

## Changing the natural history of HIV disease

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Our understanding of the pathogenesis of AIDS has advanced considerably since the disease was first reported 15 years ago. We now know that the primary damage inflicted by HIV-1 is mainly brought about by active virus replication. With the advent of sensitive tools for monitoring HIV replication *in vivo*, an individual's risk of disease progression can be assessed early in the course of the infection and the efficacy of antiviral therapies can now be determined accurately and expeditiously. When used appropriately, potent combinations of antiviral drugs seem to be able to circumvent the inherent tendency of HIV-1 to generate drug-resistant viruses, the main reason for failure of all antiviral therapies, and are significantly more effective than earlier approaches. For the first time, rational approaches to contain and perhaps eliminate HIV-1 infection can be pursued.

Since the initial reports of AIDS 15 years ago, our understanding of the disease process and the behaviour of the aetiological agent, the human immunodeficiency virus (HIV), have advanced substantially. Sensitive tools are now available to monitor levels of HIV replication *in vivo*, and have greatly illuminated the basic pathogenic mechanisms. These tools also provide us with effective means to gauge risk of disease progression and to assess the efficacy of therapeutic regimens.

Impressive advances in treatment have recently been realised with the development of more numerous and more potent inhibitors of virus replication. With the introduction of new, more effective antiviral drugs, therapeutic strategies can be designed to accomplish lengthy and near complete suppression of virus replication in many HIV-infected persons.<sup>1</sup> The inherent tendency of HIV-1 to generate drug-resistant variants remains the main obstacle limiting the ability of antiviral drugs to inhibit virus replication and delay disease progression.<sup>2</sup> However, recent insights into the biology of HIV replication and the molecular basis of antiviral drug resistance suggest approaches for the use of antiviral agents in ways that delay or even prevent the emergence of drug-resistant viruses.

Lessons from studies of the pathogenesis of HIV-1 disease provide a compelling rationale for the initiation of therapy with combinations of potent antiviral drugs early in the course of the infection, and suggest that most HIV-1-infected individuals should be treated to diminish levels of virus replication, irrespective of the stage of their disease.<sup>2-5</sup> However, the basic and applied advances that support this view have accrued faster than our ability to confirm, in well-designed clinical trials, that early and aggressive treatment of HIV-1 infection leads to predictable clinical benefits. Consequently, clinicians must struggle to balance what they think is best with what has, in rigorous clinical trials, been formally proven to be the best. Moreover, as our understanding of HIV disease and the availability of beneficial therapies have increased, so has the complexity of caring for patients. Recent evidence indicates that practitioners familiar with HIV disease provide more effective care for HIV-infected patients than do those with less experience or understanding of the disease process.<sup>6</sup>

This disparity is likely to become even more pronounced since therapeutic success will depend on a thorough understanding of pathogenesis and on familiarity with when and how to use the increasingly effective drugs to treat HIV-1 infection and its complications.

### Emerging principles of HIV pathogenesis

Infection with HIV-1 initiates a process that leads to progressive destruction of the population of CD4 T lymphocytes with roles in the generation and maintenance of host immune responses.<sup>7</sup> The target cell preference for HIV-1 infection and depletion is determined by the identity of the cell surface molecule, CD4, that is recognised by the HIV-1 envelope (*env*) glycoprotein as the virus binds to and enters host cells to initiate the virus replication cycle.<sup>7</sup> In addition, the process of cell fusion known as syncytium formation, which represents a major cytopathic consequence of HIV-1 infection of CD4 T lymphocytes, also depends on the specific interaction between CD4 and the HIV-1 *env* glycoprotein. Additional cell surface molecules that normally function as receptors for chemokines have lately been identified as essential co-receptors required for the process of HIV-1 entry to target cells, and probably for virus-induced cytopathic consequences as well.<sup>8</sup> HIV-1 variants that efficiently induce syncytium formation and that can infect established T-cell lines but not primary macrophages in culture (syncytium-inducing or SI viruses) use a co-receptor variously termed LESTR or fusin. Non-syncytium-inducing HIV-1 variants (NSI viruses) can infect both primary T cells and macrophages (but not established T-cell lines) and mainly use the CC CKR-5 receptor that binds the  $\beta$ -chemokines MIP-1 $\alpha$ , MIP-1 $\beta$ , and RANTES. The ability of these  $\beta$ -chemokines to inhibit HIV-1 infections seems to be due to competitive inhibition for binding to the CC CKR-5 receptor.

After initial infection of the human host, the pace at which immunodeficiency develops and the susceptibility to opportunistic infections and malignancies becomes manifest, is associated with the rate of decline in CD4 T-cell levels.<sup>9-12</sup> The rate of CD4 T-cell decline varies considerably from person to person and is not constant throughout all stages of HIV-1 infection. Acceleration in the rate of decline of CD4 T-cell numbers heralds the progression of disease. The point of onset of a more rapid fall in CD4 T-cell counts is known as the inflection point. The virological and immunological events that occur around the time are poorly understood but are often

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associated with increasing rates of HIV-1 replication *in vivo*, the emergence of SI viruses in tissue culture, and declining cell-mediated immune responses.<sup>13</sup>

