (Chun et al., 1997).

Even after a successful integration event, there are still multiple barriers to productive HIV-1 replication within resting CD4+ T-cells (Fig. 1). Although HIV-1 genomes are generally integrated into genes that are actively expressed within resting T-cells (Han et al., 2004), viral gene expression may be downregulated by promoter occlusion (if the provirus is integrated in the same orientation as the host gene) (Greger et al., 1998) or by collisions between RNA Pol II molecules that are travelling in opposite directions (if the provirus is integrated in the opposite orientation as the host gene) (Han et al., 2008). Two nucleosomes, named nuc-0 and nuc-1, are frequently associated with the HIV-1 5’LTR and regulate the basal transcriptional activity of the viral genome by controlling the access of transcription factors to the LTR (Verdin et al., 1993). The remodelling of the nucleosomes is regulated by the acetylation status of their constituent histones, which is in turn controlled by enzymes such as histone acetyltransferases and histone deacetylases (Van Lint et al., 1996). This allows the manipulation of HIV-1 transcriptional activity.
by pharmacological means, potentially leading to a viable method to eliminate the virus reservoir from resting CD4+ T-cells (see Novel drug discovery). The presence of cellular transcriptional repressors, for example YY1 and LSF, as well as the methylation of the two CpG islands at the HIV-1 transcription start site, can recruit histone deacetylases to the HIV-1 LTR and reinforces latency (Blazkova et al., 2009; Coull et al., 2000; Kauder et al., 2009), while the binding of the transcription factor NF-kB stimulates proviral reactivation by recruiting histone acetyltransferases to the LTR and initiating early HIV-Tat production (Lusic et al., 2003; Williams et al., 2007). The lack of NF-kB, as well as transcription factors NEAT, SP-1 and AP-1 prevents the synthesis of Tat and the subsequent Tat-dependent, high level viral gene expression (Coiras et al., 2009; Mbonye & Karn, 2011; Williams & Greene, 2007). The transcriptional activity of Tat is highly dependent on interacting with the cellular factor P-TEFb, which triggers effective RNA Pol II elongation (Parada & Roeder, 1996), and the negative regulation of P-TEFb activity in resting T-cells further impairs the expression of HIV-1 genes (Ghose et al., 2001).

Tat also interacts with several other cellular factors such as the histone acetyltransferases p300 and P/CAF to promote transactivation of viral genes (Benkirane et al., 1998). The acetylation of the RelA subunit of NF-kB by p300 increases its transcriptional activity (Chen et al., 2002) and this is countered by the cellular deacetylase SIRT1 (Yeung et al., 2004). SIRT1 activity is in turn blocked by HIV-1 Tat (Kwon et al., 2008). In addition, it has been demonstrated that HIV Tat and Rev transcripts are retained in the nuclei of resting CD4+ T-cells (Lassen et al., 2006) and that numerous host microRNAs can directly or indirectly downregulate HIV-1 gene expression, contributing to the maintenance of proviral latency (Chiang & Rice, 2012).

Due to the involvement of so many cellular factors, it has been proposed that there are different degrees of latency within the T-cell population, depending on the cell type and the extracellular environment (Pace et al., 2011). A recent in vitro study of HIV-1 latency using a central memory T-cell model system has shown that IL-7-driven homeostatic replication of infected cells can induce partial virus reactivation, while stimulation of the T-cell receptor signalling pathway with anti-CD3/anti-CD28 antibody induced full reactivation (Bosque et al., 2011). This supports the hypothesis of a dynamic reservoir of infected T-cells at various levels of cellular and viral activation.

An area of research which has, as yet, escaped the attention of the HIV-latency field is the molecular mechanism behind CD4+ T-cell quiescence. It has been known for some time that the quiescence state is actively maintained by factors such as LKLF, Tob, Foxo3a and Foxj1 (Coffer & Burgering, 2004; Tzachanis et al., 2004; Yusuf & Fruman, 2003). The role of these factors in HIV-latency has been explored by few laboratories so far (Haaland et al., 2005; van Grevenynghe et al., 2008) and further research may provide new insights into the mechanism of latency as well as potential therapeutic targets.

