ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Feb. 1997, p. 449–453 Vol. 41, No. 2
0066-4804/97/$04.00+0

Modeling of the Change in CD4 Lymphocyte Counts in Patients before and after Administration of the Human Immunodeficiency Virus Protease Inhibitor Indinavir

DANIEL S. STEIN* AND GEORGE L. DRUSANO

Departments of Medicine and Pharmacology, Albany Medical College, Albany, New York

Received 11 June 1996/Returned for modification 30 September 1996/Accepted 7 November 1996

We investigated the relationships between changes in CD4 lymphocyte counts over 24 weeks after the initiation of therapy with indinavir at dosages of ≥2.4 g/day ($n = 15$) in human immunodeficiency virus-positive patients and compared them to the baseline values. Starting CD4 counts were linked to the time-weighted average CD4 cell count (return) through a nonlinear effect model. The diminution of destruction of CD4 cells after the initiation of indinavir therapy was estimated by fitting simultaneous differential equations to the data by using a linked lymph node (LN)-blood (BL) (two-compartment) system in which there is a constant rate of generation (R), first-order transfer rate constants ($K_{LN\text{-}BL}$ and $K_{BL\text{-}LN}$) of compartment exchange, and first-order rate constants of CD4 destruction in the absence and presence of indinavir ($K_{LN\text{-}OUT1}$ and $K_{LN\text{-}OUT2}$). The half-life of CD4 lymphocytes was calculated from the rate constants by standard two-compartment methods. The CD4 lymphocyte counts at the start and return were linked in a sigmoid-E_{max} model where the maximal effect (E_{max}) was at 574.6 cells/μl and 50% of the effect occurred at 157.1 cells/μl ($r^2 = 0.94$; $P < 0.001$). The mean ± standard deviation (median) $K_{LN\text{-}OUT2}$ was 0.574 ± 0.202 (0.589), indicating that indinavir decreased the destruction of CD4 cells by circa 41 to 42%. The mean (median) CD4 half-life was 11.5 ± 5.72 day (10.3 days). In multivariate analysis, $K_{LN\text{-}OUT2}$ was significantly correlated with starting the CD4 cell count and the change in the CD4 cell count on therapy. The relationship between CD4 lymphocyte half-life and the starting CD4 lymphocyte count was hyperbolic, with a rapid increase in half-life as the CD4 count decreased. On the basis of the calculated half-life, the average production (destruction) of CD4 lymphocytes was approximately 3×10^9 cells/day, with an individual variation of 44-fold. These findings suggest that (i) the CD4 lymphocyte cell count at the start is significantly correlated to both the decrease in the destruction rate of CD4 cells and the degree of change in the CD4 lymphocytes on therapy; (ii) the lower the initial CD4 lymphocyte count, the higher the amount of CD4 lymphocyte turnover and the lower the ability of the immune system to increase absolute CD4 lymphocyte levels after viral suppression, consistent with a decreased regenerative capacity with progression of disease; and (iii) the increase in CD4 lymphocytes is likely secondary to the expansion of a proliferating pool of cells since our determinations are based on 24 weeks of effect.

CD4 lymphocytes are the cells primarily infected by human immunodeficiency virus (HIV). In the presence of an effective antiviral compound the number of circulating CD4 lymphocytes generally increases as the HIV load decreases. Previous studies have examined the relationship between baseline CD4 lymphocyte values and subsequent changes in both antiviral agent and CD4 lymphocyte levels after exposure to nucleoside agents (3, 4, 18). The degree of these changes and their relationship to drug exposure have not previously been investigated for protease inhibitors. We therefore examined the return (or time-weighted average) of CD4 lymphocytes and the inhibition of their destruction after the initiation of therapy with indinavir (L-735524; MK-639; Merck Research Laboratories) in HIV-positive patients. By investigating these relationships, we can gain further insight into the expected degree of CD4 lymphocyte changes while a patient is receiving therapy, the CD4 lymphocyte half-life at steady state, and the degree of turnover of CD4 lymphocytes per day.