For adults in developed countries, the average time to development of AIDS after initial HIV-1 infection is about 10–11 years in the absence of antiviral therapy or with older regimens of nucleoside analogue (eg, zidovudine) monotherapy.<sup>9</sup> However, some individuals ( $\approx 20\%$ ) manifest full-blown AIDS within 5 years of infection, whereas others ( $<5\%$ ) have sustained long-term ( $>10$  years) symptomless HIV-1 infection without significant decline in CD4 counts. Only about 2% or less of HIV-1-infected persons seem to be able to contain viral replication to extremely low levels and maintain stable CD4 counts within the normal range for lengthy periods ( $>12$ – $15$  years).<sup>14</sup> Within this group, very rare individuals are infected with HIV-1 variants harbouring genetic defects (eg, *nef* gene deletions).<sup>15,16</sup> However, most instances of slowly progressive or apparently non-progressive HIV-infection are believed to result from more effective host antiviral immune responses.<sup>14</sup> These individuals tend to have active and broadly reactive cytotoxic T-cell (CTL) responses against HIV-1-infected cells; whether this is the mechanism that preserves the integrity of their immune system or is merely a reflection of it is unclear. Certain combinations of major histocompatibility complex (MHC) genes (HLA in human beings) are associated with either relatively rapid or delayed progression to AIDS in infected persons; this observation suggests that specific aspects of the host immune response to HIV infection may be important determinants of the rate of disease progression but the precise nature of these genetically determined factors remains unidentified.<sup>17</sup>

Inheritance of specific genes other than HLA genes might also affect the rate of progression of HIV-1 disease. For example, we do not know whether the CD4 T cells from different individuals vary in their susceptibility to the cytopathic consequences of HIV-1 infection. However, some rare individuals who have not become infected with HIV-1 despite multiple exposures to the virus display higher levels of  $\beta$ -chemokine production and altered expression of the specific chemokine receptors (eg, CC CKR-5) that serve as co-receptors for entry to HIV-1 into target cells.<sup>18</sup> Other patterns of co-receptor expression might likewise confer relative resistance to HIV-1-induced cytopathicity.

Recognition that age of an individual at the time of HIV-1 infection can influence the subsequent rate of progression to AIDS suggests that the regenerative capacity of the host immune system (known to diminish with age) may also determine how well the immune system can resist or repair the damage caused by HIV-1 infection.<sup>12,19</sup>

Another possibility is that environmental factors, especially those leading to activation of the immune system, may affect the tempo of HIV-1-induced immunosuppression. Exposure to environmental antigens or common infections might activate HIV-1 replication, thereby increasing immune system damage and enhancing progression of HIV-1 disease.<sup>20,21</sup>

Early efforts to synthesise a coherent model of the pathogenic consequences of HIV-1 infection were based on the notion that very few cells in infected individuals harbour or express HIV-1, and that virus replication is restricted during the period of clinical latency. However, early detection methods were insensitive and newer more sensitive tools have shown that virus replication is active

throughout the course of the infection, and proceeds at levels far higher than imagined previously.<sup>2-4,22</sup> HIV-1 replication has been directly linked to the process of T-cell destruction and depletion. In addition, ongoing HIV-1 replication in the face of an active but incompletely effective host antiviral immune response is probably responsible for secondary manifestations of HIV-1 disease including wasting and dementia. Our new appreciation of the population dynamics of HIV-1 infection has profound implications for basic understanding of AIDS pathogenesis and for clinical practice.

*HIV replication rates in infected individuals can be readily and accurately gauged by measurement of plasma HIV-1 RNA concentrations*

Until recently, methods for monitoring virus replication were hampered by poor sensitivity and reproducibility.<sup>23</sup> However, new techniques for sensitive detection and accurate quantification of HIV-1 RNA in plasma provide measures of active virus replication. HIV-1 RNA in plasma is contained within circulating virus particles or virions, each virion containing two copies of HIV-1 genomic RNA. Plasma HIV-1 RNA concentrations can be quantified either by target amplification (eg, quantitative reverse transcription polymerase chain amplification [RT-PCR]; [Amplicor, Roche Molecular Systems] or nucleic acid sequence-based amplification [NASBA, Organon Teknika]) or signal amplification (branched-DNA [bDNA] assay [Quantiplex, Chiron]) methods.<sup>23-26</sup> Versions of both types of assays are now commercially available, and the Amplicor assay was recently approved by the US Food and Drug Administration for assessment of risk of disease progression and monitoring of antiviral therapy. Target amplification assays are more sensitive ( $\approx 400$  copies RNA/mL) than the first-generation bDNA assay ( $\approx 10\,000$  copies RNA/mL) but the sensitivity of the bDNA assay has recently been improved ( $\approx 500$  copies RNA/mL). Although target and signal amplification assays use different strategies to quantify viral RNA, they yield very similar results when they are applied to identical clinical specimens whose HIV-1 RNA content falls within the range that can be detected by both assay types.<sup>23,27</sup> These assays have already helped in the evaluation of new antiviral therapies, and in illuminating fundamental aspects of disease pathogenesis. Suggested guidelines for use of these new assays in clinical practice to quantify plasma HIV-1 RNA have recently been published.<sup>23</sup>

Recent data indicate that the level of viraemia, as measured by the amount of HIV-1 RNA in the plasma, accurately reflects the extent of virus replication in an infected person.<sup>2-5</sup> The terms "viraemia" and HIV-1 RNA concentrations are often combined in the convenient but somewhat imprecise concept of "viral load" that is commonly used by practitioners and patients. Although the lymphoid tissues such as lymph nodes and other compartments of the reticuloendothelial system are thought to be the largest reservoirs of virus infection *in vivo*, and the major sites of active virus production, virus produced in these tissues seems to be released, through as yet unidentified routes, into the peripheral circulation, where it can be readily sampled. Thus, plasma HIV-1 RNA concentrations seem to reflect the level of active virus replication throughout the body, although we do not know whether specific compartments (eg, the central nervous system) represent sites of infection that are not in communication with the peripheral pool of virus.