**In vitro and in vivo models of latency**

The latently infected CD4+ T-cell population within the patient is very small, thus making ex vivo experiments very difficult. The use of in vitro and in vivo models of latency has been and will continue to be vital to the understanding of HIV-1 latency and drug discovery. Early studies of lentiviral latency using cell lines such as ACH-2, U1 and J-Lat showed the involvement of host cytokine signalling pathways and chromatin reorganization in modulating latency (Folks et al., 1987, 1989; Jordon et al., 2003), but their transformed nature means their responses to treatments may not be physiologically relevant. For example, in the latently infected J-Lat cell line, HIV-1 preferentially integrates near the heterochromatin where transcriptional activity is low (Jordon et al., 2003). However, this preference is not observed within the latently infected resting CD4+ T-cells from HAART-treated patients, rather the provirus overwhelmingly favours integration into active transcriptional regions (Han et al., 2004; Schröder et al., 2002).

Most of the current in vitro models of HIV-1 latency involve the use of primary cells (Yang, 2011). These experiments are technically challenging, often taking weeks or months to complete in order to mimic the transition of activated T-cells to quiescent memory T-cells in vivo (Marini et al., 2008). Generating enough cells for experiments, especially in high-throughput screening of compounds, is another problem. Strategies such as the transduction of a survival gene into primary cells (Yang et al., 2009), using low levels of cytokines such as IL-2 or IL-7 (Bosque & Planelles, 2009; Marini et al., 2008) or co-culture with a feeder cell line (Sahu et al., 2006; Tyagi et al., 2010) have been described. Protocols to directly infect purified resting T-cells ex vivo have also been developed. To overcome the inefficient nature of infecting resting T-cells, methods such as spinoculation (O’Doherty et al., 2000) or stimulation with the chemokines CCL19 and CCL21 (Saleh et al., 2007, 2011) were used. The pros and cons of these in vitro model systems have been reviewed elsewhere (Pace et al., 2011; Wightman et al., 2012; Yang, 2011). Any model of latency would have to balance multiple conflicting demands such as maintaining the viability of the cells, while preserving a resting state and allowing viral integration without stimulating full-blown virus replication. A further complication is the fact that there are multiple types of cells that can be latently infected, such as central memory T-cells, transitional memory T-cells and naive T-cells (Chomont et al., 2009; Wightman et al., 2010); any future treatments to reactivate the latent proviruses would have to be effective in all of these subsets of latently infected T-cells.

Non-human primates, in particular rhesus macaques infected with the simian immunodeficiency virus (SIV) or chimeric SIVs containing HIV-1 reverse transcriptase have been used to model HIV-1 latency in HAART-treated patients (Dinuso et al., 2009a; North et al., 2010; Shen et al.,...
immature CD4+ cells have led to the development of other, non-primate models for studying SIV. The complexity of finding the correct host and SIV strain is such that the locations of the persistent viral reservoirs mirror those in humans (North et al., 2010), which allows for comparative in vivo studies. Also, the progression of SIV in macaques resembles HIV-1 infection in humans, with distinctive acute and chronic phases of infection that may lead to immunodeficiency (Hirsch & Paiardini, 2011). However, there are significant differences between SIV infection of non-human primates and HIV-1 infection in humans. For example, the residual viraemia for SIV in rhesus macaques during chronic infection is higher than the levels seen in humans (Brenchley & Paiardini, 2011). The progression to AIDS appears to be more rapid in rhesus macaques than in humans (North et al., 2010). In African green monkeys or sooty mangabeys, although high levels of virus replication are observed during the chronic phase of infection, this is not accompanied by the destructive chronic immune activation seen in rhesus macaques or humans (Brenchley & Paiardini, 2011; Chahrouri et al., 2012). Also, different strains of SIV can produce different pathologies in the same host (Hirsch et al., 2000).

