

# PATHOGENESIS OF HIV INFECTION

The pathogenesis of HIV infection was discussed at the New York meetings by David D. Ho, MD, from the Aaron Diamond AIDS Research Center and New York University School of Medicine, New York, New York. Dr Ho's presentation focused on recent studies by his group on the dynamics of HIV and CD4+ cell turnover in vivo and featured discussion of the implications of their findings for treatment practices.

Observational studies of HIV-1 pathogenesis have indicated that increased viral load is correlated with CD4+ lymphocyte depletion and disease progression. However, these studies have provided little information on the kinetics of HIV and CD4+ cell turnover. Dr Ho and colleagues recently performed interventional studies in which the effects of an antiretroviral agent in perturbing the balance between virus production and clearance were utilized to permit assessment of such kinetics in vivo.

## HIV Kinetics

Although it is known that disease progression is associated with increased viral load, viral load remains relatively stable over the short term in infected individuals, indicating a steady state relationship between virus production and virus clearance (Figure 1). In a phase I/II study of a potent protease inhibitor (ABT-538, ritonavir) in 20 patients (mean CD4+ cell count of 180/ $\mu$ L), viral load and CD4+ cell count responses were closely followed, with the intention of deriving kinetics data on the pretreatment steady state by observing the effects of "turning off" viral production with treatment. Figure 2A provides representative data from three subjects with regard to plasma HIV RNA response to initiation of treatment, with a similar response being observed in all patients treated: (1) prior to treatment, there is relative stability of RNA level; and (2) there is a rapid decline in viral load over the first 2 weeks of treatment. Particularly during the first 10 days of treatment, the data points exhibit a linear de-

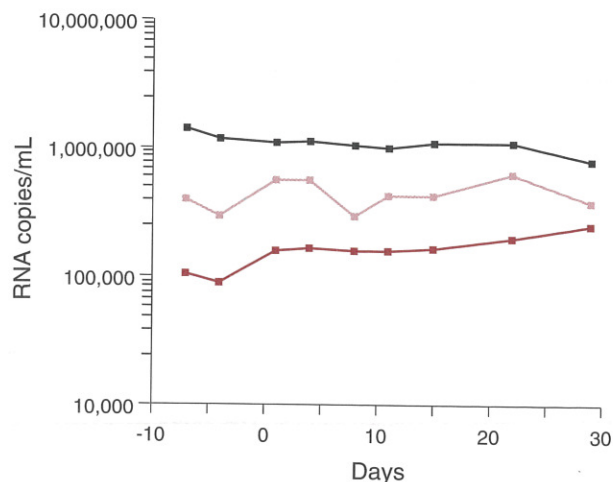


Figure 1. Relatively constant plasma HIV RNA levels in three subjects, indicating steady state of viral production and clearance during short-term monitoring.

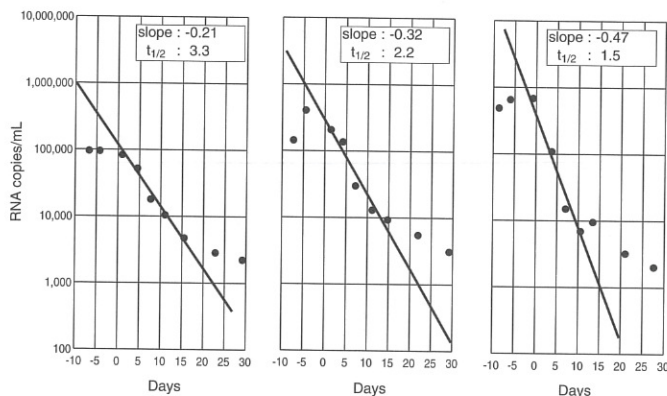
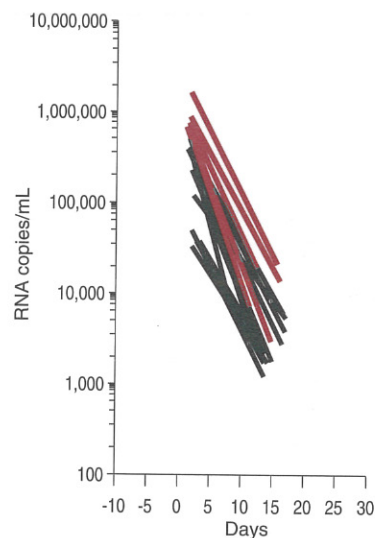