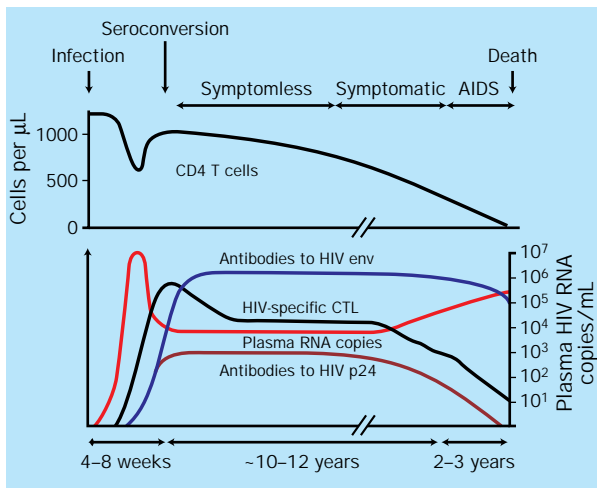


Figure 1: Schematic view of the course of HIV-1 infection and disease

Plasma HIV-1 RNA can be detected in virtually all HIV-1-infected individuals although its concentration can vary widely depending on the stage of infection and on incompletely understood aspects of the host-virus interactions unique to each HIV-infected individual (figure 1). During primary HIV-1 infection, when there are numerous susceptible target cells without a countervailing host immune response, concentrations of plasma HIV-1 RNA can exceed  $10^7$  copies/mL.<sup>22</sup> With the emergence of antiviral immune responses, of which the antiviral CTL response is believed to be the most important, concentrations of plasma HIV-1 RNA decline precipitously (by two or three logs or more) and after a period of fluctuation, often lasting 6 months or so, appear to stabilise around a so-called "set point". Different patients display different steady-state levels of viral replication, manifest as different set-points of plasma HIV-1 RNA.<sup>28-31</sup> There is an inverse correlation between plasma HIV-1 RNA and CD4 counts.<sup>22,31</sup> However, at any CD4 concentration, plasma HIV-1 RNA concentrations show wide inter-individual variation (figure 2).<sup>22,23,31</sup> Those who display higher steady-state concentrations of plasma HIV-1 RNA are at greater risk for disease progression<sup>30,31</sup> (see below). The determinants of the set point of plasma viral RNA concentrations are incompletely understood, but probably include the effectiveness of host antiviral immune responses, the number of target cells available for infection, the degree of immune activation in an individual, and potentially, the replicative vigour (or "fitness") of the infecting strain of HIV-1. In established HIV-1 infection, persistent concentrations of HIV-1 RNA range from less than 200 copies/mL in extraordinary persons with non-progressive HIV-1 infection to more than  $10^6$  copies/mL in those with advanced immunodeficiency. In most HIV-1-infected persons set-point RNA concentrations are between  $10^3$  and  $10^5$  and, once established, these concentrations remain fairly constant for months to years.<sup>28-31</sup> Progressively increasing plasma HIV-1 RNA concentrations can herald the development of advancing immunodeficiency and AIDS, irrespective of the initial set-point value.<sup>28-31</sup>

The steady-state concentration of HIV-1 RNA present in the plasma is a function of the rates of production and clearance (collectively referred to as turnover) of the virus in circulation. Assessment of kinetic rates of virus turnover requires that some disturbance of the steady-state system—

eg, that provided by the initiation of effective antiviral therapy—be used to allow measurement of viral clearance, the magnitude of virus production, and the longevity of virus-producing cells. Recent studies measuring virus turnover in this way (in persons with moderate to advanced HIV-1 disease) have revealed a very dynamic process of virus production and clearance that underlies the seemingly static steady-state level of HIV-1 virions in the plasma.<sup>2-5</sup> Measurement of the slope of the initial fall in viraemia after initiation of potent antiviral therapy permits, via mathematical modelling, calculation of the half-life of clearance of HIV-1 virions. The half-life of virions in circulation is surprisingly short—about 6 hours. Thus, on average, half of the population of plasma virions turns over every 6 hours. Clearance rates do not vary substantially between persons with different pre-treatment CD4 counts or plasma HIV-1 RNA concentrations; this observation suggests that clearance may not be dependent on specific host immune responses. Rather, virion clearance may be via the reticuloendothelial system or brought about by inherent thermodynamic instability of the virus.

With the definition of such a short and rather constant half-life of HIV-1 virions in circulation, researchers realised that the main determinant of steady-state concentrations of HIV-1 in an infected person is the amount of virus production taking place in their body at any point in time.<sup>2-5</sup> To maintain the steady-state plasma virus concentrations typically found in persons with moderate-to-advanced HIV infection,  $10^8$ – $10^9$  virions or more must be produced, released into the extracellular fluid, and cleared each day.<sup>3-5</sup> The rate of virus turnover has not yet been formally determined in individuals with earlier stage HIV-1 infection. Unless virus clearance rates prove to be different in persons with high CD4 counts or shorter duration of infection (there is no reason to suspect this is the case), the main determinant of steady-state concentrations of plasma viral load in these groups is also likely to be the rate of virus production.

