Cell Host & Microbe
Perspective

As Good As It Gets? The Problem of HIV Persistence despite Antiretroviral Drugs

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Human immunodeficienty virus (HIV) infection is suppressed but not eliminated by antiretroviral drugs. Viral persistence in the face of therapy has been explained by viral latency, lowered effectiveness of drugs in some anatomical sites and cell types, and cell-to-cell spread. These mechanisms allow for drug-sensitive virus to persist despite treatment. Understanding the persistence mechanism at work at different times after infection, including the time of initial infection immediately following transmission when reservoirs are first formed, will reveal if we are at the limit of what can be achieved with the current therapy paradigm of suppressing ongoing virus replication with drugs. We discuss some of the possible reasons why HIV persists at different points on the infection timeline, focusing on the role ongoing replication may have in maintaining the infection despite drugs at early times postexposure.

Introduction

Antiretroviral therapy (ART), which targets ongoing human immunodeficienty virus (HIV) replication, has been extremely successful at containing HIV infection. However, there is no possibility of curing an established HIV infection with ART, and the reasons for this are the cause of some debate. Have we reached the limit of what can be achieved by suppressing ongoing replication? There is growing interest in this question in the context of early infection, since it may be possible to suppress infection before the long-term reservoirs of virus, which are refractory to ART, are established. This is precisely the logic behind prophylactic administration of drugs before or immediately after virus exposure (Cardo et al., 1997; Grant et al., 2010). Even if the prophylactic window is missed, there is emerging evidence that administration of drugs very early following infection reduces the viral loads achieved subsequently (Ananworanich et al., 2012; Strain et al., 2005). Both prophylaxis and early treatment increase the relevance of understanding how HIV infection becomes established early on the infection timeline (Figure 1), which may differ from the principles underlying persistence once the infection is fully established. Understanding early infection may present opportunities for intervention aimed at either increasing the effectiveness of prophylaxis or ameliorating the course of infection.

In this Perspective, we first consider the mechanisms of HIV drug insensitivity as currently understood. These break down into mechanisms that require new cycles of viral replication and those that do not, and depend on anatomical sites and cell types with lowered drug penetration, cell-to-cell HIV spread, and viral latency. We then discuss how each of these mechanisms may play a role at different points on the HIV infection timeline as outlined in Figure 1, with particular focus on the time points before and soon after exposure.

Latent Infection and Other Types of Nonreplicating Reservoirs

Current ART is very effective and may even shut down virus replication completely (Shen et al., 2008). However, HIV has a non-replication-based mechanism for insensitivity to drugs, which consists of latently integrated provirus in long-lived cells. In this state, the integrated provirus can remain quiescent and persist for the life of the cell, many years in some cell types. The provirus will be duplicated along with the cellular DNA whenever the cell divides. The most common view is that the quiescent provirus is harbored by a population of latently infected $CD4^+$ memory T cells (Trono et al., 2010). This population may expand by homeostatic proliferation, and hence the reservoir need not decay with time (Chomont et al., 2009). Occasionally, a memory T cell containing the quiescent provirus will be activated. This will lead to transcription factors NFAT and NF-κB binding to their target sites on the viral long terminal repeat (LTR) and initiating transcription of the viral genome. Once transcription is initiated, it will be amplified by a positive feedback loop mediated by the HIV protein Tat (Trono et al., 2010), giving rise to actively infected T cells capable of reinitiating ongoing infection if drugs are discontinued.

A variation on this scenario is that infection is not truly latent or in memory T cells. Instead, long-lived cells such as hematopoietic stem cells or neurons are infected. These cells are not killed by the virus. Therefore, they may either produce a constant stream of virions from the integrated provirus or, in the case of hematopoetic cells, differentiate into actively infected cells (Trono et al., 2010). This virus population would be distinct from the virus present in the latently infected T cell pool, and such a population has been identified in some individuals on ART (Bailey et al., 2006). Like latency, no new infections occur in the presence of drugs but ongoing infection can be rekindled upon therapy interruption. Since replication is expected to

