Mechanisms of HIV-1 escape from immune responses and antiretroviral drugs
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Despite the fact that HIV-1 induces vigorous antiviral immune responses, viral replication is never completely controlled in infected individuals. Recent studies have provided insight into the mechanisms by which focused immune pressure directed at particular B or T cell epitopes leads to the rapid appearance of escape mutations. Even if anti-HIV-1 immune responses could be enhanced to the point where they inhibit viral replication to the same extent as certain combinations of antiretroviral drugs, eradication would be unlikely because of the persistence of the virus in an extremely stable latent reservoir in resting memory CD4+ T cells.

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Introduction
HIV-1 persists in infected individuals despite robust immune responses. Within weeks of exposure, rapid viral replication produces high level viremia [1], which is partially controlled by cytotoxic T lymphocyte (CTL) responses [2,3]. Antibody responses develop soon thereafter, but viremia is not reduced to zero. Rather, it falls to a 'set point', typically $10^4 - 10^5$ copies of HIV-1 RNA per milliliter of plasma. This level is maintained throughout a prolonged asymptomatic period, during which continuous viral replication drives a progressive depletion of CD4+ T cells, leading eventually to a collapse of immune responses against the virus and other pathogens, and the development of AIDS. There are also functional abnormalities in the CD4+ T-cell compartment, including an early defect in the anti-HIV-1 response [4]. However, the CD4+ T-cell defects do not prevent the emergence of vigorous and sustained B-cell and CD8+ T-cell responses to HIV-1.

This review will discuss recent work on how HIV-1 avoids eradication by these responses. Interested readers are referred to excellent reviews of earlier work in this area [5,6]. Recent work [7] on intrinsic host factors that restrict retroviral replication non-specifically will not be discussed further here.

Lessons from the response to antiretroviral therapy
Insight into how HIV-1 evades immune responses has come from studies of viral persistence in the face of the much stronger selective pressure exerted by antiretroviral drugs. Following the initiation of highly active antiretroviral therapy (HAART), plasma virus levels decay rapidly with multiphasic exponential kinetics [8–11]. This decay occurs because HAART almost completely stops the new infection of susceptible cells [8,9], thereby perturbing the set point equilibrium between virus production and virus clearance, and revealing the decay rates for free virus ($t_1/2$ = minutes), for the CD4+ lymphoblasts that produce most of the plasma virus ($t_1/2$ = 1 day), and for a minor population of infected cells that turns over slowly ($t_1/2$ = 2 weeks). These decay processes bring plasma virus levels down below the limit of detection of ultrasensitive clinical assays (50 copies per milliliter of plasma).

The short $t_1/2$ of most productively infected cells means that the set point viremia is maintained by the infection of large numbers of new cells per day to balance those that die. According to the classic model of viral dynamics [11,12], the number of new cells infected per day is given by the equation:

$$\delta T^* = \frac{cV}{N}$$

where $T^*$ is the total body number of productively infected cells, $\delta$ and $c$ are decay constants for productively infected cells and free virus, respectively, $V$ is the level of viremia, and $N$ is the burst size. Using recently revised estimates of $\delta$ and $c$ [13] and a conservatively large estimate of the burst size (50,000 virions/cell), the number of newly infected cells arising per day for a patient with typical levels of viremia is minimally $10^5$. In each newly
infected cell, the 10 kb viral genome is copied by reverse transcriptase, which introduces errors at a rate of ~3 × 10⁻⁷/nucleotide [14]. Thus ~1/3 of the 10⁵ newly copied viral genomes carry a new mutation. This number is roughly equal to the total number of possible single point mutations in the genome. Therefore, every possible point mutation in the HIV-1 genome arises on a daily basis, a result that explains the propensity of HIV-1 for escape from immune selective pressure.