For this analysis we used two approaches: classical nonlinear pharmacokinetic models of effect and two-compartment models with simultaneous first-order inhomogeneous differential equations. Nonlinear (sigmoid-E_{max} [where E_{max} is maximal effect]) models are used because the CD4 lymphocyte population, after the introduction of the protease inhibitor, is in transition between the pre-drug-effect steady state and the post-drug-effect steady state. A two-compartment model is used, in contrast to the models used in earlier studies (9, 23), since the production and trafficking of lymphocytes involve both the blood and lymphatic compartments. The rate constants of lymphocyte exchange from this model can then be used to calculate the CD4 lymphocyte half-life by standard two-compartment methods. By use of the information on half-life, the average daily turnover (production and destruction) of CD4 lymphocytes necessary to maintain steady state can then be estimated.

MATERIALS AND METHODS

All patients were in phase I/II trials, with inclusion criteria of a baseline HIV RNA load determined by PCR of ≥20,000 copies/ml, no acute medical problems, cessation of antiretroviral agents for at least 2 weeks prior to entry into the study, and adequate renal, hepatic, and bone marrow function. The CD4 lymphocyte count had to be ≤300 cells/μl for the first group (22) and between 150 and 500 cells/μl for the remaining groups. For the patients studied, all of whom had received prior nucleoside therapy, indinavir dosages of 2.4 g/day (600 mg every 6 h [q6h] [$n = 5$]; or 800 mg every 8 h [q8h] [$n = 3$]), 3 g/day (as 1,000 mg q8h

* Corresponding author. Mailing address: Departments of Medicine and Pharmacology, Albany Medical College, 47 New Scotland Ave., M/C A-142, Albany, NY 12208. Phone: (518) 262-6330. Fax: (518) 262-6333.

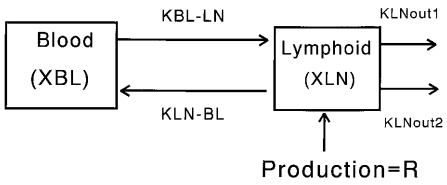

FIG. 1. Illustration of the model variables and their relationships as described in the differential equations. $K_{\text{LN-OUT1}}$ and $K_{\text{LN-OUT2}}$ are turned on and off by piecewise input functions based on the initiation of therapy.

[$n = 2$]), and 3.2 g/day (as 800 mg q6h [$n = 5$]). All dosage groups evaluated had demonstrated similar and significant antiviral activities (21, 21a). Two separate CD4 lymphocyte count determinations, but not the screening value, were averaged to establish the baseline count. All CD4 lymphocyte determinations for an individual patient were determined in the same laboratory by a standard methodology (7). No other antiviral agents or agents that could potentially interfere with the hepatic metabolism of indinavir (e.g., rifabutin and fluconazole) were allowed during the study. All except one of the patients were studied for 24 weeks; one patient in the highest-dose cohort withdrew from the protocol after 16 weeks. Counts were determined every 2 weeks for 12 weeks (24 weeks for the first group) and then every 4 weeks through week 24. Screening HIV RNA load determinations were performed by a commercially available PCR assay (12).

Average CD4 lymphocyte counts were determined by estimating the area under the CD4 lymphocyte cell-versus-time curve by using the LAGRAN program of Rocci and Jusko (17). This value was divided by time on therapy to yield a time-weighted average CD4 lymphocyte count (or average CD4 cell return). Starting CD4 counts were then linked to the average CD4 cell return through a sigmoid-$E_{\max}$ model, as had been explored earlier for the five subjects receiving the dosage of 600 mg q6h (22). Sigmoid relationships are the classical nonlinear relationships found in pharmacologic interventions (8, 10), where Return = $E_{\max} \cdot \text{Start}^H/(\text{Start}^H + \text{Start}_{50}{}^H)$, where the $E_{\max}$ is the maximal effect, the Start is the baseline or starting CD4 lymphocyte count, Start_{50} is the starting CD4 lymphocyte count at which 50% of the maximal effect occurs, and H is the sigmoidicity. The Akaike information criterion (23) was used to determine the number of parameters allowed in the model. In the model presented, the sigmoidicity was fixed at 1.0 because use of the Akaike information criterion did not support inclusion of a floating H term.