Figure 2. A (above). Slopes of log decreases in plasma HIV RNA levels during early treatment with ABT-538 monotherapy and calculated half-life values ( $t_{1/2}$ ) of viral turnover in three subjects.

B (right). Curves showing exponential decay of viral load for all 20 subjects; red curves indicate patients with initially highest viral loads. Adapted from Ho DD et al. Nature 1995.



cline on the log plot, indicating that the viral level is decreasing exponentially. Measurement of the slope of the line best fitting these data points allows direct calculation of the clearance or decay half-life of the viral population. For the subjects represented in Figure 2A, these half-life values were 3.3, 2.2, and 1.5 days. The slopes of the exponential decay in viral load for all 20 patients are shown in Figure 2B, with the magnitude of the decay also being represented by the length of each line; it can be seen that responses were similar among all subjects, with the decrease being equivalent to an approximately 2-log drop in viral load.

For each patient, the slope was derived and the decay half-life calculated. With this information, the minimum rates of viral production and viral clearance at steady state could be calculated, based on volume of distribution in accordance with patient weight and the pretreatment steady state viral load. Table 1 shows the mean and range of values for all 20 patients. The mean slope of -0.34 indicates that approximately 30% of the virus in the blood is turning over each day, with this rate being equivalent to a half-life of approximately 2 days. The mean minimum

**TABLE 1. VIRAL KINETICS IN 20 SUBJECTS**

|              | Baseline values                |   | Kinetics of HIV-1 turnover |                     |  |
|--------------|--------------------------------|---|----------------------------|---------------------|--|
|              | CD4+ count<br>(cells/ $\mu$ L) | Plasma viremia<br>(virions/mL $\times 10^9$ ) | Slope                      | Half-life<br>(days) | Minimum production and<br>clearance (virions/d $\times 10^9$ ) |
| <b>Range</b> | 36 – 490                       | 15 – 554                                      | -0.21 to -0.54             | 1.3 – 3.3           | 0.05 – 2.07  |
| <b>Mean</b>  | 180 $\pm$ 46                   | 134 $\pm$ 40                                  | -0.34 $\pm$ 0.06           | 2.1 $\pm$ 0.4       | 0.68 $\pm$ 0.13  |

production and clearance of virus calculated on the basis of these figures is 0.68 billion viral particles per day at steady state. As noted by Dr Ho, although patients with CD4+ cell counts >500/ $\mu$ L have not yet been studied in this manner, it is suspected that the viral dynamics in such patients are similar, although the numbers of particles involved might be quantitatively smaller.

Wei and colleagues at the University of Alabama at Birmingham have performed similar studies and have arrived at similar figures for half-life and production and clearance. Dr Ho stressed that the figures arrived at for production and clearance must be considered minimum estimates, since their calculation is based on particular assumptions. First, the viral decay is actually a reflection of two parameters—clearance of viral particles from the circulation and death of productively infected CD4+ lymphocytes. The calculations take into account only the former parameter and further assume that virus production is completely shut off immediately upon initiation of drug administration. Thus the actual production and clearance rates are higher than the calculated values.

