Insight into HIV-2 latency may disclose strategies for a cure for HIV-1 infection

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Abstract

HIV-1 and HIV-2 originate from two distinct zoonotic transmissions of simian immunodeficiency viruses from primate to human. Although both share similar modes of transmission and can result in the development of AIDS with similar clinical manifestations, HIV-2 infection is generally milder and less likely to progress to AIDS. HIV is currently incurable due to the presence of HIV provirus integrated into the host DNA of long-lived memory cells of the immune system without active replication. As such, the latent virus is immunologically inert and remains insensitive to the administered antiviral drugs targeting active viral replication steps. Recent evidence suggests that persistent HIV replication may occur in anatomical sanctuaries such as the lymphoid tissue due to low drug penetration. At present, different strategies are being evaluated either to completely eradicate the virus from the patient (sterilising cure) or to allow treatment interruption without viral rebound (functional cure). Because HIV-2 is naturally less pathogenic and displays a more latent phenotype than HIV-1, it may represent a valuable model that provides elementary information to cure HIV-1 infection. Insight into the viral and cellular determinants of HIV-2 replication may therefore pave the way for alternative strategies to eradicate HIV-1 or promote viral remission.

Keywords: HIV-2, HIV-1 latency, cure strategies

Introduction

HIV latency represents the major impediment towards viral eradication in patients receiving combination antiretroviral therapy (cART). The stable latent reservoir comprises cells carrying integrated proviral genomes that are transcriptionally inactive, that is, not producing viral particles. Latently infected resting memory CD4+ T cells represent the major constituents of the viral reservoir, allowing for long-term viral persistence [1,2]. Other potential contributors include naive CD4+ T cells [3,4], cells of the monocyte/macrophage lineage [5,6] and dendritic cells [7,8]. The HIV-1 latent reservoir is established early on during acute infection [9,10]. Recent evidence suggests that ongoing HIV-1 replication may occur in lymphoid tissue due to poor drug penetration [11]. In contrast to HIV-1, HIV-2 is known to be considerably less virulent and less likely to progress to AIDS [12]. HIV-2 infection is generally asymptomatic in most patients [13]. Although the amounts of proviral integrated DNA are similar in HIV-1 and HIV-2 infection, HIV-2 infection is associated with a lower accumulation of viral mRNA in the cytoplasm [14]. These data may suggest a block in viral replication at the postintegration level. Comparing HIV-1 and HIV-2 pathogenesis may provide future clues for a (functional) cure for HIV infection.

Clinical course and treatment for HIV-2

Compared with HIV-1, HIV-2 is predominantly restricted to west Africa [15] and regarded as less pathogenic [16]. On the contrary, HIV-1 is the causative agent of the current worldwide HIV pandemic. Table 1 lists some of the differences between HIV-1 and HIV-2 pathogenicity. HIV-2 and HIV-1 share a similar transmission route, followed by the same viral replication steps and overall pathogenesis. HIV-2 is less infectious than HIV-1, with a fivefold lower rate of sexual transmission and a 20- to 30-fold lower rate of vertical transmission from mother to child [12,16–18]. Compared with HIV-1, after initial infection of a focal founder population, HIV-2 more slowly progresses towards AIDS, with clinical symptoms occurring at later time points after initial infection [19,20]. Of the people infected with HIV-1, 5–15% are considered to be long-term non-progressors in contrast to 86–95% of people infected with HIV-2 [21]. Relative to HIV-1, the clinical course of HIV-2 infection is characterised by a longer asymptomatic stage (10 years or more). A cohort study showed that the mortality risk of HIV-2-infected patients is twofold higher than that of uninfected individuals [22,23]. Even during the symptomatic stage, the survival time of HIV-2-infected patients is longer than that of patients with HIV-1 AIDS (reviewed in [24], Table 1).

In contrast to the extensive knowledge and clinical information on HIV-1 treatment, no optimal treatment strategy has been defined for HIV-2. Studies of virological and immunological responses to antiretroviral therapy have demonstrated a higher CD4+ T cell increase in HIV-1-infected patients than in HIV-2-infected patients after initiation of antiretroviral therapy [25,26]. HIV-2 is intrinsically resistant to non-nucleoside reverse transcriptase inhibitors and to enfuvirtide. These factors, combined with the absence of well-controlled clinical trials using cART for HIV-2 treatment, challenge optimal HIV-2 treatment. No experimental data are available to decide that cART should be initiated at a different time point for HIV-2 compared with HIV-1-infected patients [27]. A recent, large systematic review of cART in HIV-2-infected patients (n=17 studies with 976 HIV-2-infected patients) was unable to conclude which specific regimens should be recommended [28].

