Reduction of HIV Concentration During Acute Infection: Independence from a Specific Immune Response

Andrew N. Phillips

After infection with the human immunodeficiency virus (HIV), the concentration of the virus in the person's plasma increases. The subsequent decrease in concentration a few weeks later was thought to result from an HIV-specific immune response. This purported causal relation is investigated with a model of the dynamics of early HIV infection that incorporates no increase in the rate of removal of free virions or virus-infected cells. A pattern of changes in virus concentration similar to that observed in patients is predicted by the model. Thus, the reduction in virus concentration during acute infection may not reflect the ability of the HIV-specific immune response to control virus replication.

Within a few months of infection with HIV, the concentration of the virus in plasma tends to increase transiently (*1*, *2*). It has been assumed that the concentration then falls because of the host's HIV-specific immune response, which has been demonstrated in some patients around this time (*3*). A mathematical model of the population dynamics of early HIV infection was defined to investigate this causal relation.

The model considers a notional representative quantity of tissue or blood that would on average contain 1000 CD4 lymphocytes in an uninfected individual. This could be, for example, 1 mm^3 of peripheral blood or a much smaller volume of T cell–rich areas of lymph nodes. The total (whole-body) inoculum of HIV was assumed to consist of 100 virions, but there were initially no infected cells. The model is defined by four equations describing the interrelated changes over time in the number of activated, uninfected CD4 lymphocytes (R), latently infected cells (L), actively infected cells (E), and free virions (V). These equations can be explained as follows. Activated, uninfected CD4 lymphocytes arise at a constant rate $\Gamma\tau$, where Γ is the rate at which new, uninfected CD4 lymphocytes arise and τ is the proportion that are activated, and they are removed by HIV-independent death at rate μ or by infection at rate βV

$$\frac{dR}{dt} = \Gamma\tau - \mu R - \beta RV \qquad (1)$$

Upon infection, a proportion p of cells become latently infected, and these are removed either by HIV-independent cell death or by activation at rate α

$$\frac{dL}{dt} = p\beta RV - \mu L - \alpha L \qquad (2)$$

Actively infected cells are generated immediately after infection or from the activation of latently infected cells before they die at rate δ

$$\frac{dE}{dt} = (1 - p)\beta RV + \alpha L - \delta E \qquad (3)$$

Free virions are produced at rate π by actively infected cells and removed at rate σ

$$\frac{dV}{dt} = \pi E - \sigma V \qquad (4)$$

Imporantly, the appearance of an immune response to HIV some weeks to months after infection is ignored; the rates of removal of free virions (σ) and infected cells (δ) were assumed to remain constant over time from infection.

This model is similar to basic versions of models of HIV infection previously defined by others (*4*, *5*) and is adapted from the model presented by McLean *et al.* (*4*). The model, however, has not previously been used to study primary (acute) HIV infection. The patterns generated by the model (Fig. 1, with parameter values from Table 1) approximate those actually observed in patients (*1*, *2*, *6*), with a transient peak in virus concentration (to a concentration equivalent to about 10^7 RNA copies per milliliter) a few weeks after infection and a corresponding rapid decline in CD4 lymphocyte count that then stabilizes.

The rapid decline of the virus concentration after its high peak a few weeks after initial infection (Fig. 1) was not a result of the introduction of any immune response, as no increases in the rates of removal of free virions or virus-infected cells at the time of the peak were incorporated into the model. The decrease was simply a result of population dynamics. The determinant of free-virus concentration over the short term is the number of free virions produced by an average virion during the course of a generation. This number rises rapidly in the first weeks after infection because free virions can find suitable activated, uninfected cells to infect relatively easily, and these newly infected cells in turn produce more virions; the average number of new virions produced per virion in one generation is above one, and therefore, their number increases. Numbers of infected cells and free virions thus become high. However, the decline in the number of activated uninfected cells available (as a result of the premature death of cells that become infected) means that free virions find it relatively more difficult to find suitable uninfected cells to infect and thus to reproduce themselves. At some point the number of free virions produced by an average virion during the course of a generation declines below one, and the number of free virions thus begins to decrease. After the initial peak of virus, the model predicts an equilibrium state with total CD4 lymphocyte count [$1000(1 - \tau) + R + E + L$], R, E, L, and V remaining constant for an

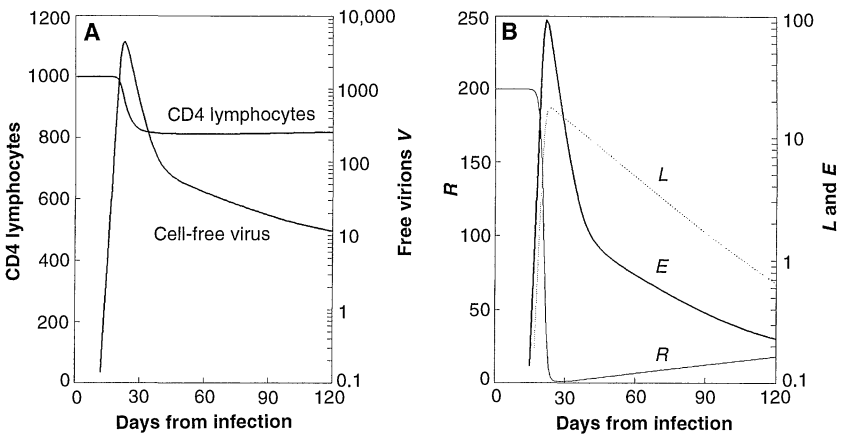

Fig. 1. Temporal changes in the number of (**A**) total CD4 lymphocytes [$1000(1 - \tau) + R + L + E$] and free virions ($V$) and (**B**) activated, uninfected CD4 lymphocytes (R), latently infected cells (L), and actively infected cells (E) in the first 120 days after HIV infection as predicted by the model on the basis of a simulation with iterations of step length 1 hour and 20 min. The total (whole-body) inoculum of HIV was assumed to consist of 100 virions (and no infected cells); thus, there were initially 4×10^{-7} virions in the quantity being considered.