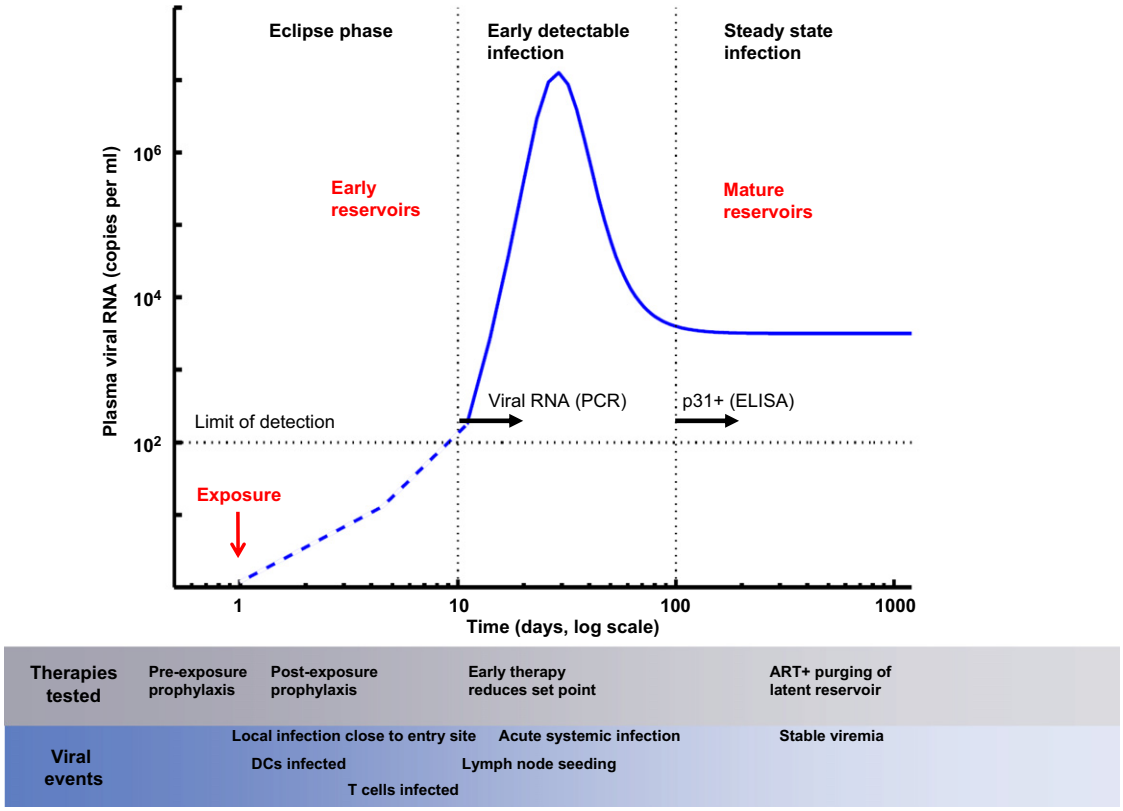

Figure 1. Timeline of HIV Infection and Treatment

After exposure, infection can be roughly staged into the eclipse phase, early detectable infection, and steady-state infection. No virus can be detected in the eclipse phase, but reservoir formation has already begun since the infection is incurable 2–3 days postexposure. After approximately 10 days, infection starts to seed the draining lymph nodes and becomes detectable by PCR for viral RNA. This period is known by Fiebig stages I–V (Fiebig et al., 2003), with each stage adding a detection modality. Early therapy that does not consist of prophylaxis is given during this time window. Infection becomes acute and peaks after about day 20, and it is at this time that the latent reservoir is thought to be seeded. After about 100 days, the infection settles into a steady state, where it is controlled by ART in most individuals, but re-emerges quickly upon therapy interruption. Purging of the latent reservoir is usually considered at this point on the infection timeline. The therapeutic modalities currently investigated for each stage of infection are shown in the gray box below the graph, with the box and graph sharing the same timeline. Viral events are in the blue box, which is also on the timeline. X axis is in time days, y axis is plasma viral RNA. Both are on a log scale. Elements in the figure are modified with permission from Keele et al. (2008a), Copyright (2008) National Academy of Sciences, USA.

rapidly create mutations in the viral sequence, the lack of detected sequence evolution during ART in virions circulating in the plasma supports the presence of a nonreplicating reservoir.