The complexity of finding the correct host and SIV strain combination that mimics HIV-1 latent infection most closely, together with issues such as ethical concerns and high cost, has led to the development of other, non-primate animal models for HIV-1 infection such as humanized SCID (SCID-hu) mouse models (Boberg et al., 2008; Brooks et al., 2001; Van Duyne et al., 2009). SCID-hu mice are created by transplanting SCID mice with human fetal thymus and liver tissues or peripheral blood lymphocytes to form SCID-hu Thy/Liv and SCID-hu PBL mice, respectively (Van Duyne et al., 2009). For example, an in vitro model of latently infected immature CD4+CD8+ thymocytes has been generated using SCID-hu Thy/Liv mice (Brooks et al., 2001; Burke et al., 2007). A major drawback of using SCID-mouse-based models is the failure to fully reconstitute the human immune system within the transplanted animals (Rossi et al., 2001; Van Duyne et al., 2009). Further improvement to efficiency of engraftment was achieved with the generation of the NOD/SCID mouse model (Hesselton et al., 2003). The major advantage of using non-human primates is that the locations of the persistent viral reservoirs mirror those in humans (North et al., 2010), which allows for comparative in vivo studies. Also, the progression of SIV in macaques resembles HIV-1 infection in humans, with distinctive acute and chronic phases of infection that may lead to immunodeficiency (Hirsch & Paiardini, 2011). However, there are significant differences between SIV infection of non-human primates and HIV-1 infection in humans. For example, the residual viraemia for SIV in rhesus macaques during chronic infection is higher than the levels seen in humans (Brenchley & Paiardini, 2011). The progression to AIDS appears to be more rapid in rhesus macaques than in humans (North et al., 2010). In African green monkeys or sooty mangabeys, although high levels of virus replication are observed during the chronic phase of infection, this is not accompanied by the destructive chronic immune activation seen in rhesus macaques or humans (Brenchley & Paiardini, 2011; Chahrouri et al., 2012). Also, different strains of SIV can produce different pathologies in the same host (Hirsch et al., 2000).

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The feline model of HIV-1 latency

Feline immunodeficiency virus (FIV) was discovered in 1986 in California (Pedersen et al., 1987). Both HIV-1 and FIV target activated CD4+ T-cells (Yamamoto et al., 1988; Zagury et al., 1986), but whereas the primary receptor for HIV-1 is CD4 (Dalglish et al., 1984), the primary receptor for FIV is CD134 (OX40) (Shimojima et al., 2004). HIV-1 utilizes CCR5 and CXCR4 as its secondary receptors (Choe et al., 1996; Deng et al., 1996; Peng et al., 1996), while FIV uses CXCR4 alone as its sole secondary receptor (Willet et al., 1997). FIV is transmitted mainly by biting (Yamamoto et al., 1989) and causes clinical signs in cats that are similar to AIDS in humans (Ackley et al., 1990; Barlough et al., 1991; Novotney et al., 1990).

Since FIV has a similar cell tropism to HIV-1, it is expected that the host response to FIV and its pathogenesis will be comparable to HIV-1. Cats mount both humoral and cytotoxic T-cell responses to FIV infection (Beatty et al., 1996; Egberink et al., 1992; Flynn et al., 2002). However, the hosts usually fail to clear the infection and may succumb to immunodeficiency. The mechanisms of pathogenesis of HIV-1 and FIV are remarkably similar. Both viruses cause massive depletion of the gastrointestinal tract CD4+ T-cell population (Brenchley et al., 2004a; Howard et al., 2010). The low fidelity of the HIV-1 and FIV reverse transcriptases results in the generation of a diverse pool of viral variants within the host, encouraging immune escape (Bebene et al., 1993; Hosie et al., 2011; Kraase et al., 2010; Mansky & Temin, 1995). All of these factors promote chronic immune activation, eventually leading to the breakdown of the host immune system (Douek et al., 2009; Tompkins & Tompkins, 2008).

Previous studies of FIV in cats have shown that activated CD4+CD25+ and resting CD4+CD25− CD4+ T-cells from peripheral blood can be latently infected ex vivo and that FIV replication can be reactivated by the application of ConA or IL-2 (Joshi et al., 2005, 2004), mirroring the crucial role of IL-2 in productive infection with HIV-1 (Oswald-Richter et al., 2004). In a separate study, cats challenged with a low-dose exposure to FIV-infected T-cells showed an aviraemic infection, and when cells from multiple tissues were stimulated by PMA, FIV gp120 production was detected (Assogba et al., 2007). More recently it has been shown that FIV establishes a latent infection within activated and resting T-cells of cats during the asymptomatic phase of infection, similar to the latent infection of the resting T-cell population by HIV-1 in humans (Murphy et al., 2012). These cells contained detectable FIV DNA but no FIV RNA. Furthermore, virus replication from these latently infected cells could be reactivated ex vivo by the mitogens PHA and PMA as well as the histone deacetylase inhibitor SAHA (McDonnel et al., 2012) (see Novel drug discovery). These findings support the proposal of using FIV-infected cats as an alternative small animal model for HIV-1 latency.