Diminution of destruction of CD4 cells after the initiation of indinavir was estimated by fitting the following simultaneous differential equations to the data by using a linked lymph node (LN)-blood (BL) (or two-compartment) system which allows for the inclusion of the majority of the lymphocytes which are not in circulation, where

$$dX_{\text{LN}}/dT = R + (K_{\text{BL-LN}} \cdot X_{\text{BL}}) - (K_{\text{LN-BL}} \cdot X_{\text{LN}}) - (K_{\text{LN-OUT1}} \cdot X_{\text{LN}}) - (K_{\text{LN-OUT2}} \cdot X_{\text{LN}})$$

$$dX_{\text{BL}}/dT = (K_{\text{LN-BL}} \cdot X_{\text{LN}}) - (K_{\text{BL-LN}} \cdot X_{\text{BL}})$$

where R is the constant rate of generation, X_{LN} is the amount of virus in the lymph node (peripheral) compartment, X_{BL} is the amount of virus in the blood, and $K_{\text{LN-BL}}$ and $K_{\text{BL-LN}}$ are the first-order transfer rate constants of compartment exchange. $K_{\text{LN-OUT1}}$ and $K_{\text{LN-OUT2}}$ are the first-order rate constants of CD4 lymphocyte cell destruction in the absence and presence of indinavir. The model is illustrated in Fig. 1. The rate constants $K_{\text{LN-OUT1}}$ and $K_{\text{LN-OUT2}}$ were turned on or off by piecewise input functions on the basis of the time of initiation of indinavir therapy. $K_{\text{LN-OUT1}}$ was fixed to 1.0 so that $K_{\text{LN-OUT2}}$ would represent the fractional rate of destruction after indinavir exposure. The relationship ($1 - K_{\text{LN-OUT2}}$) is therefore the change in destruction of CD4 lymphocytes mediated by indinavir exposure (or the downturn of the destruction rate constant). For one patient (described elsewhere [22]), secondary to a marked decrease in his CD4 lymphocyte count after 6 weeks because of newly diagnosed cytomegalovirus infection and the initiation of ganciclovir administration, the first differential equation was altered to allow a new CD4 cell generation rate which was a fraction of the initial rate. This change of rate occurred after the diagnosis of the cytomegalovirus infection and the initiation of ganciclovir and was accomplished by use of piecewise input functions that turned the first rate off as the new one was turned on.

The experimental data were used to estimate the parameters in the equations given above by use of the ADAPT II program of D'Argenio and Schumitzky (1). A weight of 1 was used for all values except for the baseline count, for which a weight of 2 was used since it represented two separate determinations. The half-life of CD4 lymphocytes was determined from the rate constants obtained from the analysis described above by using the following standard half-life formula:

$$\text{half-life of CD4 lymphocytes} = \ln(2)/\{(K_{\text{LN-OUT2}} + K_{\text{LN-BL}} + K_{\text{BL-LN}}) - \text{SQRT}\,[(K_{\text{LN-OUT2}} + K_{\text{LN-BL}} + K_{\text{BL-LN}})^2 - 4 \cdot K_{\text{LN-OUT2}} \cdot K_{\text{BL-LN}}]\}/2$$

where SQRT is square root. This half-life is a hybrid of several processes which occur simultaneously and is a value reflective of the interval required for the CD4 lymphocyte count to achieve 50% of the difference between baseline and steady-state counts. The model-estimated curves of the CD4 lymphocyte were used for the baseline and maximal change values in subsequent comparisons. Multivariate linear regression analysis was performed with the SYSTAT package of programs (SPSS, Inc., Evanston, Ill.).