**Escape from antibody-mediated neutralization**

Although antibodies effectively control many viral infections [15], HIV-1 replicates continuously in the face of a strong antibody response. The mechanisms that allow the virus to do so are best understood by considering the structural features that the virus has evolved to avoid neutralization. Neutralizing antibodies (nAbs) are directed at the HIV-1 envelope (env) protein, a heterodimer consisting of an extensively glycosylated CD4-binding subunit (gp120) and an associated transmembrane protein (gp41). The env proteins are present on the virion surface as ‘spikes’ composed of trimers of three gp120–gp41 complexes [16–18]. Although exposed on the surface, these spikes resist neutralization through occlusion of epitopes within the oligomer, extensive glycosylation, extension of variable loops from the surface of the complex, and steric and conformational blocking of receptor binding sites [5,17].

Some antibodies bind monomeric gp120 or gp41 but do not neutralize because they recognize epitopes buried within the trimeric complex [18]. The HIV-1 env complex is extensively glycosylated, with 50% of the mass of its extracellular portion made up of N-linked glycans [19]. Glycosylation blocks access to the conserved core of the protein while itself stimulating relatively little immune response [20]. The removal of N-linked glycans near the CD4 and coreceptor binding sites renders HIV-1 more sensitive to neutralization [21]. The protection afforded by glycosylation is not static, however. The precise pattern of N-linked glycosylation varies widely between isolates. Glycosylation sites can be shifted by mutation, blocking or altering epitopes and allowing escape from nAbs [22**]. Moreover, addition or loss of glycosylation at a particular site may affect distant epitopes [23].

Gp120 has five variable regions (V1–V5), four of which form loops on the surface, shielding the conserved core of the protein. When nAbs against the variable loops develop, escape mutants can usually be selected without extreme loss of viral fitness [24]. Other structural elements also protect critical receptor binding sites from nAbs. Access to the conserved coreceptor binding site is sterically restricted. Gp120 undergoes a conformational change when it binds CD4, exposing this site. Access is limited even after this conformational change, as the neutralizing activity of many antibodies to this site is greater with Fab fragments than with intact antibodies [25]. In addition, some antibodies against the CD4 binding site induce a conformational change in gp120, making binding thermodynamically unfavorable [26].

The structural features of gp120, particularly its variable loops, allow it to tolerate a vast array of mutations. This permits repeated selection of neutralization escape variants, as has been previously demonstrated in culture assays, animal models, and in infected humans [5]. Even cocktails of nAbs against highly conserved env epitopes were shown to exert little control on established HIV-1 infection in a severe combined immunodeficiency (SCID) mouse model [27]. Using single round infectivity assays with reporter viruses, two groups have recently tracked the development of nAbs and the evolution of virus in individuals with acute HIV-1 infection [22**,28**]. Neutralizing antibodies against autologous plasma virus developed within two months of seroconversion. Although these nAbs ultimately reached high titers, escape was extremely rapid, occurring while titers were still relatively low. As new variants arose, nAbs against those variants developed within three months, but by this time new viral variants resistant to neutralization by those antibodies had already arisen. Thus, the combination of structural features limiting the formation of nAbs together with the rapid selection of escape mutations explains the inability of the nAb response to completely suppress viremia.

**Escape from HIV-1-specific cytotoxic T lymphocytes**

A high frequency of HIV-1-specific CD8⁺ T cells can be found in infected individuals, even those with advanced disease [29]. That these cells reduce viral replication has been convincingly demonstrated by experimental depletion of CD8⁺ T cells in simian immunodeficiency virus (SIV)-infected macaques, which results in a marked and immediate increase in viral load [3,30]. The partial control that CD8⁺ T cells exert over viral replication is observed despite the downregulation of some class I MHC molecules by HIV-1 Nef [31] and functional defects observed in the CD8⁺ T-cell response [32]. Nevertheless, readily measurable viral replication occurs throughout the course of the infection in most patients.

Earlier work suggesting that the virus generates escape mutations to avoid the CTL response, summarized in reference [5], was confirmed in an elegant study by Allen and colleagues [33] in SIV-infected macaques. Following infection with cloned SIV, viruses with mutations in an immunodominant epitope in the Tat protein completely replaced the wild-type virus one week after the peak CTL response. Interestingly, the viral load did not increase. Thus, the virus had escaped from the dominant CD8⁺ T-cell response but not from the entire immune response. This finding could be explained by a broadening of the
SIV-specific immune response and/or the reduced fitness of escape mutants.