Pathogenesis of HIV-1 and HIV-2 infection

The lower infectivity of HIV-2 is likely to be related to lower RNA transcription levels and lower plasma viral loads [13,29]. A comparative cell culture study on the kinetics of viral replication for HIV-1 and HIV-2 primary isolates detected a similar pattern of replication in T cells derived from peripheral blood mononuclear cells (PBMCs) [30]. However, in macrophage-derived monocytes (MDMs) the titres of HIV-2 measured at 21 days post infection were significantly lower than those of HIV-1. Unlike HIV-1, HIV-2 showed an initial burst of virus production in MDMs followed by a subsequent latency phase [30]. A more recent *in vitro* study on HIV-2 infection in CD4+ T cells and monocyte-derived dendritic cells (MDDCs) showed HIV-2 to replicate efficiently in activated CD4+ T cells [31]. Unstimulated CD4+ T cells were not productively infected by HIV-2, but viral replication was triggered upon lymphocyte activation. Furthermore, MDDCs were poorly infected

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Table 1. Comparison of HIV-1 and HIV-2 clinical outcome

	HIV-1	HIV-2
Geographic distribution	Worldwide	Restricted to West African countries [15], with limited spread outside this area [114–118]. HIV-2 has also been reported in former Portuguese colonies, such as Angola, Mozambique, and Brazil, and in parts of India such as Goa and Maharashtra [18].
Viral load and CD4+ count	High in acute illness, increases steadily in cells during the asymptomatic stage of HIV infection, correlated with the loss of CD4+ cells [119].	Lower plasma viral loads [16], with matching CD4+ count [120].
Transmission	By sexual route, mother-to-child, blood-borne (through injection).	By sexual route, mother-to child, blood-borne (through injection).
Duration of asymptomatic stage	The time between HIV infection and the development of AIDS varies, ranging from a few months to many years, with an estimated median time of 9.8 years (reviewed in [121]).	Longer duration, could be over 18 years [122].
Clinical illness	If untreated, around half of people infected with HIV-1 develop AIDS within 10 years.	86–95% of people infected with HIV-2 are long-term non-progressors [21].
Treatment	cART: the combination of three antiviral drugs. Two NRTIs + integrase inhibitor or protease inhibitor	Naturally resistant to non-nucleoside analogues targeting reverse transcriptase [123] and to enfuvirtide (T20) [124]. Recommended treatment: two NRTIs plus an appropriate boosted PI, such as lopinavir, saquinavir or darunavir.

NRTI: nucleoside reverse transcriptase inhibitor; PI: protease inhibitor.

Table 2. Differential replication characteristics in primary cells

Cell type	HIV-1	HIV-2
Human thymus	HIV-1 can replicate efficiently in thymus tissue [80,125].	HIV-2 is able to infect the human thymus but this is associated with limited viral replication. The block in HIV-2 replication is at a post-transcriptional level [80].
Peripheral blood mononuclear cells (PBMCs)	HIV-1 can infect PBMCs efficiently; cells readily undergo apoptosis and necrosis (reviewed in [126].	Similar pattern of infection like HIV-1 [30]. However, infection of PBMCs with HIV-2 induces lower level of apoptosis than HIV-1 [127].
Activated CD4+ T cells	HIV-1 efficiently replicates in these cells (about 40% of the cells are Gag+ at day 4 post infection [31].	Lower level of HIV-2 replication in primary activated CD4+ T cells, with 5–25% of cells infected [31].
Resting CD4+ T cells	HIV-1 can enter resting CD4+ T cells without progression to viral production. These cells can be infected *in vivo* and serve as a latent viral reservoir (reviewed in [128]).	These cells poorly support infection; no viral replication, less than 2% of non-stimulated cells are productively infected [129]. Unstimulated CD4+ T cells are not productively infected by HIV-2, but viral replication can be triggered on lymphocyte activation [31].
Monocyte-derived dendritic cells (MDDCs)	DC can become infected and are able to effectively transmit infection to CD4+ T cells [130].	Low efficiency of infection with HIV-2 primary isolates. HIV-2 is not propagated in mDCs even after 96 hours post infection [131]. MDDCs can be efficiently infected with the laboratory-adapted HIV-2 ROD strain pseudo-typed with VSV-G [132,133].
Macrophage-derived monocytes (MDMs)	HIV-1 efficiently infects MDMs and may continue to produce virus up to 40 days [134].	Initial burst of viral production in MDMs followed by an apparent latency phase [30].

when exposed to HIV-2. Therefore HIV-2 possibly avoids an MDDC-mediated immune response trigger [31]. The difference between HIV-1 and HIV-2 in their ability to replicate in different primary cells is summarised in Table 2.