Stem cell transplantation and gene therapy approaches to curing HIV/AIDS

Recently, a HIV-1-positive patient who developed acute myeloid leukaemia was apparently cured of HIV-1
infection after receiving an HSC transplant from a donor who was homozygous for the CCR5 Δ32 allele (Hütter et al., 2009). The patient underwent intensive chemotherapy and radiotherapy to prepare for the transplant, which presumably also eliminated almost all the infected CD4+ T-cells within the body. In addition, the patient developed graft-versus-host disease after transplantation, indicating that the transplanted cells had replaced the host immune system. HAART treatment was then stopped and in the follow-up study the patient was shown to remain free from the virus (Allers et al., 2011). To subject otherwise healthy HAART-treated patients to this potentially lethal procedure is ethically questionable and practically not viable, especially in resource-poor settings. However, this unique case has raised an interesting question regarding the kind of intervention necessary to clear the body of HIV-1: was the cure achieved by the intensive chemotherapy and radiotherapy or by the transplant of the Δ32 HSCs, which gave rise to HIV-1 resistant CD4+ T-cells? Treatment of HIV-1-positive lymphoma patients with autologous stem cell transplants failed to eliminate the virus from the body (Cillo et al., 2012), which indicated the presence of residual virus or infected cells within the extracted autologous cell population or within the host. It also demonstrated the need to make the host CD4+ T-cells immune to HIV-1 infection before transplantation.

The CCR5 Δ32 mutation abrogates infection of CD4+ T-cells by R5 HIV-1 viruses (Dean et al., 1996), the strains most frequently associated with early stage infection and which are transmitted preferentially between individuals (Margolis & Shattock, 2006). Thus, the nascent CD4+ T-cells from the transplant would be resistant to new infection. Disruption of the CCR5 gene has no apparent undesirable effects on the normal functioning of HSCs (Bai et al., 2000; O’Brien & Moore, 2000). Various techniques have been developed to disrupt the CCR5 gene ex vivo, including the use of CCR5-specific siRNAs, ribozymes, intrabodies and zinc-finger nucleases (ZFNs) (Anderson et al., 2007; Bai et al., 2000; Holt et al., 2010; Kumar et al., 2008; Swan et al., 2006). Each of these treatments has been tested in mouse models and led to the production of modified HSCs which give rise to CD4+ T-cells that are resistant to R5 HIV-1 infection. ZFNs, which are engineered endonucleases containing zinc finger domains that recognize specific DNA sequences (Urnov et al., 2005), have also been used to disrupt the CCR5 gene in CD4+ T-cells in a mouse model of HIV-1 infection (Perez et al., 2008). More recently, ZFNs targeting CD4+ T-cells have been successfully tested in a phase I clinical trial, in which the treatment was well tolerated by patients, the modified CD4+ T-cells were able to persist in the body and there were improvements on the CD4+ T-cell count and CD4+:CD8+ T-cell ratio (June et al., 2012). ZFNs that can disrupt the CXCR4 gene in CD4+ T-cells have also been developed and it has been demonstrated that they confer resistance to cells against the X4-tropic HIV-1 strains associated with late-stage infection (Wilen et al., 2011; Yuan et al., 2012). Combining the disruption of CCR5 and CXCR4 may provide a viable gene therapy approach to a functional cure, in which the patient’s CD4+ T-cells are made resistant to HIV-1 ex vivo and are reintroduced back into the body. There may still be residual viremia but the virus would not cause disease after the withdrawal of HAART. Potential problems with the use of the ZFNs include the possibility of non-specific cleavage of host DNA (Gabriel et al., 2011; Pattanayak et al., 2011) and the possibility of adverse effects from disrupting CXCR4, which has not been well studied at the time of writing.

