Rapid turnover of plasma virions and CD4 lymphocytes in HIV-1 infection

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Treatment of infected patients with ABT-538, an inhibitor of the protease of human immunodeficiency virus type 1 (HIV-1), causes plasma HIV-1 levels to decrease exponentially (mean half-life, 2.1 ± 0.4 days) and CD4 lymphocyte counts to rise substantially. Minimum estimates of HIV-1 production and clearance and of CD4 lymphocyte turnover indicate that replication of HIV-1 *in vivo* is continuous and highly productive, driving the rapid turnover of CD4 lymphocytes.

In HIV-1 pathogenesis, an increased viral load correlates with CD4 lymphocyte depletion and disease progression[1–9], but relatively little information is available on the kinetics of virus and CD4 lymphocyte turnover *in vivo*. Here we administer an inhibitor of HIV-1 protease, ABT-538 (refs 10, 11), to twenty infected patients in order to perturb the balance between virus production and clearance. From serial measurements of the subsequent changes in plasma viraemia and CD4 lymphocyte counts, we have been able to infer kinetic information about the pretreatment steady state.

ABT-538 has potent antiviral activity *in vitro* and favourable pharmacokinetic and safety profiles *in vivo*[10]. It was administered orally (600–1,200 mg per day) on day 1 and daily thereafter to twenty HIV-1-infected patients, whose pretreatment CD4 lymphocyte counts and plasma viral levels ranged from 36 to 490 per mm^3 and from 15×10^3 to 554×10^3 virions per ml, respectively (Table 1). Post-treatment CD4 lymphocyte counts were monitored sequentially, as were copy numbers of particle-associated HIV-1 RNA in plasma, using an ultrasensitive assay (Fig. 1 legend) based on a modification of the branched DNA signal-amplification technique[12,13]. The trial design and clinical findings of this study will be reported elsewhere (M.M. *et al.*, manuscript in preparation).

Kinetics of HIV-1 turnover

Following ABT-538 treatment, every patient had a rapid and dramatic decline in plasma viraemia over the first two weeks. As shown using three examples in Fig. 1*a*, the initial decline in plasma viraemia was always exponential, demonstrated by a straight-line fit to the data on a log plot. The slope of this line, as defined by linear regression, permitted the half-life ($t_{1/2}$) of viral decay in plasma to be determined (Fig. 1 legend): for example, patient 409 was found to have a viral decay slope of −0.47 per day, yielding a $t_{1/2}$ of 1.5 days (Fig. 1*a*). Hence the rate and extent of decay of plasma viraemia was determined for each patient. As summarized in Fig. 1*b*, in every case there was a rapid decline, the magnitude of which ranged from 11- to 275-fold, with a mean of 66-fold (equivalent to 98.5% inhibition). The residual viraemia may be attributable to inadequate drug concentration in certain tissues, drug resistance, persistence of a small long-lived virus-producing cell population (such as macrophages), and gradual activation of a latently infected pool of cells. As summarized in Table 1, the viral decay slopes varied from −0.21 to −0.54 per day, with a mean of -0.34 ± 0.06 per day; correspondingly, $t_{1/2}$ varied from 1.3 to 3.3. days, with a mean of 2.1 ± 0.4 days. The latter value indicates that, on average, half of the plasma virions turn over every two days, showing that HIV-1 replication *in vivo* must be highly productive.

The exponential decline in plasma viraemia following ABT-538 treatment reflects both the clearance of free virions and the loss of HIV-1-producing cells as the drug substantially blocks new rounds of infection. But although drug inhibition is probably incomplete and virus-producing cells are not lost immediately, a minimum value for viral clearance can still be determined (Fig. 1 legend) by multiplying the absolute value of the viral decay slope by the initial viral load. Assuming that ABT-538 administration does not affect viral clearance, this estimate is also valid before treatment. As the viral load varies little during the pretreatment phase (Fig. 1*a*, and data not shown), we assume there exists a steady state and hence the calculated clearance rate is equal to the minimum virion production rate before drug therapy. Factoring in the patient's estimated plasma and extracellular fluid volumes based on body weight, we determined the minimum daily production and clearance rate of HIV-1 particles for each case (Table 1). These values ranged from 0.05 to 2.07×10^9 virions per day with a mean of $0.68 \pm 0.13 \times 10^9$ virions per day. Although these viral turnover rates are already high, true values may be up to a few-fold higher, depending on the $t_{1/2}$ of virus-producing lymphocytes. The precise kinetics of this additional parameter remains undefined. However, the mean $t_{1/2}$ of virus-producing cells is probably less, or in any case cannot be much larger, than the mean $t_{1/2}$ of 2.1 days observed for plasma virion elimination, demonstrating that turnover of actively infected cells is both rapid and continuous. It could also be inferred from our data that nearly all (98.5%) of the plasma virus must come from recently infected cells.