The currently proposed approach to eliminate the latent reservoir is to purge the latently infected cells by activating HIV production in these cells using inhibitors to histone deacetylases, which prevent transcription from the viral LTR promoter (Archin et al., 2012a; Lehrman et al., 2005; Sagot-Lerolle et al., 2008; Siliciano et al., 2007). The cells actively producing virus are expected to be eliminated either because the virus is cytotoxic or because cytotoxic lymphocytes will recognize and kill actively infected cells (Shan et al., 2012). Drugs are administered at the same time to prevent the actively infected cells from infecting new cells.

Reservoir Mechanisms that Require Virus Replication

I) *Decreased Drug Levels*

While HIV can be described as transmitting relatively poorly, it is the great persister. Despite the detection of potent anti-HIV antibodies in some patients (Li et al., 2007), a cellular immune response that is thought to at least be able to contain infection (Walker et al., 1986), or effective ART, no sterilizing clearance of established infection is known to occur. The immune response is a natural pressure the virus needs to deal with. Some of the strategies that HIV uses to replicate despite the immune response may also enable it to replicate in the presence of drugs.

One mechanism of persistence based on ongoing replication is infection in anatomical sites where drugs are to some degree excluded, such as the brain (Trono et al., 2010), genital tract (Cu-Uvin et al., 2010; Deleage et al., 2011), and dramatically in the gut (Stevenson et al., 2012). Suboptimal intracellular drug levels can also ensue: cells can actively pump drugs out using the P-glycoprotein transporter (Schuetz et al., 1999). For the nucleoside/nucleotide analog class of drugs included in almost all regimens, cellular phosphorylation to the triphosphate form is necessary for these inhibitors to compete with the native nucleotide pool for incorporation into the HIV cDNA and lead its premature termination. Since the kinases involved tend to be active during cell proliferation, at least some of these compounds are poorly phosphorylated in quiescent cells (Davis et al., 2001), where the initial local infection is reported to take place (Zhang et al., 1999). Both the P-glycoprotein-based efflux

mechanisms and poor phosphorylation of analogs are prominent in macrophages (Jorajuria et al., 2004; Richman et al., 1987).

II) *Cell-to-Cell Spread*

A second mechanism for drug insensitivity during ongoing replication is infection by cell-to-cell spread (Sigal et al., 2011). This is a mode of directed HIV transmission that aims the infected cell's virus load at a few nearby cells at very close range through the formation of direct interactions between the infected and uninfected cells (Dimitrov et al., 1993; Hübner et al., 2009; Jolly et al., 2011; Rudnicka et al., 2009; Sattentau, 2008; Sowinski et al., 2008). Cell-to-cell transmission takes advantage of normally occurring interactions between immune cells that form physical contacts along which the virus is delivered to the uninfected target cell. It is a receptor-mediated process and not dependent on cell fusion (Sattentau, 2008). During cell-to-cell transmission, many virus particles are transmitted to a single uninfected target cell, and this makes it more probable that at least one virus particle stochastically escapes HIV inhibitors such as drugs or antibodies and proceeds to infect the cell (Sigal et al., 2011).

Cell-to-cell spread of HIV is usually investigated between T cells but also works when the donor cell is an infected macrophage (Carr et al., 1999). Moreover, donor cells do not need to become actively infected themselves. Dendritic cells (DCs) can bind virus on their surface by interaction with DC-SIGN and transmit HIV in *trans* to T cells when the latter form immunological synapses with DCs as part of the process of antigen presentation (Geijtenbeek et al., 2000). Virus particles are not only concentrated by DCs for transmission to T cells, but also transported by DCs to lymph nodes, where the numbers of susceptible T cells is high (Embretson et al., 1993; Pantaleo et al., 1993). HIV-infected T cells are also very motile and perform a similar function for the virus (Murooka et al., 2012). At the lymph nodes, HIV opsonized by antibodies can be captured by follicular dendritic cells and remain infectious for months (Heath et al., 1995; Keele et al., 2008b). Closer to the site of exposure, the virus can be captured and presented to $CD4^+$ T cells by genital epithelial cells (Dezzutti et al., 2001; Maher et al., 2005; Wu et al., 2003). This is also true of mammary epithelial cells (Dorosko and Connor, 2010). Lastly, virus capture need not only occur by a cell: amyloid fibers in the semen can bind and efficiently transmit HIV to any susceptible cells they come in contact with (Münch et al., 2007). All these mechanisms allow for directed virus spread and reduce drug sensitivity due to the sheer number of virus particles infecting a single target cell.

