**AIDS** Rev. 2011;13:234-9

# Evolutionary Mechanisms of Retroviral Persistence

*James B. Whitney[1], So-Yon Lim[1] and Mark A. Wainberg[2]*

*[1]Division of Viral Pathogenesis, Department of Medicine, Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, USA; [2]McGill University AIDS Centre, Lady Davis Institute-Jewish General Hospital, Montreal and Department of Microbiology and Immunology, and Faculty of Medicine, McGill University, Montreal, Quebec, Canada*

## Abstract

***Retroviruses are known to exhibit remarkable genomic pliancy, a capacity that has been attributed to one or more error prone steps in the viral replication cycle. However, increasing evidence suggests that such error represents a key element in viral survival, as exemplified by studies on virus immune evasion, shifts of cellular tropism, and anatomic compartmentalization, which facilitate persistent virus reservoirs. Understanding the dynamic mechanisms that contribute to the establishment and maintenance of retroviral persistence is critical toward the goal of attaining HIV-1 eradication.*** **(AIDS Rev. 2011;13:234-9)**

*Corresponding author: James B. Whitney, jwhitne2@bidmc.harvard.edu*

## Key words

***HIV eradication. Antiretroviral therapy. Persistence. Virus evolution.***

## Introduction

Pathogens exhibit a wealth of strategies to diversify and evade host immunity as a means of persistence. This is illustrated by the adaptive potential for transmission across species exhibited by *Mycobacterium tuberculosis*, influenza virus, and hepatitis B virus. However, no pathogen shows greater propensity for persistence than HIV-1 and the closely related simian immunodeficiency virus (SIV), since the very nature and course of lentivirus infection is manifested in shifts of nucleotide diversity. This review highlights several interrelated virus mechanisms and host factors that drive rapid viral adaptation and facilitate viral persistence.

## Replicative dynamics during lentiviral infection

High-level replication is one requisite for virus sequence variation. The acute phase of HIV or SIV infection is characterized by an exponential increase in plasma viral RNA levels[1]. It is agreed that viral genetic variation within HIV-infected humans or SIV-infected monkeys is continuous[2-4]. In this scenario, steady-state virus load, and expanding viral diversity is a function of virus replication dynamics, available target cells, and host immunity[2,3,5]. Despite the fact that viremia is controlled to a large degree during set point, virus replication continues unabated throughout the protracted asymptomatic phase and even in the setting of antiretroviral therapy[1-8].

RNA viruses such as HIV-1, by virtue of their replication kinetics and plasticity, are capable of exploring an enormous sequence space in relation to other organisms, resulting in the generation of a heterogeneous virus swarm in which all possible variants are theoretically represented[9]. However, the number of variants represented phenotypically is constrained to maintain protein function and by the imposed limits of genome size[6]. Mathematical modeling has estimated that the overall rate of HIV virus production is in the order of $10^{10}$ virions/day and the maximal replication capacity of HIV-1 *in vivo* is approximately 180 generations per year[10-12]. Approximation of the base pair substitution rate of currently circulating HIV-1 variants is 0.0024 substitutions per nucleotide per year[6]. By comparison, the mutation rate of the mammalian genome is about six orders of magnitude lower[13]. Thus, the majority of mutations that are generated in HIV are deleterious, resulting in the production of suboptimal phenotypes or noninfectious virus[1].

One important outcome of this diversity is the capacity for continuous interaction between the selective host environment and the viral quasispecies. This has serious

**Correspondence to:**
James B. Whitney
Division of Viral Pathogenesis
Beth Israel Deaconess Medical Center
Center for Life Sciences, 3 Blackfan Circle
Boston, Massachusetts, 02115, USA
E-mail: jwhitne2@bidmc.harvard.edu

implications for the selection of drug resistance-conferring mutations during antiretroviral therapy[14]. Likewise, the host's immune response is under constant challenge and it has been proposed that this may result in a broadening of immune recognition[1,15]. However, the large numbers of defective particles produced may result in the diverting of the host immune responses, such that much immune capacity is expended on battling irrelevant targets[3,16,17].

## Reverse transcriptase infidelity

Lentiviral reverse transcriptases (RT) exhibit an infidelity that is significantly greater than that of other comparable polymerases involved in nucleic acid replication. The mutation rate of HIV-1 RT is approximately $10^{-4}$ nucleotides per replication cycle, an order of magnitude higher than that of cellular polymerases[18-20]. The generation of HIV-1 nucleotide diversity has been largely attributed to the lack of a 3'-5' exonuclease activity within RT. Although this lack of proofreading ability accounts for a significant proportion of the RT error rate, other attributes of this enzyme contribute to facile nucleotide change in combination with high-level replication[1,12].