Examination of Fig. 1*b* shows that the viral decay slopes (clearance rate constants) are independent of the initial viral loads. The slopes do not correlate with the initial CD4 lymphocyte counts (Fig. 2*a*), another indicator of the disease status of patients. Therefore these observations strongly suggest that the viral clearance rate constant is not dependent on the stage of HIV-1 infection. Instead, they indicate that viral load is largely a function of viral production, because clearance rate constants vary by about 2.5-fold whereas the initial loads vary by almost 40-fold (Table 1).

Kinetics of CD4 lymphocyte turnover

After ABT-538 treatment, CD4 lymphocyte counts rose in each of the 18 patients that could be evaluated. As shown in three examples in Fig. 3, some increases were dramatic (patient 409, for example) whereas others (such as patient 303) were modest. Based on the available data, it was not possible to determine

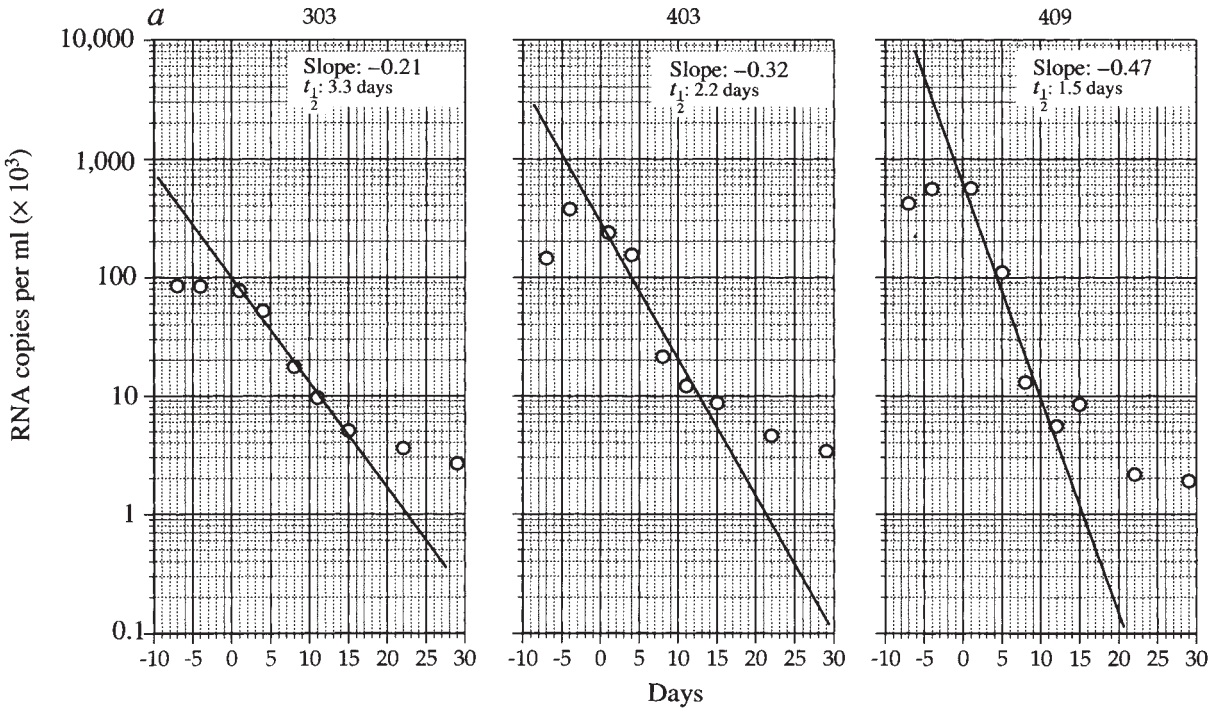
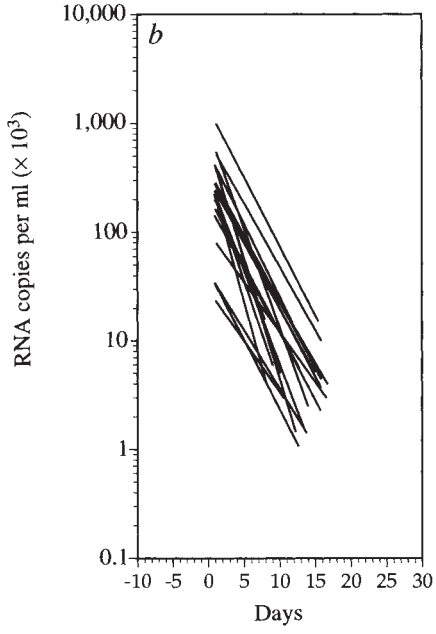