**Residual Plasma Viremia**

An important characteristic of the decrease in viral load with institution of the anti-HIV intervention is that there is a floor that is reached in reduction; as can be seen from Figure 2B, the decreases typically are from  $10^5$  to  $10^6$  to approximately  $10^3$  RNA copies/mL, with the residual viremia representing a level of approximately 0.1% to 1.0% of the initial steady state viral load. Dr Ho stated that there are a number of potential explanations for this residual viremia (Table 2). (1) There may be inadequate drug penetration to some sites where actively infected cells are sequestered. (2) There may be drug-resistant virus present at or shortly after the initiation of treatment. (3) There may be a population of long-lived cells that continue to produce virus; although such virus will be noninfectious, they would be accounted for in the HIV RNA assay. (4) There may be a pool of latently infected cells that gradually enter the population of cells that are actively

**TABLE 2. IMPORT OF RESIDUAL VIREMIA ( $\approx 1\%$ )**

Potential explanations

- Inadequate drug penetration
- Drug-resistant virus
- Long-lived, virus-producing cell population
- Gradual activation of latently infected cells

Implications

- It can be inferred from finding of residual viremia that nearly all of the plasma virus must come from recently infected cells

producing virus, a phenomenon that would go unperturbed by the intervention with the protease inhibitor. Further investigation will be necessary to identify the source or sources of the residual viremia.

An important implication of the residual viremia is that, given its low level, it can be inferred that the vast majority of virus present in the plasma comes from recently infected cells. That is, the approximately 99% of the plasma viral population—that proportion whose production is turned off by the intervention—is supplied by cells infected only a few days prior and is constantly turning over. As noted, the residual proportion may be supplied by activation from latent to producer cells or generated

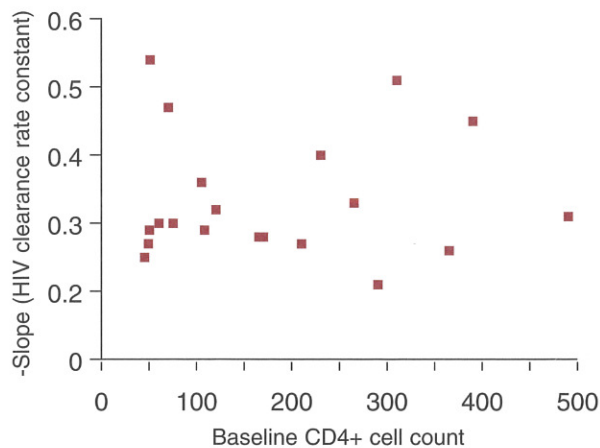


Figure 3. Analysis of viral clearance rate by baseline CD4+ cell count in subjects receiving ABT-538. Data indicate absence of correlation. Adapted from Ho DD et al. Nature 1995.

by chronically infected productive cells (eg, macrophages). The residual viremia pool must be dealt with, since it is capable of reigniting the high-level, continuous HIV-1 replication when an effective antiretroviral agent is withdrawn.

**Viral Clearance and Disease Stage**

Numerous published data demonstrate that more advanced disease is associated with higher viral load. Nevertheless, as can be seen from Figure 2B, the slopes of viral clearance in patients with higher viral load are essentially equivalent to those in patients with lower viral load at baseline. This study performed by Dr Ho and colleagues indicates that rate of viral clearance is not substantially affected by disease status. The same point is made by analysis of viral clearance rate by initial CD4+ cell count in these patients, in which no obvious correlation is evident (Figure 3). Thus it can be concluded that viral load is largely not a function of viral clearance, since clearance efficiency varies little

among individuals and since individuals can differ in viral load by several orders of magnitude. It therefore seems that viral load is primarily a function of viral production.