**Novel drug discovery**

The main strategy that is currently being pursued by many laboratories to eradicate HIV-1 from the body is to reactivate the latent virus reservoir within resting CD4+ T-cells (Marsden & Zack, 2009; Richman et al., 2009). Early attempts at reactivation using powerful cytokines such as IL-2 and TNF-α stimulated virus production (Chun et al., 1998) but also caused dangerous side effects such as the non-specific, global activation of T-cells (Prins et al., 1999). In contrast, the cytokine IL-7 has also been shown to have potent anti-HIV latency effects without inducing T-cell activation (Levy et al., 2009; Scripture-Adams et al., 2002; Wang et al., 2005). IL-7 is well-tolerated in vivo (Levy et al., 2012) and it has been used in a clinical trial to reduce the latent reservoir size (ERAMUNE 01, due to finish in January 2013. [http://clinicaltrials.gov]). However, a potential problem with the use of IL-7 to deplete the latent reservoir is that at low concentrations, IL-7 can promote the survival or induce the homeostatic proliferation of the latently infected memory T-cells without triggering activation of the virus, thus inadvertently expanding the reservoir of infected cells (Chomont et al., 2009; Marini et al., 2008).

Compounds that stimulate protein kinase C and NF-κB such as the phorbol ester prostratin and 5-hydroxynaphthalene-1,4-dione (5HN) have been shown to reactivate latent infection in vitro (Kulkosky et al., 2001; Yang et al., 2009). Intriguingly, prostratin also has anti-HIV-1 replication effects (Biancotto et al., 2004; Rullas et al., 2004) and a similar dual effect of the compound on FIV replication has been described in vitro (Chan et al., 2013). Histone deacetylase inhibitors such as valproic acid and suberoylanilide hydroxamic acid (SAHA or Vorinostat) have been shown to reverse HIV latency by remodelling the HIV-repressive nucleosome nuc-1 (Archin et al., 2009; Contreras et al., 2009; Van Lint et al., 1996; Ylisastigui et al., 2004). SAHA is a selective class I and II histone deacetylase inhibitor and is approved as a clinical treatment for cutaneous T-cell lymphoma. It has been used in a number of ex vivo and clinical studies of the latent reservoir (Archin et al., 2012; Shan et al., 2012). However, further research is required to investigate fully the long-term side effects of SAHA in terms of its potential as a mutagen and its ability to reactivate other latent viruses (Archin et al., 2012; Kerr et al., 2010; Wightman et al., 2012). Using a siRNA screen, a novel HIV replication-inhibiting host factor has been identified recently (Zhu et al., 2012). This factor, named...
bromodomain containing 4, can be inhibited by a small molecule known as JQ1 (Filippakopoulos et al., 2010) and JQ1 has been shown to have HIV-1 latency reversing activity by several laboratories (Banerjee et al., 2012; Li et al., 2013; Zhu et al., 2012). Other compounds and small molecules that have been shown recently to reactivate latent HIV-1 infection include a bacterial protein named HIV-1-reactivating factor (Wolschendorf et al., 2010), the aldehyde dehydrogenase inhibitor disulfiram (Xing et al., 2011) and a number of quinolin-8-ol derivatives (Xing et al., 2012). Curiously, a recent clinical trial has demonstrated that intensification of HAART with the CCR5 antagonist Maraviroc (MVC) caused a reduction in the size of the latently infected T-cell reservoir (Gutiérrez et al., 2011). The mechanism behind this effect of MVC is unknown, but the binding of MVC to CCR5 may lead to the stimulation of cell signalling analogous to the stimulation of the other major HIV-1 co-receptor, CXCR4, by the binding of HIV-1 Env (Wu & Yoder, 2009; Yoder et al., 2008). If the findings of this study are confirmed, a new way of reactivating latently infected cells may have been identified. Moreover, it has been shown recently that fever enhances the activity of Tat, a phenomenon mediated by the heat-shock protein Hsp-90 (Roesch et al., 2012). Using a J-Lat model, this study demonstrated that although hyperthermia by itself cannot reactivate latency, it can enhance the reactivation effect of other treatments such as the co-cultivation of the J-Lat cells with IL-2 supplemented PBMCs (Roesch et al., 2012). This suggests that the artificial induction of fever may be used to boost the effectiveness of any future latency reversing therapies.

Borrowing from the concept of HAART, a combination of different latency reversing agents may be used synergistically to enhance the effect of reactivation therapies (Burnett et al., 2010; Deeks et al., 2012; Reuse et al., 2009). The number of compounds identified is likely to increase thanks to on-going and future high-throughput screens that look for molecules which can stimulate latent HIV-1 to reactivate. One novel screening method described recently can measure the expression of cell-associated viral RNA among latently infected T-cells as soon as the cells are extracted from the patient without the need for further co-culturing (Archin et al., 2012). Using this assay, an increase in viral RNA can be demonstrated amongst the resting T-cells from patients after being treated with a single dose of SAHA. However, an important caveat to this type of experiment is that stimulation of viral transcription, viral protein synthesis or even virion production may not necessarily lead to the destruction of the latently infected cell (see below).