*HIV replication proceeds via an active process of de-novo infection of target cells that produce virus for only a short time before being destroyed*

The rapidity of decline of plasma HIV-1 RNA indicates that, when new rounds of virus infection are blocked, virus production from infected cells continues for only a short time, averaging about 2 days (with the average half-life for an infected cell of  $\approx 1.6$  days) (figure 3).<sup>2-5</sup> Virus production by infected cells seems to be limited by cell death from the

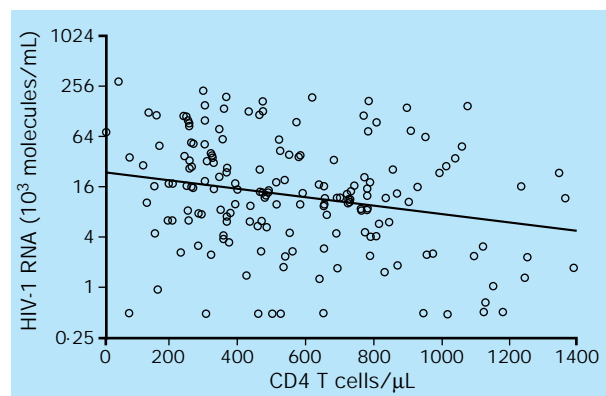


Figure 2: Relation between CD4 T-cell counts and HIV-1 RNA concentrations

From Mellors et al,<sup>31</sup> with permission.

cytopathic consequences of HIV-1 expression. The estimated average generation time of HIV-1 (the time from release of a virion from one cell until it infects another cell and results in release of a new generation of virions) is  $\approx 2.5$  days, which implies that the virus is replicating at a rate of  $\approx 140$  or more cycles per year in an infected person.<sup>4</sup> Thus, in the course of the median period between initial infection and the diagnosis of AIDS, the virus genomes present in an infected individual are removed by well over a thousand generations from the virus that initiated the infection.

These studies also show that more than 99% of virus production comes from recently infected cells rather than from long-lived chronically infected cells or from latently infected cells recently activated to produce virus.<sup>3-5</sup> Thus, 30–50% of the virions present in the plasma of an infected person at any one time are produced by CD4 cells infected within the previous day. The cells that support production of the vast majority of virions in plasma are believed to be CD4 cells. Recognition that the vast majority of HIV-1 produced *in vivo* is derived from recently infected cells has prompted a re-evaluation of the importance of chronically or latently infected cells in the persistence of HIV-1 infection. Since more CD4 T cells in both the peripheral blood and lymph nodes contain HIV-1 DNA sequences than actively synthesise detectable quantities of viral RNA,<sup>32</sup> such cells could represent latently infected cells, but most of them seem to carry HIV-1 variants that are replication-defective. Macrophages are more resistant to the cytopathic consequences of HIV-1 infection and are widely distributed throughout the body. Macrophages or their counterparts within the central nervous system, the microglial cells, may therefore provide potential sources of chronic HIV-1 production. Should they exist, chronically or latently infected cells need not be numerous to contribute in important ways to the propagation of HIV-1 infection because they might provide reservoirs of virus infection able to escape elimination by the host immune responses or antiviral therapies.

Surprisingly, the estimated lifespans of productively infected cells show little correlations with initial CD4 count, even though persons with low counts are commonly believed to have less vigorous antiviral immune responses.<sup>3-5</sup> Thus, it remains unclear whether and to what extent the host immune response can effectively interrupt HIV-1 replication by targeting newly infected cells for destruction before they produce infectious progeny virus.

Coincident with the rapid fall in plasma HIV-1 RNA in

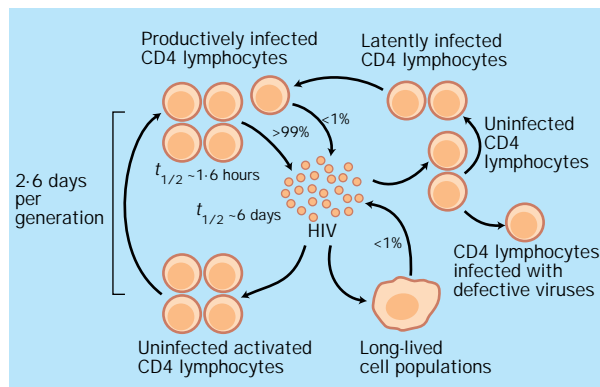


Figure 3: Schematic summary of the dynamics of HIV-1 infection *in vivo*

Shown in the centre is the cell-free virion population that is sampled when plasma HIV-1 RNA concentrations are measured. From Perelson et al,<sup>4</sup> with permission.