### Implications of High Turnover and Mutation Rates

As related by Dr Ho, the high replication rate of the virus has important implications for treatment. Based on the kinetics studies, it can be estimated that the viral population undergoes

*The quantity and genetic diversity achieved by HIV in vivo can outstrip the ability of the immune system to recognize and respond to the virus, permitting resistant mutants to emerge and, ultimately, gain predominance. This suggests that the simultaneous use of multiple potent antiretroviral agents may constitute a reasonable treatment strategy, since it would require the viral population to acquire multiple and perhaps disadvantageous mutations in the attempt to avoid susceptibility.*

between 3000 and 5000 replication cycles over the course of 10 years, producing a minimum of  $10^{12}$  virions. According to Dr Ho, given the assumptions in the kinetics analysis that render the figures minimum estimates, the number of virions produced is probably actually closer to  $10^{13}$ . Retroviruses, including HIV, have been shown to have a mutation rate of approximately  $10^{-4}$ . As stated by Dr Ho, the combination of having this many rounds of replication and this high a

transcription error rate results in "too many variants!"

In brief, the enormous quantity and great genetic diversity achieved by the virus in vivo can outstrip the ability of the immune system to recognize and respond to virus and permit resistant mutants to emerge and gain predominance. Dr Ho maintained that in the face of these figures, it must be concluded that monotherapy is not a rational strategy if a dramatic impact on the course of infection is to be achieved. He stated that a reasonable strategy is to use multiple potent agents simultaneously (eg, three or four in combination), requiring the viral genome to

simultaneously develop multiple and perhaps disadvantageous mutations in the attempt to avoid susceptibility. He also stated that given the dynamics of HIV in vivo, it makes sense to begin treatment as soon as infection is diagnosed, on the rationale that the more replicative cycles that are permitted, the more variants there will be when treatment is begun, stacking the deck in favor of the virus' ability to avoid both immune responses and effects of treatment. He suggested that with the addition of potent protease inhibitors, NNRTIs, and newer nucleoside analogues to the currently available agents, the ability to provide potent multiple-agent therapy may be on the horizon.

### CD4+ Lymphocyte Kinetics in HIV Infection

Dr Ho also provided data on CD4+ cell kinetics in the subjects in the intervention study. In the study, there was a reciprocal relationship between change in viral load and CD4+ cell count with initiation of ABT-538 treatment. Derivation of the slopes of the CD4+ cell increases permitted calculation of the doubling time of the CD4+ cell population. It was calculated that approximately 5% of the CD4+ cells are being regenerated per day, indicating a destruction rate of approximately 5% per day during steady state (Table 3). The 5% figure is equivalent to a daily turnover of approximately 35 million cells in the bloodstream. Since the bloodstream accounts for approximately 2% of the total lymphoid population, it can be estimated that the total population minimum production and destruction rate is approximately 1.8 billion CD4+ cells per day. When the individual rates of CD4+ cell repopulation were plotted against initial CD4+ cell counts for the 20 subjects, an inverse correlation was found, with those subjects with the lowest counts exhibiting the greatest production rates (Figure 4).

This finding was somewhat surprising, given the expectation based on prior literature that patients with more advanced illness would have the lowest regenerative capacity. The implication is that the body is trying extremely hard in late-stage disease to counter the greater destruction rates associated with the greater quantity of actively replicating virus. The regenerative processes may indeed be maximally turned on in late-stage disease.

Using the analogy of a faucet and basin, Dr Ho likened the water level in the basin to the CD4+ cell count, the faucet to cell replenishment, and the drain to viral destruction of cells. In the analogy, the basin drains just slightly faster than replenishment occurs, with the faucet turned full on in late-stage disease. Dr Ho maintained that the major objective of treatment should be to

TABLE 3. KINETICS OF CD4+ LYMPHOCYTE TURNOVER IN 20 SUBJECTS

|       | Slope                         | Minimum production and destruction |                           |
|-------|-------------------------------|------------------------------------|---------------------------|
|       |                               | Blood (cells/d x $10^6$ )          | Total (cells/d x $10^9$ ) |
| Range | 0.004 – 0.088<br>(0.5 – 25.7) | 4.3 – 108.00<br>(2.7 – 157.0)      | 0.2 – 5.4<br>(0.1 – 7.8)  |
| Mean  | 0.047<br>(8.6)                | 35.1<br>(53.2)                     | 1.8<br>(2.6)              |