RESULTS

The population enrolled in the study (Table 1) had a group mean CD4 lymphocyte count of 174.7 ± 103.4 cells/μl (median, 175 cells/μl), with a mean $\log_{10}$ number of HIV RNA copies/ml of 4.8974 ± 0.2906 (geometric mean, 78,971 copies/ml). The modeled increase in the CD4 lymphocyte count was 91.6% (range, 13.2 to 295%) over the baseline, or an absolute change of 106.5 ± 49 cells/μl (from a modeled starting mean of 183.2 cells/μl to an average steady-state mean of 289.7 cells/μl). The baseline CD4 lymphocyte count and its return (Fig. 2) were significantly linked ($r^2 = 0.94$; $P < 0.0001$); the $E_{\max}$ attributable to the drug intervention was 574.6 cells/μl, and Start_{50} was 157.1 cells/μl with H fixed at 1 (Hill's model of the general sigmoid-$E_{\max}$ equation). These data indicate that CD4 cell return is determined in large part by the starting CD4 cell count over the dose range of indinavir examined. The fit of the CD4 counts over time to the differential equations were acceptable, with the variance explained by the relationship (r^2), ranging from 0.13 to 0.89 ($r = 0.37$ to 0.94). The mean ± standard deviation (median) $K_{\text{LN-OUT2}}$ was 0.574 ± 0.202 (0.589), indicating that indinavir decreased the destruction of CD4 cells by circa 41 to 43% (Table 1). By using the rate constants obtained from the equations given above and the equation for half-life determination, the mean half-life of the CD4 lymphocytes was 11.5 ± 5.72 days (median, 10.3 days).

As determined by univariate analysis, $K_{\text{LN-OUT2}}$ was correlated with the size of the CD4 cell count change as percent change ($F = 82.463$; $P < 0.0001$), with a trend as absolute change from the baseline ($F = 3.536$; $P = 0.08$), the baseline CD4 count ($F = 48.96$; $P < 0.0001$), and, as expected from the known relationship, CD4 half-life ($F = 26.013$; $P < 0.0001$); but a correlation was not observed by using the screening $\log_{10}$ number of HIV RNA copies per milliliter. On multivariate analysis, the CD4 count change from the baseline, either as percent change ($P = 0.003$) or as absolute change ($P = 0.003$), and the baseline CD4 cell count remained significantly correlated (overall $F = 94.134$; $P < 0.0001$). The CD4 lymphocyte half-life had, in addition to the relationship with $K_{\text{LN-OUT2}}$ discussed above, significant correlations with the baseline CD4 lymphocyte count ($F = 10.328$, $P = 0.007$), and the percent increase in CD4 lymphocyte cell count from the baseline ($F = 45.852$; $P < 0.0001$), but not the absolute cell change from baseline or screening of the $\log_{10}$ number of HIV RNA copies per milliliter. On multivariate analysis only the percent increase in CD4 cell count from the baseline remained significantly associated ($F = 45.852$; $P < 0.0001$) with the CD4 cell half-life. The relationship between CD4 cell half-life and the starting CD4 lymphocyte count, as seen in Fig. 3, is not linear but is hyberbolic, which explains its removal in multivariate linear regression analysis. The CD4 cell half-life rapidly increases as the starting CD4 lymphocyte cell count decreases. The relationship best fits an equation derived from the calculation of half-life: $\ln(2)/[0.000163 \cdot (\text{CD4 at start}) + 0.034569]$; $r^2 = 0.42$; $P < 0.005$. This is consistent with a significant impairment in the ability of the CD4 lymphocyte population to expand by the time that the baseline CD4 cell count is severely decreased.

From our calculation of CD4 cell half-life, it is possible to

TABLE 1. Data for patients included in the analysis[a]

Patient no.	Baseline CD4 count (cells/μl)	$\log_{10}$ no. of HIV RNA copies/ml	$K_{LN\text{-}OUT2}$	Downturn	CD4 cell turnover (cells/day)	Half-life (days)
1	56	4.6675	0.315	0.685	5.42×10^8	15.5
2	169	5.1033	0.454	0.546	1.54×10^9	16.5
3	64	5.3539	0.44	0.56	8.73×10^8	11.0
4	14	4.3304	0.458	0.542	1.71×10^8	12.3
5	27.5	5.2625	0.183	0.817	1.56×10^8	26.5
6	265	5.1396	0.717	0.283	5.76×10^9	6.9
7	120	4.9548	0.351	0.649	1.30×10^9	13.9
8	180	4.8035	0.553	0.447	1.54×10^9	17.5
9	260	4.9851	0.701	0.299	5.65×10^9	6.9
10	175	4.9046	0.767	0.223	4.10×10^9	6.4
11	300	5.1893	0.74	0.26	6.82×10^9	6.6
12	280	4.6776	0.794	0.206	6.89×10^9	6.1
13	345	4.8067	0.883	0.117	5.02×10^9	10.3
14	155	4.4509	0.657	0.343	3.14×10^9	7.4
15	210	4.8324	0.589	0.411	3.80×10^9	8.3
Mean ± SD	174.7 ± 103.4	4.8974 ± 0.2906	0.574 ± 0.202	0.427	3.15×10^9	11.5 ± 5.72
Median	175	4.9046	0.589	0.411	3.14×10^9	10.3