treated patients, there is a surge in the level of circulating CD4 cells.<sup>3,5</sup> This rapid increase in CD4 T cells probably represents cells that were spared from death due to HIV-1 infection and proliferated in an attempt to restore appropriate levels of host T-cell numbers. Based on the magnitude and rate of increased CD4 cell numbers in treated patients, it was estimated that as many as  $2 \times 10^9$  cells, or about 5% of the total body CD4 T-cell population, are being destroyed each day.<sup>3,5</sup> To maintain stable CD4 numbers, an equal number of T cells must therefore be produced each day. Thus the regenerative capacity of the immune system is constantly being stressed to keep up with the damage inflicted by HIV-1 infection. Some researchers have questioned whether the observed surges in CD4 T cells in the peripheral blood actually reflect active replenishment of T-cell numbers or merely their redistribution from lymphoid organs into the peripheral circulation. Redistribution of even a small portion of the vast reservoir (an estimated 98% of total T cells) of CD4 T cells present in lymphoid tissues could produce large changes in peripheral blood T-cell numbers. However, recent identification of increased numbers of proliferating T cells in HIV-1 infected persons with decreased CD4 counts accords with kinetic measurements indicating high levels of destruction and compensatory attempts to replace lost T cells in advancing HIV-1 infection (D Ho, personal communication). Additional studies are needed to establish the magnitude of the T cell pool and turnover in HIV-1-infected persons with different steady state levels of virus replication. Rates of T-cell recovery tended to be higher in patients with lower baseline CD4 counts, which suggests that the decline in CD4 numbers that precedes ultimate immune system collapse may largely reflect increases in the magnitude of HIV-1 replication rather than an irreversible compromise of the host's capacity for regeneration of lymphocyte populations.<sup>3</sup> Thus, HIV-1 infection involves a dynamic, continuous process of new rounds of susceptible CD4 T cells that are rapidly consumed in the process of viral replication.

These findings raise three issues of great practical importance. First, because of the error-prone nature of the HIV-1 reverse transcriptase used to replicate the viral RNA genome into a DNA copy with each cycle of virus infection, the rate of appearance of genetic variants of HIV-1 within infected persons is a function of the number of cycles of virus replication that takes place during the course of an individual's infection.<sup>2</sup> That numerous rounds of replication are taking place every day in infected persons provides the opportunity to generate large numbers of variant viruses, including those that may display diminished sensitivity to antiviral drugs. A mutation is probably introduced at every position in the HIV-1 genome many times each day within an infected person.<sup>2</sup> As a result of the great genetic diversity of the resident virus population, viruses harbouring mutations that confer resistance to an antiviral drug are often present in infected individuals before therapy is initiated. Once drug treatment is initiated, the pre-existing population of drug-resistant viruses can rapidly predominate. The likelihood that drug-resistant variants are present in an infected person decreases as the number of non-cross-resistant antiviral drugs used in combination is increased.

Second, should a therapy effectively inhibit HIV-1 replication in an infected person, a fall in plasma HIV-1 RNA should be observed within the first few days of treatment. Likewise, if drug-resistant variants are present

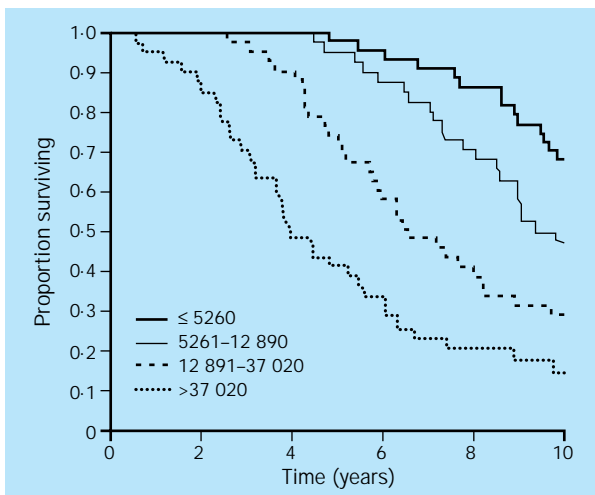


Figure 4: Association between HIV-1 RNA concentrations (copies/mL) obtained between 6 and 12 months after initial infection and subsequent risk of disease progression  
From Mellors et al,<sup>31</sup> with permission.

and possess sufficient replicative ability, decreases in plasma HIV-1 RNA will not be seen or will be of very short duration. With potent combinations of antiviral drugs now available—eg, zidovudine, lamivudine, and indinavir—plasma HIV-1 RNA concentrations become undetectable in most (~90%) of treated patients within 2–3 months of initiation of therapy, and have remained undetectable throughout follow-up of up to 48 weeks.<sup>1</sup> Should drug-resistant viruses not appear and HIV-1 RNA levels remain below detectable levels for months, drug-resistant viruses are unlikely to emerge as long as therapy is continued at effective doses. Incomplete suppression of HIV-1 replication (as indicated by the continued presence of detectable plasma HIV-1 RNA) will afford the opportunity for continued accumulation of mutations that may confer high-level drug resistance, and thereby facilitate the eventual outgrowth of the resistant virus population during continued therapy. Thus, initiation and maintenance of therapy with optimum doses of combinations of potent antiviral drugs provides the most promising strategy to forestall (or prevent) the emergence of drug-resistant viruses and achieve maximum protection from HIV-1-induced immune system damage.

Third, now that we know that most HIV-1-infected cells live for only a short time, it is essential to determine whether chronically or latently infected populations of cells exist, and, if so, to define how long they live. If HIV-1 infection is completely sustained by ongoing cycles of de-novo infection of target cells, populations of already infected cells should decline and ultimately disappear within a limited period of time once new cycles of replication are effectively blocked by potent antiviral drugs. If latently or chronically infected cells do not exist, or live for only a short time, pharmacological cure might be feasible if combinations of antiviral drugs can be identified that completely block new rounds of HIV-1 infection in all body compartments where replication is taking place. However, if chronically or latently infected cells do exist and live for prolonged periods, or if there are compartments in the body where antiviral drugs do not penetrate in sufficient concentrations to block virus replication completely (the central nervous system being the most worrisome reservoir), antiviral therapy might be able to suppress but is unlikely to eliminate infection.