Several case reports have shown that when the CTL response is focused on a single immunodominant epitope the appearance of escape mutants leads to an increase in viral replication [34–36]. A highly focused response, however, may be the exception rather than the rule. An important recent advance is the development of methods for assessing the CTL response in a global fashion, so that the memory and HIV-1 latency. (a) Normal T-cell homeostasis. Most of the CD4+ T cells in the body are small resting cells that circulate throughout the lymphoid tissues poised to respond to a specific antigen (Ag). Approximately half are naïve cells (blue) that have not encountered an Ag since emerging from the thymus. The remainder are memory cells (green) that have previously responded to Ag. Following encounter with Ag, resting cells undergo blast transformation and begin to proliferate. These lymphoblasts (red) undergo several rounds of cell division, giving rise to effector cells. Most effector cells eventually die, but a fraction revert to a resting memory state. The memory pool is maintained by the long lifespan of the cells and a gradual process of proliferative renewal. (b) Establishment of a latent reservoir in resting memory CD4+ T cells. CD4+ lymphoblasts (red cells) are highly susceptible to productive infection and usually die within a few days after infection. Latently infected cells with integrated HIV-1 DNA may be generated when lymphoblasts that are in the process of reverting to a resting state become infected. When latently infected cells subsequently encounter the relevant Ag, they become permissive for virus gene expression and virus production. Latently infected cells may be maintained by intrinsic stability, and by the process of proliferative renewal if they do not become susceptible to HIV-1-induced cytopathic effects or host cytolytic mechanisms during this process. Reproduced with permission from [63].
responses to the relevant forms of all viral proteins are detected [37,38*]. A recent comprehensive study demonstrated that HIV-1-infected patients targeted a median of 14 distinct viral epitopes [38*]. Thus, complete escape from the CTL response may require mutations in multiple epitopes. Recent work has also suggested that escape mutations may result in reduced fitness [39**,40**]. Transmission of escape mutants to hosts that do not generate an immune response to the epitope (due to the lack of the relevant MHC molecule) results in eventual reversion to wild-type sequence. These two factors may explain why escape mutations do not commonly lead to the complete loss of control of viral replication in most individuals.

**HIV-1 latency**

Although HAART can suppress detectable viremia for prolonged periods, eradication has not been achieved due, in part, to a latent form of the virus that persists in resting memory CD4\(^+\) T cells [41,42*]. This latent reservoir may be generated through infection of activated CD4\(^+\) T cells, with integration of viral DNA into the host genome. If the cell survives long enough to revert back to a resting state, then the integrated viral DNA persists in the resulting memory cell for the lifetime of the cell (Figure 1). During this time, viral gene expression is limited by several mechanisms (see below). Thus, latent infection allows the virus to survive free from the selective pressure exerted by antiretroviral drugs or the immune response. Evidence for this model comes from studies of viral persistence in patients on HAART who have no detectable free virus in the plasma. Resting CD4\(^+\) T cells from the peripheral blood of these patients do not release virus [43*], but they can be induced to do so by cellular activation [41,42**,44–46]. The size of the pool of latently infected resting CD4\(^+\) T cells does not decline substantially even in patients who have had suppression of measurable viremia for as long as seven years [42*]. Analysis of the persistence of wild-type and drug-resistant viruses supports the notion that at least a fraction of the latent pool is extremely stable [47,48].

Several recent studies have addressed the mechanisms of HIV-1 latency. Resting CD4\(^+\) T cells do not contain the activated forms of host transcription factors required to produce new virus from the integrated provirus [49–52]. The HIV-1 long terminal repeat (LTR) contains binding sites for nuclear factor-κB (NF-κB), a cellular transcription factor that is sequestered in the cytoplasm of resting CD4\(^+\) T cells by virtue of its interaction with the inhibitor of κB (IκB) complex. Activation-induced phosphorylation and proteasomal degradation of IκB allows NF-κB to translocate to the nucleus. The recently characterized Murr1 protein inhibits proteasomal degradation of IκB and may thereby restrict HIV-1 gene expression in infected resting CD4\(^+\) T cells [53*]. Other studies suggest that, in resting cells, HIV-1 transcription initiates but terminates prematurely because of a lack of Tat and Tat-associated host factors that promote transcriptional elongation [54–57]. Although elegant *in vitro* studies suggest that integration into regions of heterochromatin is another potential mechanism of latency [58], a recent study has shown that most of the HIV-1 DNA in resting CD4\(^+\) T cells from patients on HAART is found within the introns of genes that are actively expressed in resting CD4\(^+\) T cells [59*].