[a] $K_{LN\text{-}OUT2}$ is the rate constant of destruction in the presence of protease inhibitor. The relationship $(1 - K_{LN\text{-}OUT2})$ is the change in the level of destruction of CD4 lymphocytes mediated by indinavir exposure (or downturn of destruction rate constant). The CD4 cell turnover per day is based on the following equation: {[(baseline CD4 cells/μl) · (1,000 μl/ml) · (1,000 ml/liter) (6 liters of blood volume)/(0.02 of total CD4 cells are in circulation)]/2}/half-life.

determine the turnover of CD4 lymphocytes. As can be seen in Table 1, if our average subject had a CD4 cell count of approximately 200 cells/μl, the CD4 cells had an average half-life of 10 to 11 days, and the subject had a blood volume of 6 liters and assuming that 98% of the body's lymphocytes are not in circulation, then by using these assumptions and standard conversion factors (see the equation in footnote *a* of Table 1), approximately 3×10^{10} cells are made in one half-life, which is approximately 3×10^9 cells per day at steady state. The range for the daily turnover of CD4 cells is 1.56×10^8 to 6.89×10^9, a 44-fold variation.

The decrease in the destruction rate of CD4 cells was best explained by the following equation: (CD4 cell count at baseline) · (−0.002) + ($\log_{10}$ number of HIV RNA copies) · (0.199) − 0.214 ($r^2 = 0.978$; $P < 0.0001$). This, however, was only marginally better and not statistically different from the equation (CD4 cell count at baseline) · (−0.002) + 0.747 ($r^2 = 0.964$; $P < 0.0001$). Use of the Akaike information criterion would not support the use of the two-parameter model over the simpler one-parameter model.

DISCUSSION

Our data from a study involving 24 weeks of intervention, based on two different methods of mathematical modeling, indicate that the CD4 lymphocyte cell count at the start is significantly correlated to both the decrease in the destruction rate of CD4 cells and the degree of change in the CD4 cell count on therapy. These findings indicate that the lower the initial CD4 lymphocyte count, the higher the amount of CD4 lymphocyte turnover and the lower the ability of the immune system to regenerate absolute CD4 lymphocyte levels after viral suppression. This finding from the sigmoid-E_{max} model

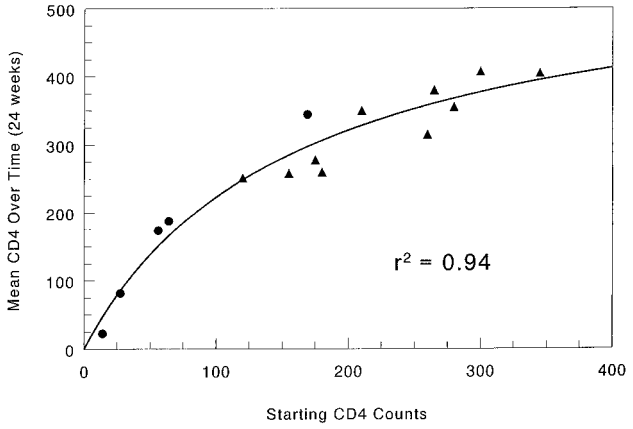

FIG. 2. Sigmoid-E_{max} relationship of starting CD4 cell count to mean CD4 cell count over time. The subjects received one of four doses of indinavir, the lowest of which (●) was already known to have significant antiviral activity. The higher doses are all represented by closed triangles. See Materials and Methods for a description of the entry criteria for the trials, the equations, and calculation of the relationship.