#### Steady-state concentrations of plasma HIV-1 RNA predict the rate of progression to AIDS and death

The need for a laboratory marker that can predict the rate of progression in HIV-1-infected individuals has long been appreciated. Several surrogate markers—variables that are followed when the “true” variable of primary interest cannot be observed in a direct manner—have been advanced for this purpose. However, until lately, no laboratory test has proven satisfactory and no direct marker of the fundamental pathogenic processes of HIV-1 disease has been available. CD4 counts (expressed as the percentage of total CD4 T cells or the absolute number of circulating CD4 T cells/ $\mu$ L of blood) have provided the best predictor of risk of progression to AIDS or death.<sup>9,12</sup> CD4 counts also provide valuable information about a patient’s risk of developing opportunistic infections and the consequent need for prophylaxis.<sup>10,11</sup> Unfortunately, CD4 counts show substantial biological and measurement variations that limit substantially the predictive value of single determinations.<sup>12</sup> CD4 counts in patients presenting for initial medical care after diagnosis of HIV infection therefore do not predict time to development of AIDS or death.<sup>31</sup> Although trends of CD4 decline over time predict disease progression, interventions to prevent immune system damage cannot be based effectively on CD4 measurements.

Now that we know that plasma HIV-1 RNA concentrations reflect rates of active virus replication and CD4 T-cell destruction, plasma HIV-1 RNA concentrations might be expected to provide a more powerful predictor of disease progression than CD4 count alone.<sup>26-31,33,34</sup> Importantly, the prognostic value of the initial post-seroconversion HIV-1 RNA measurement in a group of individuals with defined dates of infection has now been convincingly shown by Mellors et al<sup>31</sup> (most baseline HIV-1 RNA measurements obtained in this study probably reflect set-point concentrations). In this study, there was a clear gradient of disease progression and death with increasing baseline concentrations of plasma HIV-1 RNA (figure 4). Thus, a single plasma HIV-1 RNA determination obtained shortly after initial infection can predict clinical events years later. Accumulating evidence also indicates that for individuals with undefined dates of infection, individual plasma HIV-1 measurements are predictive of rates and risks of disease progression. However, plasma HIV-1 RNA concentrations are imperfect predictors of disease progression in late-stage disease,<sup>35,36</sup> perhaps because severe damage to the immune system in these individuals has already occurred.

That plasma HIV-1 RNA concentrations measured within 6 months of initial infection are predictive of the rate of CD4 decline and of time to AIDS and death indicates that fundamental aspects of the host-virus relation for each infected person are established early in the course of the infection. That additional clinical and laboratory characteristics of an individual’s initial encounter with HIV-1 in the course of their primary infection may herald the subsequent rate of disease progression accords with this observation.<sup>37</sup> Individuals with severe symptoms of primary HIV-1 infection seem more likely to progress rapidly to AIDS than those with symptomless primary infections. Rapid progression may be more likely in patients with a high level of CD8 T lymphocytosis, those who have an impaired or clonally restricted cellular antiviral immune response, or those who mount a limited serological

response to the virus.<sup>14,37</sup> Delineation of the events occur early in infection and the underlying host-specific factors are essential topics for future research. Likewise, the ability with which antiviral therapy initiated during primary HIV-1 infection, or during the stabilisation period that follows, might alter the set point of virus replication and ameliorate the progression of HIV-1 disease is also a critically important issue.<sup>38,39</sup>

Earlier notions of initiating antiviral therapy at specific CD4 counts can now be seen as fundamentally flawed. In view of the heterogeneity of biology and clinical course within groups of patients with initially similar CD4 counts, it is not surprising that many of the early antiviral drug studies yielded conflicting results. The combination of plasma HIV-1 RNA concentrations and CD4 counts should help to refine the precision with which both short-term and long-term risk of disease progression can be assessed in individual patients. By means of straightforward laboratory tests, we can now "stage" HIV-1 disease with a precision that is as good, as if not better than, the clinical and pathological staging methods used in the management of malignant diseases.<sup>31</sup> Moreover, increasing numbers of clinical trials of antiviral drugs are showing consistently that drug-induced reductions in plasma HIV-1 RNA concentrations are predictive of clinical benefits including decreased risks for disease progression and death.

*CD4 cells and lymphoid tissues destroyed in the course of HIV infection are especially important for maintenance of immune function*

Although studies of the kinetics of T-cell turnover in HIV-1 infected individuals provide critical insights into the magnitude of CD4 T-cell destruction, they do not indicate the nature or function of the T cells lost. The ability of HIV-1 and related primate lentiviruses to infect CD4 T lymphocytes depends on the target cell being activated and progressing through the cell cycle. HIV-1 can enter resting T cells, but efficient progression through the virus replication cycle does not occur unless the target cells are activated by exposure to their cognate antigens or to stimulatory cytokines and proliferating.<sup>7</sup> Once infected, production of HIV-1 RNA, protein, and virions are augmented by activation of the target T cells. As a result, HIV-1 infection in vivo is likely to be focused on those host CD4 T lymphocytes that are actively proliferating. Unfortunately, since these cells are responding to antigens present in the environment or proliferating in an attempt to compensate for T cells already lost to HIV-induced injury, HIV-1 infection may be preferentially targeted to especially important populations of CD4 helper T cells.