First, the RT of HIV-1 displays marked infidelity even in comparison to other retroviral RT[1,21-23]. A partial explanation for this can be gleaned from retroviral RT sequence and structural data. The catalytic domains of HIV-1, HIV-2, and SIV RT each encode a highly conserved YMDD motif, one that is generally found in all RNA-dependent polymerases. Yet, comparison of the crystal structure of HIV-1 RT and its *E. coli* homolog indicate that the polymerase active site of HIV-1 RT is partially responsible for low-fidelity nucleotide discrimination because it is physically larger. Moreover, structural analysis of HIV-1 RT complexed with a DNA duplex has shown that it is more fluid within the catalytic core and primer grip region than other RT[24,25].

Second, several biochemical studies have shown reduced nucleotide discrimination by HIV-1 RT. The importance of nucleotide sequence recognition by RT appears to be highly dependent on local template sequence, whereby repetitive nucleotide regions of As or Ts are hotspots for substitutions or insertions, resulting in frameshifts[18]. HIV-1 RT can incorporate and extend mispairs frequently, i.e. > 50 fold compared with eukaryotic DNA polymerases[20]. Moreover, highly structured lentiviral genomes can induce transcriptional pausing, highlighting the relationship between enzyme processivity and fidelity[18]. Additionally, the HIV-1 RT is capable of introducing non-template errors into RNA and DNA, with a preference for the insertion of errors into DNA templates[23]. Collectively, these findings point to an unequal contribution of the overall mutation rate during the RNA-dependent versus DNA-dependent polymerization steps of reverse transcription. A consequence is that mutations are not equally distributed in the viral genome. For example, mutations in the variable domains of the *env* gene, particularly G→A transversions, are more frequent than errors generated in the *gag* or *pol* genes, indicative of strong selective pressure at this locus[26].

## Lentivirus recombination

Both theoretical and experimental studies have shown that HIV-1 nucleotide diversity is a consequence of high-level replication and enzyme infidelity[1]. However, the significant contribution of recombination should not be overlooked. Recombination offers HIV the ability to overcome environmental stress through a re-assortment of virus alleles that combine genomic permutations under selection, ultimately resulting in the selection of recombinants with increased fitness[16,27-30]. The number of non-synonymous mutations induced by RT necessitates an ability to rapidly combine mutations into various beneficial permutations, a mechanistic requirement that can only be efficiently accomplished through recombination. Temin, et al. suggested that retroviruses exhibit features such as pseudo-diploidy and low processing rates to facilitate recombination[31-33]. Without the advantage of recombination, viral variants would be relegated to increase fitness in an iterative fashion, risking the collapse of the virus population due to error catastrophe[34,35].

Recombination events follow the copy choice model established by Negroni and Buc[36,37]. During reverse transcription, recombination has been shown to occur frequently[38]. When assessed under single cycle conditions, the recombination frequency was found to be up to three recombination events per replication event or $2.4 \times 10^{-4}$ bp per cycle; however this calculation excludes "recombination hotspots"[39].

Events of reverse transcription, genome dimerization, and recombination are linked, mutually dependent, and complementary[40,41]. The impact of recombination on viral diversity can be best appreciated at the population level by the worldwide distribution of numerous HIV subtypes and circulating recombinant forms (CRF)[42-46].

## Viral latency

Both virus integration and transcriptional latency allow HIV-1 and SIV to persist under the guise of a native host gene. Proviral integration affords HIV or SIV an enormous advantage by archiving potentially unique variants whose genotypes are maintained through cell division[16,17]. Moreover, the unique ability of lentiviruses to infect naive inactivated T-cells can impact virus sequence evolution as substrate availability can be limiting for viral replication in this cell subset[47,48]. Several studies have shown that the

frequency of G→A hypermutation is increased in the presence of low intracellular dCTP concentrations[26]. Similarly, selective pressure stemming from fluctuations of cellular dNTP pools as a result of nucleoside analog therapy can similarly alter base substitution frequency[18,38]. For example, antiviral regimen efficacy can result in variations that are partially dependent on the cellular activation state. For example, both didanosine (ddI) and dideoxycytidine (ddC) can exert greater antiviral effect in resting as compared to activated cells. However, zidovudine (ZDV) can preferentially protect activated cell subsets against HIV[49-51].