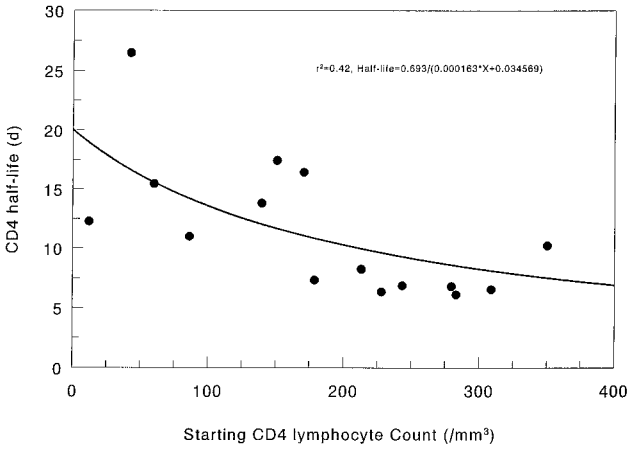

FIG. 3. Relationship between starting CD4 lymphocyte count and CD4 cell half-life. Note the rapid increase in CD4 cell half-life as the starting CD4 lymphocyte count decreases. See Materials and Methods for calculation of the relationship.

was also found in the relationship of CD4 cell half-life to starting CD4 lymphocyte count. In both cases, a nonlinear relationship indicated significant impairment of the expansion of the CD4 lymphocyte population with progressive depletion of the population at the baseline, consistent with the immunopathophysiology of HIV disease (5, 6, 13–15). Another implication of these models is that earlier therapeutic intervention would be associated with less of a change in the CD4 cell count from that at the baseline on a percentage basis because the degree of CD4 lymphocyte turnover is less but the absolute values over time would tend to be higher.

The average decrease in the rate of destruction of CD4 cells after the initiation of treatment with the protease inhibitor was 41% in a population with a mean CD4 count at the baseline of 174.7 ± 103.4 cells/μl. While the degree of change in the CD4 cell count was greater after inhibition by the protease inhibitor indinavir than those after inhibition by nucleoside agents, the significant relationship between starting CD4 cell counts and the time-averaged CD4 cell counts (return) while the subject is on therapy is consistent with previous observations on nucleoside therapy and our prior observation for a subgroup of five patients receiving indinavir (3, 4, 18, 21a). The lack of an association between a change in the CD4 lymphocyte count and the baseline viral load may be secondary to the study limitation of enrolling only those subjects with viral loads of ≥20,000 copies/ml at the baseline, although the association has been observed by others (9).

We determined the CD4 lymphocyte half-life to be an average of 11.5 days. It was significantly associated with the percent increase in CD4 lymphocyte count on therapy but not with other parameters by multivariate linear regression. As we demonstrate in Fig. 3, the relationship between half-life and starting CD4 lymphocyte count, similar to the case for starting CD4 cell count and CD4 cell return, is nonlinear. A linear relationship between half-life and starting CD4 lymphocyte count, found on univariate analysis, would still result in an increasing CD4 cell half-life because the starting CD4 lymphocyte count declined but was rejected by multivariate analysis and would not be consistent with the formula for the calculation of half-life. Therefore, only the significant nonlinear relationship between half-life and starting CD4 cell count fits both the analysis and the inverse function formula for half-life determination. This CD4 lymphocyte half-life is likely a reflection of the effect of the decrease in the destruction of CD4 lymphocytes by inhibition of viral replication. Therefore, several processes and assumptions are part of its determination. The generation rate of CD4 lymphocytes was assumed to be constant. Whether the stimuli that drive this process would decrease as the rate of destruction of the cells decreases is unknown. While an antiviral therapeutic intervention would decrease the amount of viral replication and therefore decrease the amount of cell destruction, it would not be expected to affect the rate of cell replication or exchange between the tissue and blood compartments. We modeled the lymphocyte changes as a two-compartment system since the lymphocytes are exchanged between the circulating compartment, where their levels are measured, and the tissue compartment, where their levels are not measured. This is essential, since the majority (98%) of lymphocytes reside outside the circulation and prior investigations have indicated significant viral replication and cell destruction in the tissue compartment (5, 6, 13, 14, 19). The measured half-life is a conservative measure of a system in steady state that is subjected to an external perturbation that eventually results in a new steady state. This is physiologic, since the effect of the protease inhibitor is to markedly decrease the rate of destruction of the CD4 lymphocytes; however, there is still an ongoing rate of destruction (and production) after the intervention, which suggests that the true half-life of the CD4 cells is probably shorter than the one that we calculated.