Several factors might be responsible for the reduced pathogenicity of HIV-2 *in vivo*. One of the determinants is the host cell tropism and preference for cellular co-receptors. CCR5 and CXCR4 form the major HIV-1 co-receptors and usually require an initial interaction of the viral envelope glycoproteins with the CD4+ receptor [32]. HIV-2 strains are also capable of interacting with the CCR5 and/or CXCR4 co-receptors to enter into CD4+ cells. Compared with HIV-1 strains, however, many primary HIV-2 strains utilise a broader range of co-receptors. Other co-receptors that mediate the entry of some M-tropic HIV-1 and HIV-2 strains include Bonzo/STRL33, Bob/GPR15, US28, CCR8, CX3CR1/V28 and CCR9 [33–35]. Moreover, HIV-2 and also simian immunodeficiency virus strains are able to interact with co-receptors quite efficiently in the absence of the CD4+ receptor [36–38]. A study of the HIV-2 tropism in the largest available antiretroviral-naive population showed that X4-tropic viruses represented only 13% of isolates in this population but were associated with a lower CD4+ cell count [39]. In HIV-1 infection, the shift from R5 to X4-tropic viruses during disease progression is generally associated with a decline in CD4+ cell counts and faster disease progression [40]. A similar association between X4 variants and disease has been observed for HIV-2 [34,41,42].

A stronger immunological control of HIV-2 infection

Significant differences in immune response to HIV-1 versus HIV-2 have been pinpointed in several studies [19]. The humoral immune response is more efficient in controlling HIV-2 than HIV-1 replication. Studies showed that compared with HIV-1, the HIV-2 envelope is highly immunogenic, exposing multiple cross-reactive epitopes with fewer glycosylation sites in the V3 domain [43–45]. A study in ART-naive patients, with a focus on heterologous neutralisation, suggested that HIV-2 induces a broader range of neutralising antibodies but with a lower potency than those induced by HIV-1 [46]. However, a more recent study showed that these HIV-2 responses may be more potent than previously suggested [44]. *Ex vivo*, plasma from HIV-2-infected subjects was

shown to neutralise a greater proportion of HIV-2 viruses than plasma from HIV-1-infected subjects [47].

In addition to the humoral response, virus-specific immune responses are strongly associated with a better viral control in HIV-2 infection. HIV-2-infected subjects preserve polyfunctional virus-specific T cell responses better than their HIV-1-infected counterparts [48–50]. Assessing interferon (IFN)-γ and interleukin (IL)-2 production by HIV-1- and HIV-2-specific CD4+ T cells suggested that HIV-2-specific CD4+ T cells are capable of producing IFN-γ, IL-2 or both, whereas HIV-1-specific CD4+ T cells are capable of producing only IFN-γ [48]. In-depth analysis of multiple T cell functions from asymptomatic individuals or individuals at a non-progressive stage of the infection indicated that HIV-2-infected individuals mount a functionally superior HIV-specific T cell response characterised by highly polyfunctional HIV-specific CD4+ and CD8+ T cells [49]. This polyfunctional HIV-2-specific T cell response is a hallmark of non-progressive HIV-2 infection and may play a role in maintaining HIV-2 viral loads below undetectable levels and delaying disease progression seen in HIV-2 infection [48,49]. The HIV-2-specific CD4+ T cell response is also characterised by 25% of responding cells producing the CCR5-binding chemokine MIP-1β. MIP-1β contributes a small but substantial proportion of the HIV-2-specific CD4+ T cell response, but is almost entirely absent in the HIV-1-specific CD4+ T cell response. MIP-1β produced by HIV-2-specific CD4+ T cells competes with HIV-2 for the CCR5 receptor and may be controlling HIV-2 replication by blocking infection of susceptible [51] cells and contributing to a better clinical outcome [49]. In the asymptomatic phase of infection, the natural killer cell response is more pronounced in HIV-2-infected people than in HIV-1-infected people with a normal CD4+ cell count [52]. The Nef protein of HIV-2 was shown to downregulate the T cell CD3 receptor within infected cells and to block the response to T cell activation [53], thereby suppressing T cell responsiveness to activation and activation-induced cell death. Asymptomatic HIV-1 patients appear to have higher levels of cells in apoptosis and cell death than asymptomatic HIV-2 patients [54]. In addition, the HIV-2 envelope protein (gp105/gp36) has stronger inhibitory properties on T-cell receptor–mediated lymphoproliferative responses than that of HIV-1 [55]. The adaptive immunity gets activated on acute HIV infection. Sousa *et al.* [51] reported that in HIV-2 and HIV-1 patients there was a comparable degree of CD4+ depletion, and the up-regulation of CD4+ and CD8+ cell activation markers (HLA-DR, CD38, CD69, Fas molecules) was similar, even though the viral load in the plasma of HIV-2-infected patients is two orders of magnitude lower than in HIV-1-infected patients. HIV-2 non-progressors have low rates of T cell turnover (both CD4+ and CD8+) and minimal immune activation. The primary phenotypic difference between T cells in HIV-2 non-progressors and progressors therefore appears to relate to their very disparate levels of immune activation [56].