Because of the fast mutation rate of HIV, replication in the context of either an anatomical reservoir or cell-to-cell spread should lead to some accumulation of neutral mutations unless replication is not continuous (Sigal et al., 2011). However, there are several reasons why mutations in the viral sequence may not necessarily lead to drug resistance and the associated failure of the ART regimen (virologic failure). One reason may be that drug-resistance mutants may arise but the fitness cost of mutations to combination therapy (Bangsberg et al., 2006) may make them unable to spread. A second reason may be that a small population size of infected cells introduces high levels of drift, which counters the selection of more fit mutants—the variant that survives is determined more by chance than fitness (Brown and Richman, 1997). Hence, it is possible for reservoirs based on ongoing replication to be a cause of persistence of low-level infection, and yet not lead to virologic failure and infection breakout to high viral titers.

It is also likely that anatomical reservoir mechanisms cooperate with cell-to-cell spread to further increase insensitivity to drugs: shear flow, which inhibits cell-to-cell spread (Sourisseau et al., 2007), is usually absent from areas proposed to be anatomical reservoirs. Niches where cells can be in close contact for extended periods should also be readily available at these anatomical sites.

Drug Insensitivity in the Prophylaxis Treatment Window

In the first hours to days after HIV exposure, infection is thought to be local, close to the site of virus entry. The first cells reported to be infected are DCs or Langerhans cells (Hu et al., 2000), followed by resting $CD4^+$ T cells (Zhang et al., 1999). After infection establishes at the site of entry, it seeds the surrounding lymph nodes. This leads to the transition to a systemic infection that proceeds to become acute, with high levels of virus in the plasma (for Review, see Haase, 2010). The infection now initiates a strong immune response and subsides into an apparently asymptomatic phase with a stable level of viremia that generally lasts years.

Very early infection may be cleared by pre- and post-exposure prophylaxis (Figure 1), which attempts to clear the infection before it forms drug insensitive reservoirs. Several clinical trials have investigated the effectiveness of pre-exposure prophylaxis (PrEP). The results are encouraging but highly variable. Two studies (CAPRISA 004 and VOICE) tested a gel containing the antiretroviral tenofovir that is applied by women before and after intercourse. Five studies (VOICE, Partners PrEP, iPrEX, TDF2, and FEM-PrEP) tested a regimen of one or two antiretroviral drugs taken orally on a constant basis. The CAPRISA 004 trial found the gel to be protective (Abdool Karim et al., 2010), while the VOICE trial did not (MTN Statement on Decision to Discontinue Use of Tenofovir, http://www.mtnstopshiv.org/node/3909). The Partners PrEP (Baeten et al., 2012), iPrEX (Grant et al., 2010), and TDF2 (Thigpen et al., 2012) trials found the oral PrEP regimen effective, while FEM-PrEP (Van Damme et al., 2012) and VOICE (IH modifies 'VOICE' HIV prevention study in women: oral tenofovir discontinued in clinical trial; http://www.nih.gov/news/health/sep2011/niaid-28.htm) were stopped due to futility. In the trials where protection was observed, the reduction in the number of infections was itself variable: 44% (iPrEX), 54% (CAPRISA 004, highest adherers), 63% (TDF2), and 73% (PrEP). Except for CAPRISA 004, all percentages are for the coformulated oral tenofovir and emtricitibine regimen. Differences may reflect the different populations that were enrolled in the studies. Partners PrEP, which showed the best protection, enrolled established discordant couples. The choice of study population may affect multiple parameters. A very important parameter is adherence, which may determine if the drugs are present at all during exposure. However, it is likely not the only parameter, and others, such as how the transmission takes place, may also play an important role (Cohen and Baden, 2012).