The CD4 cells of our average subject, with a CD4 cell count of 200 cells/μl, had an average half-life of 10 to 11 days, and assuming that 98% of the body's lymphocytes are not in circulation (19), then approximately 3×10^{10} cells are made in one half-life, which is approximately 3×10^9 cells per day at steady state. These results are similar to those estimated by Ho et al. (9) and Wei et al. (22), despite differences in methodologies, and to estimates of the total number of HIV type 1 RNA-expressing lymphocytes in the body, as determined by in situ PCR and hybridization (5). The previous (9, 22) one-compartment linear and log-linear estimates gave an approximate half-life of 15 days, with approximately 2×10^9 cells produced and destroyed each day on the basis of short-term induced changes in CD4 lymphocyte populations. However, unlike the relationship suggested by Ho et al. (9), we found a strong relationship between the starting CD4 lymphocyte count and the change in CD4 lymphocyte count over time.

Several investigators have disputed the finding of a high rate of CD4 turnover per day (2, 11, 16, 20) and have suggested instead that the previously described turnover rates based on the first 28 days of observation could also be consistent with the mobilization of peripheral lymphocytes and a reduction in the level of trapping in lymphoid tissue. In our model we used 24 weeks of data for all except one person, for whom 16 weeks of data were used. Since the half-life determined was based on this long period of observation, it is unlikely that changes in mobilization or trapping in lymphoid tissue could explain the high degree of cellular turnover. Even if fewer than 98% of the lymphocytes reside outside the blood in HIV-infected individuals, a possibility raised by others (2, 11, 16, 20), the degree of CD4 lymphocyte turnover measured here would still be high. For example, as a limiting case, if none of the lymphocytes resided outside the circulation, the CD4 lymphocyte turnover would still be at least 6×10^7 cells/day, assuming a 10-day half-life and a starting CD4 lymphocyte count of 200 cells/μl. Therefore, our data support the conclusion that this increase is largely due to the proliferation of CD4 lymphocytes, supporting an immunopathology of HIV infection in which the viral infection overwhelms the high regenerative capacity of the lymphoid system until it can no longer maintain itself.

REFERENCES

1. **D'Argenio, D. Z., and A. Schumitzky.** 1992. ADAPT II. A program package for simulation, identification, and optimal experimental design. Users manual. Biomedical Simulations Resource, University of Southern California, Los Angeles.
2. **Dimitrov, D. S., and M. A. Martin.** 1995. $CD4^+$ cell turnover. Nature **375:** 194–195. (Letter.)
3. **Drusano, G. L., F. M. Balis, S. R. Gitterman, and P. A. Pizzo.** 1994. Quantitative relationships between zidovudine exposure exposure and efficacy and toxicity. Antimicrob. Agents Chemother. **38:**1726–1731.
4. **Drusano, G. L., G. J. Yuen, J. S. Lambert, M. Seidlin, R. Dolin, and F. T. Valentine.** 1992. Quantitative relationships between dideoxyinosine exposure and surrogate markers of response in a phase I trial. Ann. Intern. Med. **116:**562–566.
5. **Embretson, J., M. Zupancic, J. L. Ribas, A. Burke, P. Racz, K. Tenner-Racz, and A. T. Hause.** 1993. Massive covert infection of helper T lymphocytes and macrophages by HIV during the incubation period of AIDS. Nature **362:** 359–362.
6. **Fox, C. H., K. Tenner-Racz, P. Racz, A. Firpo, P. A. Pizzo, and A. S. Fauci.** 1991. Lymphoid germinal centers are reservoirs of human immunodeficiency virus type 1 RNA. J. Infect. Dis. **164:**1051–1057.
7. **Gelman, R., S. C. Cheng, P. Kidd, M. Waxdal, and J. Kagan.** 1993. Assessment of the effects of instrumentation, monoclonal antibody, and fluorochrome on flow cytometric immunophenotyping: a report based on 2 years of the NIAID DAIDS flow cytometry quality assessment program. Clin. Immunol. Immunopathol. **66:**150–162.
8. **Hill, A. V.** 1910. The possible effects of the aggregation of the molecules of