The role of the innate immune response to HIV-1 and HIV-2 infection of plasmacytoid dendritic cells (pDC) has been recently examined using genome-wide expression analysis [57]. Whereas HIV-1 is known to induce a rapid dysregulation of innate immune responses, promoting the excessive and prolonged production of IFN-I [58,59], HIV-2 induces a gene expression pattern distinct from HIV-1, characterised by a lower expression of type I IFN (IFN-I) genes and a lower secretion of IFN-I [57]. HIV-2 favoured pDC differentiation into cells with an antigen-presenting cell (APC) phenotype rather than IFN-α-producing cells. This preferential induction of an APC phenotype may critically contribute to the lower pathogenicity observed during HIV-2 infection [57]. The host innate immune system might also control HIV-2 infection through the tripartite motif-containing protein 5 (TRIM5α) pathway. TRIM5α acts by binding to a motif on the viral capsid protein and interferes with later steps of infection by altering the intracellular trafficking of infecting virions [60]. A recent study showed that HIV-2 capsids have higher susceptibility to hTRIM5α than observed for HIV-1 [61]. The susceptibility of HIV-2 to hTRIM5α does not appear to play a determinant role in the differences in pathogenic profiles observed among HIV-2-infected patients. However, it may contribute to the overall reduction in replication and propagation of this virus in humans. A recent study showed that the HIV-2 accessory protein, Vpx, inhibits IFN regulatory factor family member 5 (IRF5)-mediated transactivation *in vitro*. IRF5 have been pinpointed as critical transcription factors functioning in immune responses. Overexpression of Vpx reduces the production of IL-6, IL12p40 and TNF-α [62]. These data suggest a role for the Vpx–IRF5 interplay in the innate immune response, providing an additional level of viral control.

HIV-2 has a higher tendency for latency than HIV-1

HIV latency can be subdivided into two forms: pre-integration and postintegration latency. Pre-integration latency refers to presence of unintegrated HIV-1 DNA located in the host cell in the form of a pre-integration complex (PIC). The PIC will eventually either degrade or integrate into the host cell genome, usually following cell activation [63,64]. The occurrence of pre-integration latency has been shown to be a common latent form *in vivo* [63,65] and may represent the majority of viral DNA [65,66]. Macrophages, which are a naturally non-dividing cell population, are able to sustain large amounts of unintegrated HIV-1 DNA for up to 30 days. This unintegrated HIV-1 DNA in macrophages may significantly contribute to viral pathogenesis in infected individuals [67]. Unintegrated HIV-1 DNA is likely to present a stable reservoir in slowly dividing or non-dividing cells and can reside near the centromere of the resting cells for weeks [68]. This unintegrated virus can replicate, although not very efficiently [69]. Not much is known about the contribution of pre-integration latency to HIV-2 infection. The nuclear transport of the HIV-2 PIC is efficient due to the presence of Vpx [70]. Vpx is important for optimal nuclear translocation of HIV-2 PIC DNA not only in quiescent MDMs [71], but also in dividing lymphocytic cells, in contrast to HIV-1 Vpr [33,72]. Unintegrated viral DNA in the nucleus of infected cells includes both linear and circular forms [1-long terminal repeat (1-LTR) and 2-long terminal repeat (2-LTR) circles] [66,73]. The circular forms of viral DNA are often used as a marker for nuclear import of viral DNA during virus replication [74]. HIV-1 and HIV-2 2-LTR circular DNA production was compared in PBMCs and two cell lines (MT4-CXCR4 cells and HeLa-CXCR4-CCR5 cells) [27]. Although in HIV-2, 2-LTR circles appeared at later time points than observed for HIV-1, they rapidly became more abundant. A recent *in vitro* study on 2-LTR circles of HIV-2 suggests that this form of unintegrated proviral DNA is stable but does not necessarily reflect on ongoing replication [75].