Interestingly, the replication-competent virus present within the pool of latently infected resting T-cells is genetically distinct from the rebounding plasma virus that is observed after treatment interruption[52,53]. The phylogeny of this recrudescent virus can be partially inferred from HIV episomal genomes[54].

## Sequestered virus reservoirs

Within infected individuals, HIV exists as a diverse population of related genetic variants that infect $CD4^+$ lymphocytes, macrophages, and monocytes. Conceptually, there are two types of reservoirs for HIV-1: cellular and anatomical. However, virus-infected cell populations within the peripheral blood represent only a minority of the total lymphocyte pool in the body. Thus, the majority of all HIV-infected cells reside in anatomic compartments[55-60]. Indeed, the genetic compartmentalization of HIV-1 occurs in numerous anatomic regions and each tissue-specific quasispecies is a result of multiple selective forces acting on resident viral populations[1,30,61-65]. The central nervous system, gut, and male genital tract remain the best-described virus reservoirs, and their distinction from systemic virus indicates that local immune responses shape resident virus populations[66-70]. The specific contribution of each anatomic reservoir to virus persistence, residual or rebound viremia is only starting to be appreciated[60,70-72].

Each anatomic niche environment offers its own series of constraints that are enforced by selection, favoring the maintenance or extinction of specific variants. Latently infected cells within these compartments can provide a persistent source of archived virus genotypes and a potential source of residual viremia on therapy[16,17,55-59,73]. Thus, the genetically distinct rebounding viruses after treatment interruption in four of six, and five of eight patients in two independent studies may be from compartmentalized reservoirs and exhibit tissue-specific tropism[30,52,53,60,72,74].

## HIV persistence and host genetics

Host genetic factors play an important role in determining the clinical outcome of HIV-1 infection, and their role in the epigenetic regulation of virus latency is well described[75]. Numerous studies have shown that extensive interindividual variability exists in response to HIV-1 infection. This includes susceptibility to virus, its transmission, and the levels of set-point viremia. Thus, the range of phenotypes spans elite control of infection to rapid disease progression[76]. Some studies have demonstrated that levels of residual viremia while on therapy may be correlated with pretherapy viral set point, underscoring the importance of host genetics in virus persistence[7,8].

Select major histocompatibility complex (MHC) alleles are associated with altered disease progression following infection by HIV or SIV[77-80]. Moreover, several human alleles have been shown to influence immune responses against HIV-1 replication. Such host elements include the MHC alleles, HLA B*5701, HLA B*27[81], HLA C alleles[82,83], and HLA Bw4-8OI in association with KIR3DS1[84]. Similarly, patients with a delta 32 allelic form of the *CCR5* coreceptor exhibit diminished susceptibility to HIV-1[85-87].

Conversely, there is evidence for association of *HLA-B*35Px* with rapid clinical decline and progression to AIDS[88,89]. Chemokines can be natural ligands for the same receptors that HIV-1 uses to enter its target cell. Polymorphisms in chemokine genes such as "regulated on activation normal T-cell expressed" (RANTES)[90,91], macrophage inflammatory proteins (MIP-1α and MIP-1αP)[92,93], and stromal cell-derived factor (SDF-1)[94-96] have been reported to play a role in differential susceptibility to HIV-1.

Allelic polymorphisms in other host genes can also impact HIV-1 or SIV infection and disease progression[97]. Both TRIM5α and APOBEC3G have been shown to be potent inhibitors of retroviral replication[98-101]. One prominent example is the cytoplasmic tripartite motif proteins (TRIM)[98] that have been shown to restrict retroviruses in a species-specific manner[98]. Yet, the TRIM sequences of both humans and monkeys are highly polymorphic and this variation is associated with differences in TRIM-mediated viral restriction[98-99].

A number of groups have evaluated the contribution of common TRIM5α polymorphisms to HIV disease progression in HIV-1-infected humans[102-106] and have found associations of single nucleotide polymorphisms (SNP) in TRIM5 domains and modest effects on HIV-1 susceptibility or clinical progression[102-105]. For example, the single SPRY domain non-synonymous SNP H419Y was shown to have a modest effect on clinical outcome[106]. By comparison, the simian TRIM5 ortholog exerts antiretroviral effects ranging from reduced levels of set-point viral load to complete suppression of infection[98,99]. However, there is little evidence for such a robust association between human TRIM5α and HIV-1-associated clinical endpoints.