haemoglobin on its dissociation curves. J. Physiol. **40:**4–7.
9. **Ho, D. D., A. U. Neumann, A. S. Perelson, W. Chen, J. M. Leonard, and M. Markowitz.** 1995. Rapid turnover of plasma virions and CD4 lymphocytes in HIV-1 infection. Nature **373:**123–126.
10. **Holford, N. H. G., and L. B. Sheiner.** 1981. Understanding the dose-effect relationship: clinical application of pharmacokinetic-pharmacodynamic models. Clin. Pharmacokinet. **6:**429–453.
11. **Mosier, D. E.** 1995. $CD4^+$ cell turnover. Nature **375:**193–194. (Letter.)
12. **Mulder, J., N. McKinney, C. Christopheron, J. Sninsky, L. Greenfield, and S. Kwok.** 1994. Rapid and simple PCR assay for quantitation of human immunodeficiency virus type 1 RNA in plasma: application to acute retroviral infection. J. Clin. Microbiol. **32:**292–300.
13. **Pantaleo, G., C. Graziosi, L. Butini, P. A. Pizzo, S. M. Schnittman, D. P. Kotler, and A. S. Fauci.** 1991. Lymphoid organs as the major reservoirs for human immunodeficiency virus. Proc. Natl. Acad. Sci. USA **88:**9838–9842.
14. **Pantaleo, G., C. Graziosi, J. F. Demarest, L. Butini, M. Montroni, C. H. Fox, J. M. Orenstein, D. P. Kotler, and A. S. Fauci.** 1993. HIV infection is active and progressive in lymphoid tissue during the clinically latent stage of disease. Nature **362:**355–358.
15. **Pantaleo, G., C. Graziosi, and A. S. Fauci.** 1993. The immunopathogenesis of human immunodeficiency virus infection. N. Engl. J. Med. **328:**327–335.
16. **Phillips, A. N., C. A. Sabin, A. Mocroft, and G. Janossy.** 1995. $CD4^+$ cell turnover. Nature **375:**195. (Letter.)
17. **Rocci, M. L., and W. J. Jusko.** 1983. LAGRAN program area and moments in pharmacokinetic analysis. Comput. Programs Biomed. **16:**203–216.
18. **Sale, M., L. B. Sheiner, P. Volberding, and T. F. Blaschke.** 1993. Zidovudine response relationships in early human immunodeficiency virus infection. Clin. Pharmacol. Ther. **54:**556–566.
19. **Sprent, J., and D. F. Tough.** 1994. Lymphocyte life-span and memory. Science **265:**1395–1400.
20. **Sprent, J., and D. Tough.** 1995. $CD4^+$ cell turnover. Nature **375:**194. (Letter.)
21. **Steigbigel, R. T., P. Berry, J. Mellors, D. McMahon, H. Teppler, D. Stein, G. Drusano, P. Deutsch, K. Yeh, C. Hildebrand, M. Nessly, E. Emini, and J. Chodakewitz.** 1996. Efficacy and safety of the HIV protease inhibitor Indinavir sulfate (MK-639) at escalating dose, abstr. 146. *In* Abstracts of the 3rd Conference on Human Retroviruses and Opportunistic Infections.

21a. **Stein, D. S., D. G. Fish, J. A. Bilello, S. L. Preston, G. L. Martineau, and G. L. Drusano.** 1996. A 24 week open label phase 1 evaluation of the HIV protease inhibitor MK-639 (indinavir). AIDS **10:**485–492.

22. **Wei, X., S. K. Ghosh, M. E. Taylor, V. A. Johnson, E. A. Emini, P. Deutsch, J. D. Lifson, S. Donhoeffer, M. A. Nowak, B. H. Hahn, M. S. Saag, and G. M. Shaw.** 1995. Viral dynamics in human immunodeficiency virus type 1 infection. Nature **373:**117–122.
23. **Yamaoka, K., T. Nakagawa, and T. Uno.** 1978. Application of Akaike's information criterion (AIC) in the evaluation of linear pharmacokinetic equations. J. Pharmacokinet. Biopharm. **6:**165–175.