Postintegration latency refers to the presence of integrated retroviral DNA in cells that are not actively producing viral particles. Postintegration latency contributes to the persistence of the virus under a cART regimen and represents one of the major barriers towards a complete eradication of HIV infection. Postintegration latency may occur following HIV-1 infection of activated memory CD4+ T cells and subsequent cellular relaxation to a quiescent state [76,77]. Postintegration latency can also occur when CD4+ T cells that are transitioning from an activated to a resting memory state are infected by HIV, where the cellular environment still supports viral integration but does not support proviral transcription, or by direct infection of resting CD4+ T cells [78]. An older study showed that HIV-2 was able to establish a stable integrated proviral DNA within the PBMCs of patients without active replication [14]. This study suggests the possibility of a higher tendency for HIV-2 to establish latent infection *in vivo* [14]. Another study showed that

proviral DNA levels are similar in patients with HIV-1 and HIV-2, suggesting that the slower progression of HIV-2 disease is not due to a difference in the rate of infection [13]. Postintegration HIV-2 latency has also been described after *in vitro* infection of MDMs. Addition of lipopolysaccharide, a potent LTR activator, resulted in re-stimulation of this latent virus and the production of fully infectious virions [79]. Assessment of the direct impact of HIV-2 infection on the human thymus has shown that HIV-2 is able to infect the thymus but HIV-2 replication is impaired after viral transcription [80]. These data highlight the potential importance of post-transcriptional control of viral replication in specific subsets of target cells.

In the next section, we will focus on the different mechanisms responsible for controlling postintegration latency and highlight the differences between HIV-1 and HIV-2. Differential regulation of the LTR-driven transcription might play a role in the distinct pathogenicity observed for both viruses.

Proviral transcription and latency

After integration, proviral transcription is initiated through interactions of the 5′ of the HIV LTR with Tat and the cellular transcription machinery. The HIV-2 LTR, similar to the HIV-1 LTR, is divided structurally into the U3, R and U5 regions. The 5′ LTR of HIV-2 contains the trans-activation responsive region (TAR) located downstream of the transcriptional initiation site in the R region [81,82]. Unlike the HIV-1 TAR element, which contains a single stem-loop, the HIV-2 LTR is significantly larger than HIV-1, as it contains a duplicated TAR RNA stem-loop structure (Figure 1A). A previous *in vitro* study showed that HIV-2 was able to inhibit HIV-1 replication by suppression of the HIV-1 LTR, whereas HIV-1 has no obvious effect on HIV-2 replication. The inhibitory effect appears to be related to the differences in the TAR elements [83]. In addition, the TAR structure in HIV-2 creates a block to translation [84]. The HIV-2 5′-UTR and specifically the TAR RNA structure were recently shown to slow down translation, resulting in low levels of Gag production. This sharply contrasts with protein synthesis from the HIV-1 gRNA, which occurs very efficiently [84].

The HIV-2 LTR is less responsive than the HIV-1 LTR to CD4+ T cell activation signals [85]. The HIV-2 transcriptional enhancer regions lack the nuclear factor of activated T cells binding sites and the negative regulatory elements (Figure 1B) present upstream from the promoter region in the HIV-1 LTR [85]. Subtle differences in transcriptional control elements present in the HIV-2 LTR promoter together with an altered regulation by the Tat feedback loop may distinctly affect basal transcription levels and responsiveness to environmental stimulatory agents [86]. These different mechanisms affecting the transcriptional activity and subsequent virion production may, possibly, correlate with differences in the pathogenesis between the two viruses.

HIV integration and latency

Retroviral integration site preference is genus dependent and is catalysed by the viral integrase enzyme that is tethered to the host cell chromatin by co-opting endogenous cofactors [87,88]. Retroviruses, in general, favour integration into transcriptionally active units (reviewed in [88,89]). *In vitro* studies have shown that both HIV-1 and HIV-2 have a preference for proviral integration into coding regions of the genome and actively transcribed host genes [79,90,91]. Several reports have illustrated the effect of integration site distribution and the surrounding chromatin environment on HIV-1 transcriptional activity [91–93]. Integration of HIV-1 in heterochromatin regions can result in a block in viral transcription [94,95].