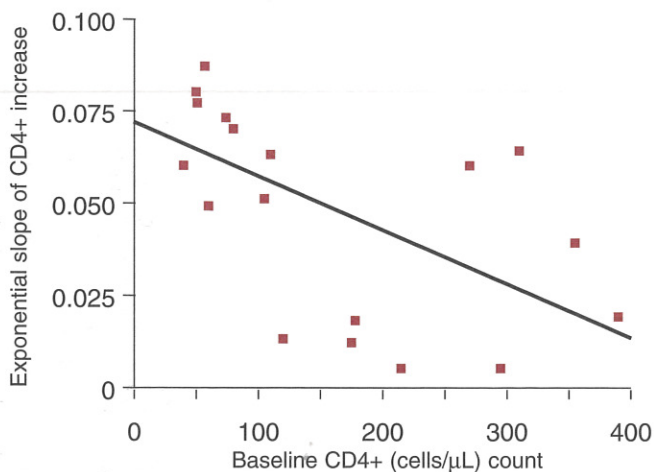


Figure 4. Analysis of exponential slope of CD4+ cell count increase during early ABT-538 treatment by baseline CD4+ cell count. Data show that patients with lower CD4+ cell counts exhibited greatest production rates. Adapted from Ho DD et al. Nature 1995.

plug the drain rather than solely attempt to increase the amount of water going into the basin—eg, through immune-based therapies designed to boost CD4+ cell production. He suggested that it is difficult to imagine how CD4+ cell production rate could be augmented beyond that observed in late-stage disease. He noted, however, that the functional capacity of regenerating CD4+ lymphocytes may be limited; thus, efforts to restore immune function are still necessary.

### Implications for Pathogenesis and Treatment

In summarizing the findings of these kinetics studies (Table

*One implication of the kinetics data is that since rapid turnover of HIV results in increasing viral diversity with time, aggressive treatment should be initiated early if dramatic clinical impact is desired.*

drawn from the findings: (1) continuous, high-level replication of HIV is the engine that drives the pathogenesis of infection; and

(2) the CD4+ lymphocyte depletion seen in AIDS is primarily a consequence of the destruction of these cells induced by HIV, not a lack of their production. At least two important implications for pathogenesis of HIV disease can be

(2) the CD4+ lymphocyte replenishment process is highly stressed, especially in later stages.

The implications for treatment are several fold. First, the primary effort must be to attack the virus. As noted by Dr Ho, this does not mean that treatment aimed at reconstitution of the immune system will not have additional important applications. Secondly, since rapid turnover of HIV results in increasing viral diversity with time, aggressive treatment should be initiated early if dramatic clinical impact is desired. Thirdly, at least with re-

### TABLE 4. KINETICS STUDIES SUMMARY

- CD4+ lymphocyte depletion seen in AIDS is primarily a consequence of the destruction of these cells induced by HIV-1, not a lack of their production
- Pathogenesis implications
  - (1) Continuous, high-level replication of HIV is the engine that drives the pathogenesis of infection
  - (2) CD4+ lymphocyte replenishment process is highly stressed (working overtime), especially in later stages
- Treatment implications
  - (1) Our primary effort must be to attack the virus
  - (2) Because rapid turnover of HIV results in increasing viral diversity with time, aggressive treatment should be initiated early if we wish to achieve a dramatic clinical impact
  - (3) Rapid turnover of plasma virus suggests that monitoring must be early and frequent

gard to the clinical trial setting, the rapid turnover of plasma virus suggests that monitoring must be early and frequent. Dr Ho suggested that with the information currently at hand, new antiretrovirals could be assessed for activity in vivo over the course of 10 to 14 days and eliminated from consideration if they do not show sufficient activity over this time period. In addition, the acute potency of various regimens could be appropriately ranked with early and frequent monitoring. However, the durability of antiviral effect of the regimens would still require long-term study. ■

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