FIG. 1 *a*, Plasma viral load before and after ABT-538 treatment was begun on day 1 for three representative cases. Plasma samples were tested with the branched DNA signal-amplification assay as previously described[12,13]. Those samples with RNA levels below the detection sensitivity of 10,000 copies per ml were then tested using a modified assay differing from the original in two ways: hybridization of the bDNA amplification system is mediated by binding to overhangs on contiguous target probes; and the enzymatic amplification system has been enhanced by modification of wash buffers and the solution in which the alkaline phosphatase probe is diluted. The results of these changes are a diminution of background signals, an enhancement of alkaline phosphatase activity, and thus a greater detection sensitivity (500 copies per ml). Linear regression was used to obtain the best-fitting straight line for 3–5 data points between day 1 and the inflection point before the plateau of the new steady-state level. The slope, S, of each line represents the rate of exponential decrease; that is, the straight-line fit indicates that the viral load decreases according to $V(t) = V(0) \exp(-St)$. Given the exponential decay, $V(t_{1/2}) = V(0)/2 = V(0) \exp(-St_{1/2})$, and hence the viral half life, $t_{1/2} = \ln(2)/S$. Before drug administration, the change in viral load with time can be expressed by the differential equation, $dV/dt = P - cV$, where P is the viral production rate, c is the viral clearance rate constant, and V is the number of plasma virions. During the pretreatment steady state, $dV/dt = 0$, and hence $P = cV$. We have also tested more intricate models, in which the viral decay is governed by two or three exponential rates, namely the viral clearance rate, the decay rate of virus-producing cells, and the decay rate of latently infected cells. But there are insufficient data at this time to estimate the multiple parameters separately. Nonetheless, using the model in ref. 14, we find that if the death rate of latently infected cells is very small compared with the other two rates, then viral decay follows a simple exponential decline, with $S = c$, because the slow activation of a large number of latently infected cells offsets the loss of actively infected cells. Irrespective of the model, on a log plot, $S = -d(\ln V)/dt = c - P/V$. If drug inhibition is complete and virus-producing cells are rapidly lost (so $P = 0$), then $S = c$. If viral production continues, S is still $\leqslant c$, so the slope is a minimum estimate of the viral clearance efficiency. *b*, Decline of plasma viral load after ABT-538 treatment in all 20 patients. The slope for each case was obtained as already discussed, and the length of each line was determined by the initial viral load and the new steady-state level.



with confidence whether the rise was strictly exponential (Fig. 3, top) or linear (Fig. 3, bottom). An exponential increase would be consistent with proliferation of CD4 lymphocytes in the periphery, particularly in secondary lymphoid organs, whereas a linear increase would indicate cellular production from a precursor source such as the thymus[14]. Given that the thymus involutes with age and becomes further depleted with HIV-1 infection[15], it is more likely that the rise in CD4 lymphocytes is largely due to proliferation. Nevertheless, as both components may contribute, we analysed the obseved CD4 lymphocyte data by modelling both exponential and linear increases.

The slope of the line depicting the rise in CD4 lymphocyte counts on a log plot was determined for each case (Fig. 3, top). Individual slopes varied considerably, ranging from 0.004 to 0.088 per day, with a mean of 0.047 per day (Table 1), corresponding to a mean doubling time of ~15 days (Fig. 3 legend). On average, the entire population of peripheral CD4 lymphocytes was turning over every 15 days in our patients during the pretreatment steady state when CD4 lymphocyte production and destruction were balanced. Moreover, the slopes were inversely correlated with baseline CD4 lymphocyte counts (Fig. 2*b*) in that patients with lower initial CD4 cell counts had more pro-

TABLE 1 Summary data of HIV-1 and CD4 lymphocyte turnover during the pretreatment steady state