Additionally, proviral integration orientation could affect HIV transcription by differentially interfering with the transcription of neighbouring genes, adding complexity to the maintenance of latency. Using a system in which HIV-1 proviruses were inserted in precisely the same position within an active host gene in either orientation, Han *et al.* demonstrated that there is orientation-dependent *cis* regulation of transcription of integrated HIV-1 by the read-through transcription of the host gene [96]. Transcriptional interference is observed when HIV-1 is inserted in the opposite orientation of the host gene, while enhancement of viral gene expression occurs when HIV-1 is in the same orientation. Orientation had a >10-fold effect on HIV-1 gene expression. For those integrations occurring within transcription units, HIV-2 was found to be integrated significantly more in the opposite direction relative to the transcriptional direction of the corresponding gene; a finding that differed from that of HIV-1 [91]. The direction of proviral integration in the reverse direction of the cellular transcript leads to transcriptional silencing and could possibly contribute to the explanation of why HIV-2 displays a more latent phenotype. When HIV-1 is integrated into the gene in the reverse orientation, it is transcribed at a low level via transcriptional interferences [97].

Epigenetic DNA methylation is yet another mechanism for transcriptional regulation. DNA methylation occurs on cytosine residues and is preferentially observed in CpG-rich sequences [98,99]. CpG islands are enriched in the rare dinucleotide CG and are often associated with gene regulatory regions containing clustered transcription factor binding sites [100]. The distance of methylated CpG islands from the transcription start site affects the gene expression regulation [101]. Data indicate that the promoter region of HIV-1 is epigenetically regulated by CpG methylation [102]. Heavily methylated promoter regions contribute to a more repressed chromatin state in HIV-1 latency [102,103]. HIV-1 favours viral integration in non-methylated chromatin characterised by a relevant transcriptional activity [104]. HIV broadly favours gene-dense chromosomal regions that contain a mixture of favourable clusters of active genes and unfavourable CpG islands [105]. In contrast to HIV-1, Moloney murine leukaemia virus (MLV) strongly favours integration near CpG islands, with 16.8% of integration found within ±1 kb of CpG islands compared with 2.1% of the randomly generated sites [105]. A more recent study showed that a strong association was observed between MLV sites and CpG islands, with 22.5% (7345) of the sites located within ±2.5 kb from ≥1 of the CpG island, compared with 4.1% of HIV-1 and 3.3% of random sites [106]. For HIV-2, integration frequency within ±1 kb of a CpG island was estimated at 2% [79,107], indicating that like HIV-1, HIV-2 disfavours integration near CpG islands and has the tendency towards integration near transcriptional start sites [79].

Role of LEDGF/p75 as epigenetic reader of the chromatin environment

The cellular transcriptional coactivator lens epithelium-derived growth factor (LEDGF)/p75 is the major cellular cofactor directing lentiviral integration. LEDGF/p75 acts as a molecular tether between integrase and chromatin, and directs lentiviral integration into active transcription units [108–110]. Recently, a cellular protein called bromodomain and extra terminal (BET) protein has been identified as the LEDGF/p75 equivalent targeting integration of gammaretroviruses. Identification of these two chromatin readers, LEDGF/p75 and BET, as tethering factors respectively for HIV and MLV integration, point to a direct link between epigenetics and efficient retroviral replication [88]. Overexpression of LEDGF fusion proteins in cells depleted for LEDGF/p75 redirected integration to the target sites of their respective chromatin-binding domains [111]. In addition, LEDGF/p75 hybrids in which the N-terminus is replaced by an alternative chromatin interaction domain, such as the heterochromatin binding element CBX1, have been shown