Can drug insensitivity mechanisms play a role in HIV breakthrough despite PrEP and account for some of the variability observed in these studies? There are several ways drug

insensitivity can arise. First, drug penetration may reduce antiviral concentrations at the site of exposure. This may be especially relevant to future prophylactic modalities such as gene therapy-based antibody expression (Balazs et al., 2012). Here, the challenge would be to accumulate sufficiently high-antibody titers in vaginal or rectal mucosa, the sites of initial infection. A second way for drug insensitivity to occur is if incoming cell-free virus is captured and efficiently transmitted to T cells or macrophages by resident DCs at the exposure site. A third, and extensively debated, possibility is that initial infection is mediated not only by cell-free virus, but also by cell-associated virus (for Review, see Anderson et al., 2010). This may lead to cell-to-cell spread, which would reduce sensitivity to drugs. In addition, it would confer the benefit—to the virus—of potentially transporting it to productive sites of infection.

Evidence that cell-to-cell transmission may occur during the initial exposure to the virus includes the following: (1) unprotected sex leads to immune cells from the infected partner making contact with T cells from the uninfected partner (Kingsley et al., 2009; Peters et al., 2004); (2) HIV can be readily transmitted in this type of mixed lymphocyte reaction (Sigal et al., 2011); (3) cell-associated infection was demonstrated in SIV vaginal challenge of macaques (Sallé et al., 2010; Weiler et al., 2008); (4) the concentration of infected cells required for transmission in the macaque model was within the physiological range, while infection with cell-free viruses by vaginal SIV challenge required superphysiological doses; and (5) for mother-to-child transmissions, the number of infected cells in breast milk and vagina has been demonstrated to correlate with the probability of infection (John et al., 2001; Rousseau et al., 2004; Tuomala et al., 2003).

If drug insensitivity through cell-to-cell spread is involved in the initial avoidance of drug-mediated suppression and underlies the variable efficacy of PrEP therapies, the effect need not be large since PrEP does not decrease successful transmission by orders of magnitude in studies to date. Therefore, a 2-fold decrease in sensitivity may result in a similar increase in the number of infected individuals. In this case, microbicidal and other PrEP therapies may need to include or be combined with ways to inhibit cell-to-cell HIV spread. Screening for host and chemical factors that interfere with the interaction between the incoming infected donor cells and the resident uninfected target cells could help arrive at the necessary therapeutic combination.

Drug-Insensitive Infection after Prophylaxis No Longer Works

Postexposure, the time window during which infection can be cured by drugs ranges from 24 hours (intravenous injection [Tsai et al., 1998]) to 48 hours (vaginal challenge [Otten et al., 2000]), with the limitation that the data is by necessity from a simian infection model and the number of animals used in the studies is small. Bearing in mind this limitation, it seems that after the first few days have elapsed, the curative window closes and the first HIV drug-insensitive reservoirs are formed. What is the nature of these reservoirs?

Since resting T cells are infected within a 2–3 day window after vaginal challenge, it is possible that a latent reservoir is established and can become self-sustaining by homeostatic expansion. However, it is more likely that the latent reservoir needs the acute phase of infection to seed sufficient cells (Chun et al., 1998) and the reservoir may continue to fill thereafter. Therefore, even if the latent reservoir is present in the first 2–3 days after infection, it is likely to be not fully formed. In support of this, early treatment initiated within approximately the first month postexposure reduced HIV set-point levels during mature infection (Ananworanich et al., 2012; Strain et al., 2005).