Baseline values			Kinetics of HIV-1 turnover			Kinetics of CD4 lymphocyte turnover*		
							Minimum production and destruction	
Patient	CD4 cell count (mm^{-3})	Plasma viraemia (virions per $ml \times 10^3$)†	Slope	$t_{1/2}$ (days)	Minimum production and clearance‡ (virions per day $\times 10^9$)	Slope	Blood§ (cells per day $\times 10^6$)	Total‖ (cells per day $\times 10^9$)
301	76	193	−0.30	2.3	0.56	0.070 (6.9)	21.7 (28.1)	1.1 (1.4)
302	209	80	−0.27	2.6	0.26	0.004 (0.5)	4.3 (2.7)	0.2 (0.1)
303	293	41	−0.21	3.3	0.11	0.005 (1.4)	9.9 (9.5)	0.5 (0.5)
304	174	121	−0.28	2.5	0.54	0.019 (1.9)	22.2 (13.0)	1.1 (0.6)
305	269	88	−0.33	2.1	0.50	0.055 (21.5)	108.0 (157.0)	5.4 (7.8)
306	312	175	−0.52	1.3	1.27	0.058 (25.7)	105.0 (150.0)	5.3 (7.5)
308	386	185	−0.46	1.5	1.48	0.020 (9.1)	55.9 (65.8)	2.8 (3.3)
309	49	554	−0.29	2.4	1.85	0.088 (11.8)	20.7 (56.6)	1.0 (2.8)
310	357	15	−0.26	2.7	0.05	0.038 (15.6)	71.0 (81.9)	3.6 (4.1)
311	107	130	−0.29	2.4	0.51	0.064 (11.0)	38.9 (62.8)	2.0 (3.1)
312	59	70	−0.30	2.3	0.30	0.048 (4.5)	17.0 (26.9)	0.8 (1.4)
313	47	100	−0.54	1.3	0.88	0.077 (5.9)	24.7 (40.5)	1.2 (2.0)
401	228	101	−0.40	1.7	0.47	NA	NA	NA
402	169	55	−0.28	2.5	0.21	0.014 (3.1)	13.4 (17.4)	0.7 (0.9)
403	120	126	−0.32	2.2	0.74	0.015 (2.4)	13.8 (18.7)	0.7 (0.9)
404	46	244	−0.27	2.6	1.06	0.080 (8.5)	24.6 (57.5)	1.2 (2.9)
406	490	18	−0.31	2.2	0.08	NA	NA	NA
408	36	23	−0.25	2.8	0.08	0.059 (3.4)	12.5 (19.7)	0.6 (1.0)
409	67	256	−0.47	1.5	2.07	0.073 (15.9)	35.3 (115.0)	1.8 (5.7)
410	103	99	−0.36	1.9	0.53	0.051 (5.6)	32.4 (34.5)	1.6 (1.7)
Range	36–490	15–554	−0.21 to −0.54	1.3–3.3	0.05–2.07	0.004–0.088 (0.5–25.7)	4.3–108.0 (2.7–157.0)	0.2–5.4 (0.1–7.8)
Mean	180 ± 46	134 ± 40	−0.34 ± 0.06	2.1 ± 0.4	0.68 ± 0.13	0.047 (8.6)	35.1 (53.2)	1.8 (2.6)

* The results for the kinetics of CD4 lymphocyte turnover generated by an exponential growth model are shown without parentheses; results generated by a linear production model are shown in parentheses.

† Each virion contains two RNA copies.

‡ Calculated using plasma and extracellular fluid volumes estimated from body weights, and assuming that plasma and extracellular fluid compartments are in equilibrium.

§ Calculated using blood volumes estimated from body weights.

‖ Calculated on the assumption that the lymphocyte pool in blood represents 2% of the total population[16]. NA, not analysed owing to large fluctuations in CD4 cell counts.

minent rises. This demonstrates convincingly that the 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.

As ABT-538 treatment reduces virus-mediated destruction of CD4 lymphocytes, the observed increase in CD4 cells provides a minimum estimate (Fig. 3 legend) of the pretreatment CD4 lymphocyte production rate, which in turn equals the destruction rate during the steady state. Minimum production (destruction) rates were calculated for each case by multiplying the slope, the initial CD4 cell count, and the estimated blood volume. The minimum numbers of CD4 cells in blood produced or destroyed each day ranged from 4.3×10^6 to 108×10^6, with a mean of 35.1×10^6 (Table 1). Given that the blood lymphocyte pool is about 2% of the total population[16], the overall CD4 lymphocyte turnover in our patients was calculated to vary from 0.2×10^9 to 5.4×10^9 cells per day, with a mean of 1.8×10^9 cells per day.