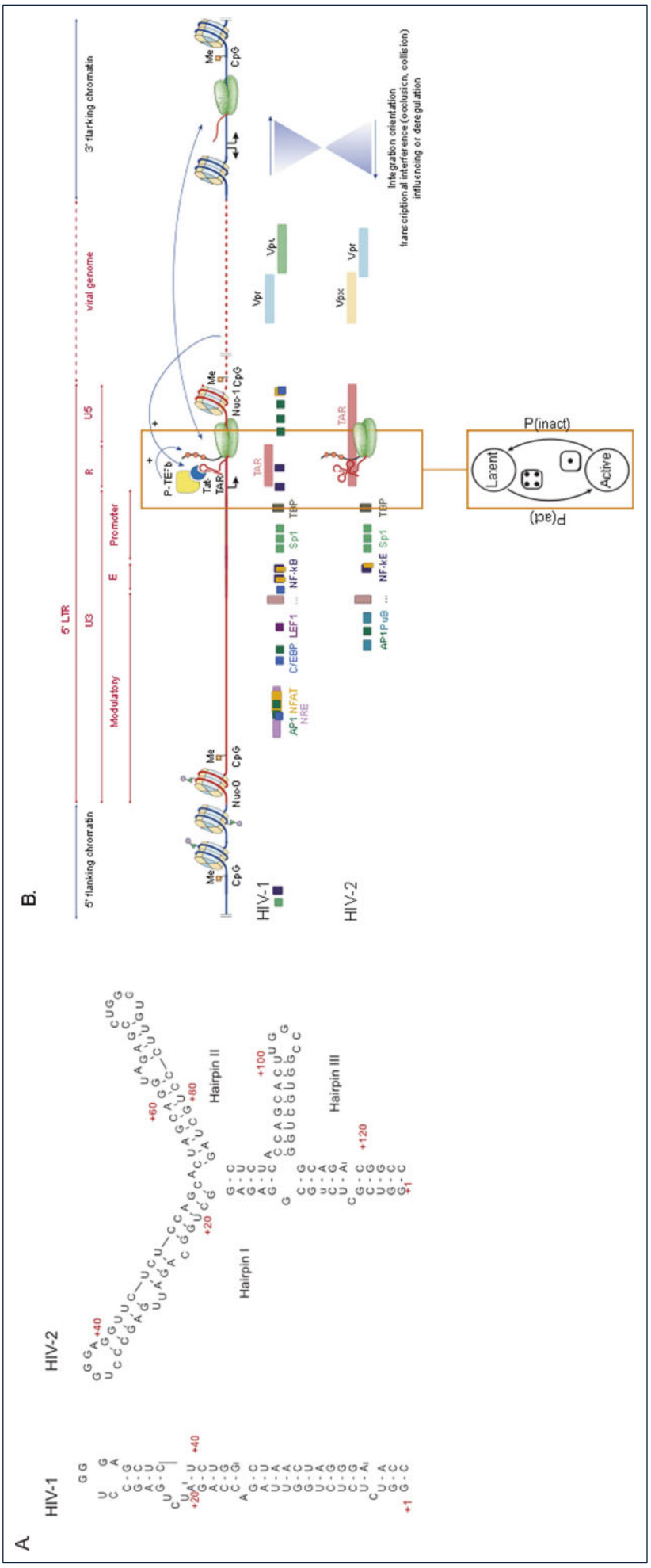

Figure 1. Intrinsic differences between HIV-1 and HIV-2. The figure highlights some of the intrinsic differences in genomic composition between HIV-1 and HIV-2 affecting transcriptional regulation. (A) Differential complexity in secondary loop structure of the trans-activation responsive region (TAR) between HIV-1 and HIV-2. The intrinsic Tat-regulatory circuitry is one of the key determinants and decision makers in driving HIV in a productive or latent infection by recruiting the positive transcription elongation complex (pTEFβ). (B) Detailed comparison of the HIV LTR structures and other factors affecting proviral transcription. Major transcription factor binding sites identified in the viral LTR are shown with respect to the structural and functional LTR divisions (compiled from multiple reviews and publications). HIV-2 displays a higher tendency to integrate in the opposite orientation with respect the open reading frame. AP 1: activator protein 1; CCAAT-enhancer-binding protein: C/EBP; LEF-1: lymphoid enhancer-binding factor 1; LTR: long terminal repeat; NFAT: nuclear factor of activated T cells; NF-κB: nuclear factor κB; NRE: negative regulatory element; pTEFβ: positive transcription elongation factor β; PuB: purine box; Sp 1: specificity protein 1; TAR: trans-activation responsive region; TBP: TATA-binding protein; Vpr: viral protein R; Vpu: viral protein unique; Vpx: viral protein X