The number of activated T cells in an infected individual seems to be an important determinant of the magnitude of ongoing HIV-1 replication. Immunisations and infections that give rise to increased numbers of activated CD4 T cells, and thereby increase the size of the pool of target cells that are susceptible to HIV-1 infection, lead to increased concentrations of plasma HIV-1 RNA.<sup>20,21</sup> These increases are usually transient, lasting only as long as the inciting immune stimulus persists. We do not know whether the CD4 T cells that respond to specific antigens in these settings are preferentially targeted for virus infection and depletion. If so, exposure to exogenous antigens (such as those prevalent in the environment) or endogenous antigens (such as those produced by latent pathogens) might result in the frequent activation of antigen-specific T cells, thereby increasing their susceptibility to HIV-1

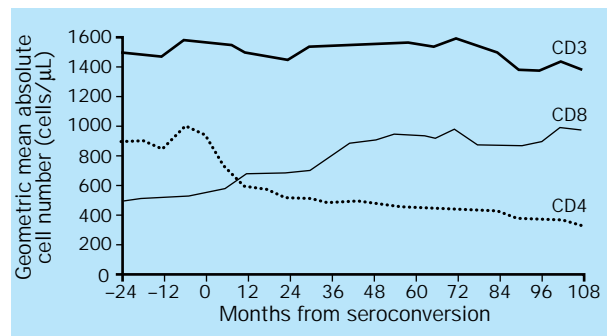


Figure 5: Mechanisms governing homeostasis of T-cell populations maintain total CD3 T-cell concentrations (CD4 plus CD8 T cells) in early HIV-1 infection

From Margolick et al,<sup>47</sup> with permission.

infection. With time, gradual attrition of CD4-T-cell-dependent immune responses may limit the repertoire of immune responses that can be mounted effectively and so predispose the host to infection with opportunistic pathogens. Memory T cells are preferential targets for HIV-1 infection, and early loss of CD4 memory T-cell responses is observed, even before there are significant decreases in total T cell numbers.<sup>40,41</sup> Pathogens that affect HIV-1-infected individuals early in the course of infection include those that are either prevalent in the environment (eg, *Candida*) or cause persistent infections (*Mycobacterium tuberculosis*, herpes simplex virus, and varicella zoster virus). In the setting of HIV-1 infection, acyclovir therapy in individuals with a history of herpesvirus infection, and isoniazid therapy in tuberculin-positive individuals, confer survival advantage and delay disease progression, respectively.<sup>42,43</sup> These observations suggest that reducing the chronic antigen stimulation caused by these pathogens is beneficial. Recent studies also indicate that active pulmonary tuberculosis is associated with progression of HIV-1 disease, despite provision of adequate antituberculosis treatment.<sup>44</sup> However, further study is needed to determine whether and how activation of HIV-1 replication by antigens or infections may lead to a faster rate of disease.

Although most of the immunological and virological assessments of HIV-1-infected individuals have focused on peripheral blood lymphocytes, these cells represent only about 2% of the total lymphocyte population in the body. The importance of the lymphoid organs, which contain the vast majority of CD4 T cells at any one time, has been highlighted by the finding that the percentage of HIV-1-infected CD4 T cells may be ten times (or more) higher in lymph nodes than in peripheral blood.<sup>32,45</sup> Moreover, the HIV-1 infected lymphocytes present in the lymphoid organs express substantially higher concentrations of viral RNA than do their counterparts in the peripheral bloodstream.<sup>32,45</sup> Thus, although the depletion of CD4 T cells after HIV-1 infection is most readily revealed by sampling of peripheral blood, damage to the immune system is exacted in lymphoid organs throughout the body. Even in the early stages of HIV-1 disease, surprisingly large amounts of virus particles are found in lymph nodes in association with follicular dendritic cells (FDCs) that play an essential part in processing and presentation of antigen to CD4 T lymphocytes.<sup>32,45</sup> For as yet unidentified reasons, gradual destruction of the FDC network occurs with time. As HIV-1 disease progresses, normal lymph node architecture is increasingly disrupted, and this probably compromises normal immune function further.<sup>45</sup>

The immune system has an impressive, but ultimately limited, capacity to replace T cells lost to HIV-1 infection. In view of the dynamic nature of HIV-1 infection and immune system damage, the regenerative capacity of the human immune system must be substantial. To understand the slowly progressive nature of HIV-1-induced immunodeficiency, the depletion of CD4 T cells after HIV-1 infection must be considered in the context of T-cell population dynamics—ie, the balance between production and destruction of T cells. Unfortunately, the T-cell population's capacity for self-renewal is poorly understood.<sup>46</sup> We do not know to what extent the peripheral expansion of mature T cells or the maturation of new T cells from progenitors contributes to maintenance of the total T-cell pool in adults. However, recent evidence suggests that the contribution of the thymus to maintaining T-cell concentrations is greatly limited by about 20 years of age.<sup>46</sup> Within the peripheral compartment of T cells where population size is maintained by proliferation of mature T cells, there also seems to be a decreasing capacity for self-renewal with advancing age.<sup>46</sup> Decreased capacity for lymphocyte reconstitution may account for the accelerated loss of CD4 T cells and the more rapid progression of disease observed in older persons infected with HIV-1.<sup>12,19</sup> The thymus is an early target of HIV-1 infection and cytopathology, thereby likely limiting the continuation of effective T-cell production even in younger individuals. Thus, in both adults and children, HIV-1 infection probably compromises both of the potential sources of T-cell production, so cell replenishment cannot match cell loss. Without adequate T-cell replacement, total CD4 T cell numbers may decline inexorably.