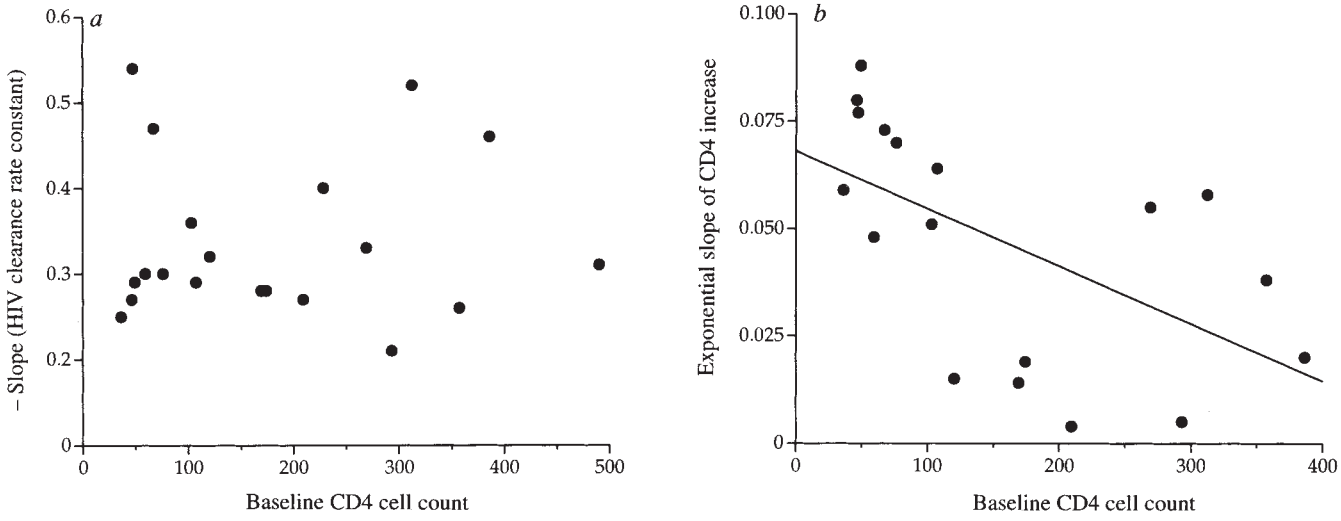


FIG. 2 *a*, Lack of correlation between viral decay slopes and disease status as indicated by baseline CD4 cell counts. Correlation coefficient = 0.05 (*P* value >0.1). *b*, Inverse correlation between the exponential CD4 increase slopes and baseline CD4 cell counts. Correlation coefficient = −0.57 (*P* value <0.01). Such an inverse correlation would be expected if T-cell proliferation were governed by a density-dependent growth function (logistic, for example), in which the growth rate decreases with increasing population level, if T cells were produced from precursors at a constant rate or from a combination of these two effects.