Stimulation of latent virus replication may not lead to the depletion of the viral reservoir

It has been assumed that once the latent provirus is reactivated inside a resting T-cell, the cell would die by HIV-induced cytopathic effects or be killed by the host immune response (Richman et al., 2009). However, this view has recently been challenged (Shan et al., 2012). Stimulation of resting CD4+ T-cells from HAART-treated patients with the SAHA did not reduce the size of the latent reservoir (Shan et al., 2012). Furthermore, latently infected resting T-cells reactivated by SAHA were killed neither by viral cytopathic effects, nor by autologous CD8+ T-cells isolated from the same patients. Only after antigen-specific stimulation of the autologous CD8+ T-cells was efficient killing of the SAHA-reactivated, infected resting CD4+ T-cells restored. The transduction of the survival gene Bcl-2 into the resting T-cells during the establishment of the latent infection assay, as well as the use of modified reporter viruses may have increased the survival rate during the study. However, reactivation by SAHA of the latent wild-type virus within unmodified resting T-cells isolated from patients also did not lead to a contraction of the latent virus reservoir. These findings showed that any future therapeutic regimen to eliminate the latent reservoir would require the boosting of anti-HIV cytotoxic T-lymphocyte (CTL) responses, which would likely be in a state of exhaustion after years of chronic activation (Trautmann et al., 2006). In addition to stimulating the CD8+ T-cells with viral antigens and cytokines, inhibiting the function of immunoregulatory molecule such as PD-1 may be another option for the restoration of full CTL function in the patient against HIV-1 (Eichbaum, 2011). Also, it is known that resting T-cells are less vulnerable to cell death compared with their activated counterparts (van Leeuwen et al., 2009). Would the use of drugs that stimulate cellular activation, such as prostratin, lead to the death of the reactivated infected T-cells? Alternatively, is it possible to use novel technologies such as nanoparticles (Peer et al., 2007), intrabodies (Pérez-Martínez et al., 2010) or RNA aptamers (Burnett & Rossi, 2012) to target the reactivated infected T-cells for destruction (Fig. 2)?

Another observation that may be a cause for concern among the ever growing literature on latency-reversing compounds is that even the most promising molecules such as prostratin, SAHA and JQ1 cannot reliably stimulate productive infection from all HAART-treated patients' samples, despite being very successful in reactivating latent viruses from in vitro models (Contreras et al., 2009; Kulksosy et al., 2001; Zhu et al., 2012). The variable performances of these compounds may be due to sampling errors as a result of the fact that there are so few latently infected cells within the patient (hence the need for in vitro model systems), or the underlying activation status of the cells, as in the case for prostratin (Chan et al., 2012; Kulksosy et al., 2001). Alternatively, this may indicate that the current in vitro models do not represent all the subset of CD4+ T-cells that are latently infected. Also can we assume that our current methods of handling CD4+ T-cells accurately reproduce in vivo conditions? Nevertheless, the potential for false negatives and false positives in the current assays demands further research into the basic molecular biology of HIV-1, T-cell biology and improvements to existing HIV-latency models.
Conclusion
After more than two decades of research we are only beginning to appreciate the full complexity of the problem of HIV-1 persistence and latency. Recent research suggests that there are multiple reservoirs of replication-competent virus which contribute to viral persistence. To achieve a sterilizing cure of HIV-1 requires significant disruption or even elimination of all these reservoirs. In addition, there are still many unanswered questions regarding HIV-1 latency remaining. For example, what is the source of the persistent low-level viremia? What is the contribution of direct infection of resting T-cells to the overall size of the viral reservoir? Can gene therapy lead to a functional cure of HIV-1? How do we eliminate the infected T-cells once they are reactivated? Research into novel small animal models of HIV-infection may speed up the drug development process but their relevance to the clinic needs to be established. Further research into these issues is needed urgently in order to stop the global HIV/AIDS epidemic, which continues to be a serious global threat to public health almost 30 years after its discovery.

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