The mechanisms by which the human immune system regulates the size of the T-cell population, and senses when too few or too many of a certain type of T cell are present, are not understood. Recent data suggest that, rather than independently regulating the numbers of T cells of each subset (CD4 and CD8), the human immune system strives to maintain homeostasis of T-cell numbers with a poorly understood gauge that senses total numbers (all CD3 T cells) (figure 5).<sup>47</sup> This type of regulation has been called "blind homeostasis" since it does not discriminate between specific T-cell subsets. In HIV-1 infection, where continuous cycles of virus replication lead to ongoing destruction of CD4 T cells, the CD4 T cells dying as a result of HIV-1 infection would be replaced by both CD4 and CD8 T cells. Because the immune system's homeostatic sensors are set to total T-cell numbers, the progressive expansion of CD8 T-cell compartment might lead to a diminished stimulus to replenish CD4 T cells and inadvertently contribute to the gradual depletion of CD4 T cells over the course of HIV-1 infection. Total CD3 T-cell counts in the early and intermediate stages of HIV-1 infection remain nearly stable owing to increased concentrations of CD8 T cells (figure 5).<sup>47</sup>

For unknown reasons, blind homeostasis eventually breaks down in many HIV-infected persons about 18 months before the development of AIDS, resulting in a net loss of total T-cell numbers in the peripheral blood and signalling impeding immune system collapse (figure 6).<sup>47</sup> The failure of homeostasis may represent the limit of compensatory increases in lymphocyte proliferation, which probably results from several processes, including exhaustion of the proliferative capacity of individual lymphocytes, inability of the immune system to keep up

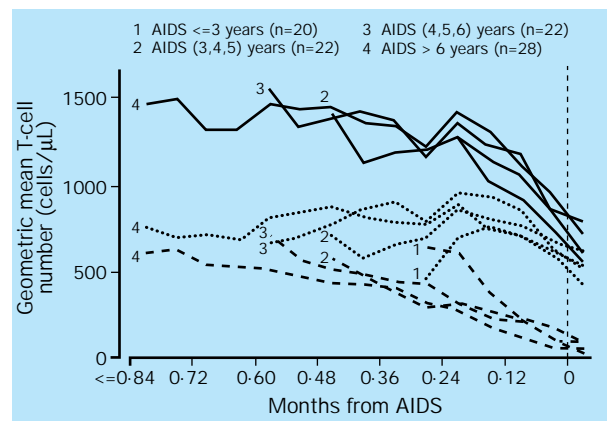


Figure 6: Mechanisms governing homeostasis of T-cell populations break down about 18 months before the development of AIDS, leading to decline in total T-cell (CD4 plus CD8 T cells) concentrations

Geometric means of absolute numbers of circulating CD3, CD4, and CD8 lymphocytes are shown as a function of months before ( $t < 0$ ) or after ( $t > 0$ ) the onset of AIDS ( $t = 0$ ; vertical dashed line) in HIV-1-infected persons with known dates of seroconversion and development of AIDS. Numbers 1–4 indicate the values for four groups of seroconverters defined by variable intervals between seroconversion and the onset of AIDS. The top (solid) group of lines represents CD3 lymphocyte concentrations, the middle (dotted) lines indicate CD8 lymphocyte concentrations, and the bottom (dashed) lines indicate CD4 lymphocyte concentrations. From Margolick et al,<sup>47</sup> with permission.

with an accelerated rate of T-cell turnover as the rate of virus replication increases with disease progression, progressive deterioration of the architecture of the lymphoid organs, and depletion of CD4 T cells to concentrations that can no longer produce sufficient quantities of the inductive growth factors necessary to support continued T-cell proliferation.

## Conclusion

HIV-1 induced damage to the immune system begins with the first cycles of replication within the newly infected host and continues until their death. The damage inflicted by HIV-1 is mainly the direct result of active virus replication. HIV-1 destroys its host target cells, the immunologically essential CD4 T cells, with each and every cycle of replication. Although the human immune system seems to have substantial regenerative potential, it is not unlimited. Decline in CD4 T-cell numbers proceeds inexorably in most HIV-1-infected individuals, but sequential loss of specific types of immune responses probably occur as well. Because of the clonal nature of the antigen-specific immune response, in the absence of generation of immunocompetent T cells from immature progenitor cells, T-cell responses may not be regained once lost, even if new rounds of virus infection can be stopped with antiviral drugs or CD4 T-cell populations increased with lymphokines such as interleukin IL-2.<sup>48</sup> Moreover, should the residual proliferative potential of CD4 and CD8 T cells decline with increased duration of HIV-1 infection and magnitude of the cumulative loss and regeneration of the lymphocyte populations, late introduction of antiviral therapy may have limited ability to reconstitute adequate numbers of functional CD4 T cells. In addition, we do not know how much, if any, of the damaged architecture of the lymphatic organs in persons with moderate to advanced HIV-1 infection can be repaired on cessation of active virus replication by potent antiviral therapies.

Recent insights into the pathogenesis of HIV-1 disease strongly suggest that the earlier effective antiviral therapy is

initiated in the course of HIV-1 infection, the better. Although we cannot yet say that suppression of HIV-1 replication to levels below the detection limits of sensitive assays will halt progression of HIV-1 disease, this is clearly the hope. By further defining the reservoirs of persistent HIV-1 infection and determining their longevity once new cycles of replication are stopped with potent drugs, we will better understand how difficult it will be to eliminate rather than contain an established HIV-1 infection. This does not mean that virological cure of HIV-1 infection will soon be possible, but we now have sufficient understanding of the disease process to frame and test hypotheses to achieve this goal.

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