Table 3. Evidence-based mechanisms of differential pathogenesis

Mechanisms of different pathogenesis	HIV-1	HIV-2
Mode of entry	Both CCR5 and CXCR4 are the major HIV-1 co-receptors and usually require an initial interaction of the viral envelope glycoproteins with the CD4+ receptor [32].	HIV-2 interacts efficiently with a broad range of co-receptors even in the absence of the CD4+ receptor [33–35,38]. The ability to infect host cells independent of CD4+ interaction might enhance the sensitivity to neutralising antibodies and enhance the capacity of the host to control virus replication [38].
Susceptibility to the cellular restriction factor hTRIM5α	The HIV-1 capsid is less susceptible to hTRIM5α [61].	The HIV-2 capsid is highly susceptible to hTRIM5α which might contribute in part to the lower replication and pathogenicity of this virus in humans [61].
Suppression of transcription activator IRF5	It is still unknown how HIV-1 infection affects IRF5 activation, and whether HIV-1 suppression of IRF5 enhances permissiveness of infection.	Vpx reduces the production of IL-6, IL12p40 and TNF-α, by inhibiting the function of IRF5 as a transcription activator [62]. These data suggest a role for the Vpx-IRF5 interplay in the innate immune response, providing an additional level of viral control.
LTR structure	The HIV-1 TAR element contains a single stem-loop [135]. The LTR of HIV-1 contains DNA binding sites for several cellular transcription factors including the one that is missing in HIV-2 LTR [136].	The HIV-2 LTR is significantly larger than that of HIV-1, as it contains a duplicated TAR RNA stem-loop structure [81]. HIV-2 inhibits HIV-1 replication by suppression of the HIV-1 LTR. The inhibitory effect appears to be related to the differences in the TAR elements [83]. The HIV-2 transcriptional enhancer lacks the NFAT binding site and the negative regulatory elements present upstream from the promoter region in the HIV-1 LTR [13,85], which make it less responsive to cellular activation signals [13].
Integration in the opposite orientation	HIV-1 integration in the opposite direction of the host genome is less common than for HIV-2 [91].	HIV-2 was found to be integrated significantly more in the opposite direction relative to the transcriptional direction of the corresponding gene [91]. The directionality of proviral integration in the reverse direction of the cellular transcript could possibly contribute to latent phenotype of HIV-2 [79].
LEDGF/HRP2 role in tethering of the proviral DNA into host genome	In the absence of LEDGF/p75, the related HRP2 can substitute for LEDGF/p75 as molecular tether [113].	Any role of HRP2 as molecular tether in HIV-2 is still unknown.

IL: interleukin; LEDGF: lens epithelium–derived growth factor; LTR: long terminal repeat; NFAT: nuclear factor of activated T cells; TAR: trans-activation responsive region; TNF-α: tumour necrosis factor-α; TRIM5α: tripartite motif-containing protein 5.

to re-target HIV-1 integration out of transcription units and towards heterochromatic regions [112]. In the absence of LEDGF/p75, the related hepatoma-derived growth factor-related protein 2 (HRP-2) can substitute for LEDGF/p75 as a molecular tether [113]. It remains to be studied whether HIV-1 and HIV-2 depend to the same extent on LEDGF/p75 and/or HRP-2 for integration site selection. In theory, a differential integration site selection may contribute to distinct states of latency and reduced levels of RNA transcription.

Differences in integration site selection together with an altered LTR promoter constitution and differences in integration orientation can all contribute to the distinct transcriptional phenotype between HIV-1 and HIV-2.

Conclusions

The existence of a viral reservoir of latently infected cells represents the main obstacle towards the finding of a cure for HIV-1 infection. A sterilising cure will be difficult to achieve. Therefore, a functional cure for HIV-1 infection should be considered as an attractive alternative. We have reviewed the experimental and clinical evidence for the underlying mechanisms regulating pathogenesis of HIV-1 and HIV-2. Contributing factors are the following: (1) The lower infectivity of HIV-2 in comparison with HIV-1 is likely related to lower RNA levels in the infected cells [14]. (2) Despite the lower RNA transcription levels and low plasma HIV-2 load, the level of proviral DNA in PBMC was shown to be similar in patients infected either with HIV-1 or with HIV-2 [17,79], suggesting that HIV-2 has more tendency for latency. (3) A high proportion of HIV-2 genomic proviral observed in HIV-2 infection is present in a latent form in long-lived cellular compartments such as macrophages [30]; in addition, higher tendency for latency may result from preservation of CD4+ T cells in HIV-2 infection [48]. (4) HIV-2 is more sensitive to immune control than HIV-1, probably because HIV-2 Env proteins expose multiple cross-reactive epitopes and have fewer glycosylation sites in the V3 loop than HIV-1 [43–45]. (5) HIV-2 infection causes lower rates of T cell activation and enhanced virus-specific immune responses leading to viral control in HIV-2 infections [53,55], thereby suppressing T cell responsiveness to activation and activation-induced cell death, leading to viral persistence and latency. (6) The ability of HIV-2 to infect its host cell independent of CD4+ interaction might enhance the sensitivity of HIV-2 to neutralisation compared with HIV-1 [38]. (7) The HIV-2 LTR appears less responsive to cellular activation signals [13]. (8) A differential tendency to integrate in the opposite orientation relative to the host gene may interfere with HIV-2 transcription [96]. The mechanisms of different pathogenesis between HIV-1 and HIV-2 are summarised in Table 3.

In-depth understanding of reduced HIV-2 replication *in vivo* can provide valuable clues to achieve a cure for HIV/AIDS. HIV-2 may well provide a natural model to study HIV latency and understand the viral pathogenesis of HIV-1.

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