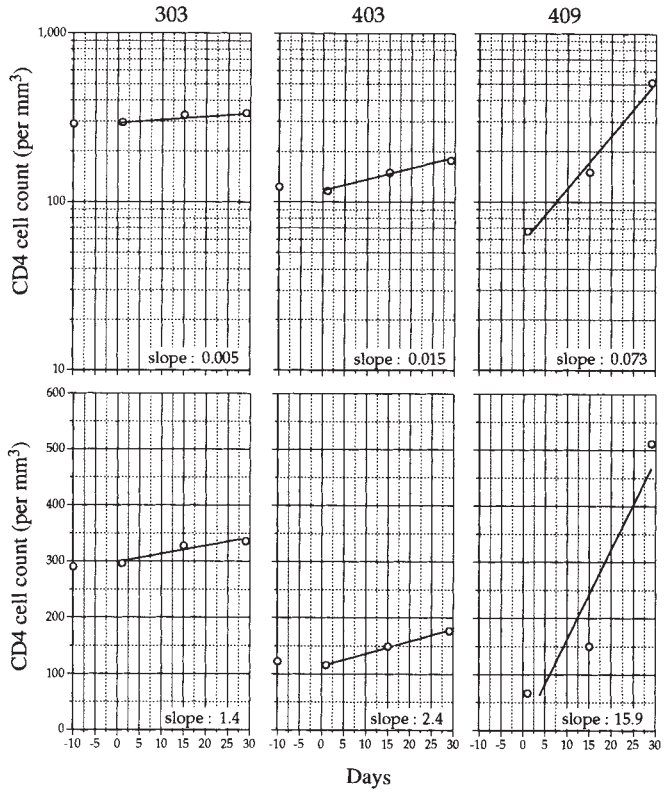


FIG. 3 Increase in CD4 cell counts after ABT-538 treatment plotted on a logarithmic (top) or linear (bottom) scale. Each slope was obtained from the best-fit line derived from linear regression on 2–4 data points. In the model for exponential increase, the doubling time was determined by dividing ln (2) by the slope. From the slope, we obtained minimum estimates of the CD4 lymphocyte production rate. The change in CD4 cell number over time can be described by the equation, $dT/dt = P - \mu T$, where T is the cell count, P is the cell production rate, and μ is the cell decay rate. The slope, S, on a log plot is thus = $d(\ln T)/dt = P/T - \mu$. Hence, $S \times T$ must be less than P, showing that our estimates indeed represent minimum CD4 lymphocyte production rates. Using a similar argument, slopes derived from a model of linear increases are also minimum estimates of CD4 lymphocyte production.

The increase in CD4 lymphocyte counts following ABT-538 administration was also modelled linearly (Fig. 3, bottom). The slope of the line depicting the increase for each case was determined, and the values varied from 0.5 to 25.7 cells per mm^3 per day, with a mean of 8.6 cells per mm^3 per day (Table 1). Using the same argument as for the exponential case, minimum estimates of total CD4 production (or destruction) rates at baseline were determined to vary from 0.1×10^9 to 7.8×10^9 cells per day, with a mean of 2.6×10^9 cells per day.

Although our two sets of CD4 lymphocyte analyses do not yield identical numerical results, they are in close agreement and emphasize the same qualitative points about HIV-1 pathogenesis. The number of CD4 lymphocyte destroyed and replenished each day is of the order of 10^9, which is strikingly close to estimates of the total number of HIV-1 RNA-expressing lymphocytes in the body determined using *in situ* polymerase chain reaction and hybridization methods[8,17]. In addition, CD4 replenishment appears to be highly stressed in many patients in that the faster production rates are ~25–78-fold higher than the slowest rate (Table 1), which is presumably still higher than the as-yet-undefined normal CD4 turnover rate. The precise mechanisms of CD4 lymphocyte repopulation, however, will have to be addressed in the future by studies on phenotypic markers and functional status of the regenerating cells. Nonetheless, the rapid CD4 lymphocyte turnover has several implications. First, the apoptosis commonly observed in the setting of HIV-1 infection[18] may simply be an expected consequence of an active lymphocyte regenerative process. Second, the CD4 lymphocyte depletion seen in advanced HIV-1 infection may be likened to a sink containing a low water level, with the tap and drain both equally wide open. As the regenerative capacity of the immune system is not infinite, it is not difficult to see why the sink eventually empties. It is also evident from this analogy that our primary strategy to reverse the immunodeficiency ought to be to target virally mediated destruction (plug the drain) rather than to emphasize lymphocyte reconstitution (put in a second tap).

Discussion

We believe our new kinetic data have important implications for HIV-1 therapy and pathogenesis. It is self evident that, with rapid turnover of HIV-1, generation of viral diversity and the attendant increased opportunities for viral escape from therapeutic agents are unavoidable sequelae[19,20]. Treatment strategies, if they are to have a dramatic clinical impact, must therefore be initiated as early in the infection course as possible, perhaps even during seroconversion. The rapid turnover of HIV-1 in plasma also suggests that current protocols for monitoring the acute antiviral activity of novel compounds must be modified to focus on the first few days following drug initiation. Our interventional approach to AIDS pathogenesis has shown that HIV-1 production and clearance are delicately balanced but highly dynamic processes. Taken together, our findings strongly support the view that AIDS is primarily a consequence of continuous, high-level replication of HIV-1, leading to virus- and immune-mediated killing of CD4 lymphocytes. □

Received 16 November; accepted 15 December 1994.

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ACKNOWLEDGEMENTS. We thank the patients for their participation, Y. Cao and J. Wilbur for assistance with branched DNA assays, A. Hsu and J. Valdes for input on trial design, and J. Moore and R. Koup for helpful discussions. This work was supported by grants from Abbott Laboratories, the NIH and NYU Center for AIDS Research, the Joseph P. and Jeanne M. Sullivan Foundation, Los Alamos National Laboratory LRDR Program and The Aaron Diamond Foundation.