It is important to understand whether the early reservoirs that exist at this point in the presence of ART involve drug-insensitive ongoing cycles of replication. Early reservoirs may fill other, possibly more stable reservoirs, such as the latent reservoir. Can we determine if ongoing replication takes place during this early phase of infection despite the presence of antiretroviral drugs? If all replication were shut down with ART, the rate of decay in the number of infected cells after therapy has been started would not affect the number of latent cells later, since no new cells would be infected in this phase. At least one study shows that this is not the case: the number of infected $CD4^+$ resting cells a year postinfection does correlate with the area under the viral load curve *after* ART has been initiated (Archin et al., 2012b). Such transient reservoirs, possibly based on ongoing replication, await further confirmation. If they exist, they may mediate the transition between a curable infection and an infection that had rooted itself by drug insensitive reservoirs.

Mature Infection

There is widespread agreement that latently infected cells are present during the long phase of stable viremia in HIV infection. There is some debate whether latency is the only reservoir mechanism at this stage. Intensification of therapy with more drugs does not lead to further declines in plasma virus levels (Trono et al., 2010), consistent with the idea that drugs at current levels shut down all replication. However, infected cells at this point on the infection timeline do contain detectable unintegrated DNA (Chomont et al., 2009), which is labile and not expected to persist over long periods without new infection. The unintegrated HIV DNA is similar in sequence to rebounding virus after therapy interruption, indicating that it is the source of the rebounding virus (Sharkey et al., 2011). Further, intensification of ART by addition of the integrase inhibitor raltegravir gives rise to an increase in unintegrated DNA, consistent with new infections occurring (Buzón et al., 2010).

One way to explain these divergent results is that replication does not occur in the plasma but happens in other anatomical compartments. In simian models, the virus during ART is mostly concentrated in the lymphoid tissues and the GI tract (North et al., 2010). If ongoing replication occurs in the GI tract and not in the plasma, it implies that the GI tract would not recover $CD4^+$ cell numbers with ART to the same degree as the plasma. This seems to be the case: human-gut-associated lymphoid tissue does not recover its $CD4^+$ cells despite prolonged suppression by ART (Chun et al., 2008). In some individuals on ART with clinically undetectable plasma viral loads, virus levels remain detectable in the cervix (Cu-Uvin et al., 2010) and semen (Politch et al., 2012). Infected macrophages have been detected during ART in the semen (Deleage et al., 2011), which is only possible with new cycles of infection since macrophages are too short lived to form a stable latent reservoir. Evidence for

replication also comes from the respiratory tract, where virus sequence divergence and the frequency of resistance mutations is elevated relative to peripheral blood in individuals on ART (Wagner et al., 2009).

If some ongoing replication does take place in the face of ART during mature infection, its causes—low-drug penetration, cell-to-cell spread, or other mechanisms—should be tackled before the approach to purge the latent reservoir is expected to bear fruit. Otherwise, new infections during the purging process will be created, which may replenish the reservoir and thwart the purging efforts.

Conclusion

Barring a stem-cell transplant from a donor who lacks the CCR5 HIV coreceptor, antiretroviral drugs that inhibit virus replication have been the only effective treatment so far to control HIV infection. But though they suppress the infection, they do not cure it unless used very near the time of exposure, and even in this case they often fail. Have we reached the limit of what can be achieved with treatments that suppress viral replication because the limiting factors now are nonreplicating reservoirs such as the latent reservoir and adherence to therapies on the part of the patients? Or, is there still work to be done by therapies that prevent viral replication (Cohen, 2011)? Results from studies that use histone deacetylase inhibitors to purge the latent reservoir by activating the virus (Archin et al., 2012a; Lehrman et al., 2005; Sagot-Lerolle et al., 2008; Siliciano et al., 2007) did not, as of yet, convincingly show that they can reduce this reservoir. If they succeed in doing so, then the true importance of the latent reservoir can be finally evaluated. Conversely, if anatomical reservoirs and cell-to-cell spread are also involved in viral persistence in the face of ART, treatments that prevent ongoing replication in these reservoirs will be effective in further reducing or eliminating infection. But, the treatments may need to be substantially different from what is currently used, since simply treating with more drugs does not seem to increase efficacy. Early treatment is one place where drugs may have additional therapeutic value, and looking at how HIV infection establishes itself at those points on the infection timeline may reveal the nature of the mechanisms that this virus uses to persist.

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