APOBEC3G is a cytidine deaminase able to restrict replication of HIV-1 that lacks the accessory protein Vif

by introducing G→A hypermutations into HIV-1 DNA[100]. An H186R coding change observed mainly in black populations has been reported to be associated with accelerated disease progression[101], but no such association between polymorphisms APOBEC3G and viremia has been observed in Caucasians[107], highlighting the need for further investigation to delineate the contribution of host genetics to HIV persistence.

## HIV persistence on therapy

The advent of potent HAART demonstrated that HIV-1-infected patients could maintain near undetectable levels of viremia over protracted periods. This led to the notion that virus eradication might be a clinically achievable goal, if complete suppression of virus replication could be maintained and if no long-lived HIV reservoirs were present[108]. However, the detection of a stable latent reservoir in resting CD4+ memory cells contradicts this hypothesis[109-114] since this reservoir harbors provirus that are refractory to antiretroviral drugs and HIV-1-specific immune responses[115]. Thus replication-competent viruses can be archived over near-indefinite periods[17].

The latent reservoir persists in patients on HAART[18-20], decays extremely slowly, and has an estimated half-life up to 44 months[21,22]. Additional studies have suggested the presence of at least one additional reservoir, not found in the population of HIV-1 permissive cells in blood. This reservoir may be a potential source of residual viremia (i.e. viral RNA levels < 50 HIV RNA copies/ml) observed in patients on long-term HAART regimens[113].

The factors influencing this low-level viremia and its relative contribution to persistence have not been fully elucidated. It has been proposed that current HAART regimens are not completely suppressive and that HIV-1 might continue to replicate in anatomical sanctuary sites that have poor drug penetrance such as the central nervous system and the male genital tract[61,116,117]. However, antiretroviral therapy intensification studies have been shown to have little impact on residual HIV RNA levels, indicating that *de novo* replication is not the predominant source of residual viremia[118]. Maldarelli, et al. demonstrated that > 80% of patients on HAART had quantifiable viremia (median of 3.1 copies/ml) for at least two years after initiation of therapy, and that the level of persistent viremia correlated with the pretherapy set point but not with any specific treatment regimen[8]. These observations suggest that persistent low-level viremia is derived from reservoirs that were established prior to initiation of therapy and not from ongoing viral replication during therapy.

A common explanation for residual viremia is that HIV-1 establishes a state of latent infection in resting memory CD4+ T-cells[15,16] and that virus is released when these cells are reactivated by a variety of mechanisms[119]. In some patients a single homogenous but distinct viral sequence (predominant plasma clone) dominated the residual plasma virus, but could not be readily found in the patient's resting CD4+ T-cells in peripheral blood[120]. Others have concluded that residual viremia is genetically distinct from proviruses in activated CD4+ T-cells, monocytes, and peripheral blood mononuclear cells, implicating an unidentified cellular source[121]. One unique feature of residual virus is that it is oligoclonal or monotypic at a sequence level. Several groups have suggested that the source of residual virus may be an HIV-infected CD34+ monocyte/macrophage progenitor cell[60,120-123].

Presently, one indication of the source of residual HIV-1 RNA is the observation of transient viremic ''blips'' above the limit of viral RNA detection in patients on HAART[124]. Viral blips appear to be relatively rare events, although sampling at high frequencies is difficult in human subjects. Thus the frequency, duration, and magnitude of blips are not well understood[119,125]. Immune activation of latently infected cells likely influences the onset of blips, but this mechanism of "blipping" has defied precise characterization due to issues with HIV RNA assay variation[26,27]. Results of recent intensification studies using raltegravir in addition to HAART indicate that immune activation is an important mechanism of maintaining the viral reservoir and that perturbation of the reservoir could be achieved through this regimen[126].

## Conclusions

Persistent HIV-1 reservoirs represent the major barrier to the eradication of HIV infection. In many HAART-treated patients low-level viremia continues unabated and can replenish HIV reservoirs and aid the development of HIV drug resistance, thus compromising long-term viral control[14,127,128]. Understanding the mechanisms that control the establishment, diversification, and maintenance of HIV reservoirs is crucial if we are to succeed in eradicating HIV from infected patients.

## Acknowledgment

This work was supported the National Institute of Allergy and Infectious Diseases (NIAID) R56AI091514-01 awarded to J.B.W.

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