Although latency prevents eradication in patients on HAART, it is not clear if HIV-1 latency evolved to promote viral persistence. Classical forms of viral latency, such as the programmed latency of herpes simplex virus (HSV), allow persistence when viral replication has been arrested elsewhere in the host [60]. For individuals with untreated HIV-1 infection, active viral replication continues throughout the course of the disease. The same is true for the simian viruses from which HIV-1 evolved [61**]. Thus, latency is not essential for HIV-1 persistence. In addition, unlike HSV, HIV-1 does not have a clear genetic program of latency. There is some evidence that HIV-1 Nef might function to establish conditions that allow the direct infection of resting CD4\(^+\) T cells [62*], but whether this leads to latency in vivo remains unclear. It remains possible that latency is an unfortunate accident of the fact that HIV-1 is trophic for activated CD4\(^+\) T cells, which can undergo a profound and reversible change to a quiescent state which happens not to be permissive for viral replication. In any event, latency occurs and is likely to prevent immune-mediated clearance should it become possible to enhance immune responses to the point where they exert as strong an antiviral effect as HAART.

**Conclusions**

In the vast majority of infected individuals, active replication of the virus continues throughout the course of the infection at a level that reflects a balance between immunological control (through nAbs and CTLs) and viral escape. Viral escape occurs through the evolution of escape mutants with alterations in key regions of the env protein and in CTL epitopes. Levels of viral replication in many untreated individuals are high enough that every possible point mutation in the entire viral genome arises on a daily basis. Structural features of the env protein allow the accumulation of mutations at a rate that permits the virus to become resistant to neutralization by contemporaneous antibodies. Escape from CTL responses is more difficult because the responses are directed at epitopes in multiple viral proteins, some of which cannot tolerate mutations without a significant loss of viral fitness. Thus, pressure exerted by CTLs holds levels of replication down.

Future approaches to the treatment HIV-1 infection might include efforts to enhance anti-HIV-1 immune
responses to the point where they can suppress viral replication to levels low enough to halt viral evolution. This degree of suppression can be achieved by antiretroviral drugs, but even in patients on combination antiretroviral therapy, the virus can persist for life in a stable latent reservoir in resting CD4+ T cells.

References and recommended reading
Papers of particular interest, published within the annual period of review, have been highlighted as:

• of special interest
• • of outstanding interest


This study shows a repetitive pattern of neutralizing antibody development and rapid viral escape that could generally be attributed to evolving patterns of N-linked glycosylation. Site-directed mutagenesis studies confirmed that specific patterns of N-linked glycosylation conveyed sensitivity or resistance to neutralization by autologous plasma.


This work emphasizes the powerful selective pressure exerted by neutralizing antibody, as well as the relative ease of viral escape from that neutralizing antibody response with the initial control of viremia in primary human immunodeficiency virus infection by CD8+ lymphocytes. Science 1999, 283:857-860.


This paper provides a good illustration of the power of comprehensive approaches to the analysis of HIV-1-specific immunity.


One of two recent studies showing the reversion to wild-type sequence after the transmission of CTL escape mutants into hosts not expressing the relevant MHC molecules.


See annotation to [39]**.


This study measured the t(1/2) of the latent reservoir as 44 months, using long-term follow-up of patients receiving HAART with no detectable viremia for up to seven years.

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This study shows that HIV-1 is preferentially integrated into the introns of actively transcribed genes in resting CD4+ T cells, indicating that the
absence of virus production in these cells is not due to integration of the
provirus into areas of the genome repressive for transcription.

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