HIV Research Unit, Department of Primary Care and Population Sciences, Royal Free Hospital School of Medicine, Rowland Hill Street, London NW3 2PF, UK.

indefinite period. Thus, although this simple model appears to capture the essential elements of primary HIV infection, it does not explain (nor attempt to explain) how HIV infection leads to gradual CD4 lymphocyte depletion after acute HIV infection.

The underlying pattern of changes in virus concentration is broadly similar to that in Fig. 1 for parameter values such that the number of free virions produced by an average virion during the course of one generation is initially greater than one. If this quantity is below one at infection, the number of free virions falls to zero, and the virus is eradicated. As a test of the robustness of the model, the magnitude and time span of the decline in free-virus concentration from its peak to the equilibrium value was determined for various values of key parameters (Table 2). Extreme low and high estimates of Γ, σ, δ, and π were chosen, and each permutation was assessed. In each case, a value of β was chosen so that the number of virions produced by an average virion during one generation was large enough for the infection to persist. For each set of parameter values, the pattern of changes in virus concentration was essentially similar to that shown in Fig. 1, with a high peak soon after infection followed by a fall to a relatively low equilibrium value. Changes in the value of p, the proportion of cells that become latently rather than actively infected, also do not affect the underlying pattern of changes in virus concentration (including $p = 0$). Changes in the size and constitution of the inoculum make only a small difference to the pattern, tending only to change slightly the timing of the peak.

It was assumed that only a proportion τ of uninfected CD4 lymphocytes are susceptible to infection by HIV at the time of initial infection. This reflects the belief that the infection rate of activated cells is far greater than that of resting cells (*7*). Because the model only covers the short period around primary infection and does not attempt to model the long-term effects of HIV, it has simply been assumed that a constant proportion ($\tau = 0.2$) of cells existing at the time of infection and newly generated cells are activated. This apparently rather high value was chosen on the basis that HIV increases T cell activation (*8*). However, as might be anticipated, the pattern of virus concentration generated was similar if a lower value ($\tau = 0.1$) was chosen. More sophisticated models allowing for transient activation of cells (which increases as a function of HIV concentration) and separate models that explicitly account for the proliferation of activated cells have also been evaluated, and the changes in virus concentration during the primary infection period remain similar to those presented here. A further refinement was to allow the rate of generation of new cells to increase as the total count fell, to reflect the homeostatic mechanism of the immune system. Again, this did not markedly affect the situation within the time span of primary infection.

The magnitude of decline in virus concentration during acute HIV infection appears to vary substantially between patients, with some patients showing less than a 10-fold decline and others over a 1000-fold decline (*1*, *2*, *9*, *10*). It is difficult to estimate this quantity, however, because one never knows how close the concentration is to its peak value at the time a sample is taken, and so underestimates are likely. The report based on the largest study of patients ($n = 53$) in primary HIV infection suggested a mean decline of 10- to 100-fold in

Table 1. The meaning of the parameters in the model and their values used in Fig. 1. All values are for a small quantity of tissue that normally contains 1000 CD4 lymphocytes. Because there are a total of about 2.5×10^{11} CD4 lymphocytes in the average uninfected individual (there are about 1000 cells/mm^3, so there are 5×10^9 cells in peripheral blood; only about 2% of cells are in peripheral blood (*15*, *17*), thus the total number is approximately 2.5×10^{11}), we are considering around $1/(2.5 \times 10^8)$ of the whole body.

Symbol	Interpretation	Value (per day)	Commments
Γ	Rate at which uninfected CD4 lymphocytes arise	1.36	Equivalent to 3.4×10^8 cells per day in whole body; chosen to be slightly lower than estimates of 2×10^9 for patients with more advanced disease (*15*, *16*)
μ	HIV-independent death rate of uninfected CD4 lymphocytes	1.36×10^{-3}	Average life span of uninfected CD4 lymphocytes, 2 years (*18*) ($\Gamma = 1000\mu$, so normally rate of production equals rate of death)
τ	Proportion of cells activated	0.2	
β	Rate of infection of CD4 lymphocytes per virion	0.00027 per virion	Cannot be directly estimated
ρ	Proportion of cells becoming latently infected upon infection	0.1	
α	Activation rate of latently infected cells	3.6×10^{-2}	
σ	Removal rate of cell-free virus	2	Average life span, 1/2 day
δ	Removal (death) rate of actively infected CD4	0.33	Average life span, 3 days; suggested that lymphocytes' average composite life span of actively infected cells and free virions is 2 days in the presence of HIV-specific immune response (*15*, *16*)
π	Rate of production of virions by an actively infected cell	100	(*19*)

Table 2. Decline in virus concentration (V) from peak for various permutations of extreme parameter values illustrating the robustness of this phenomenon to different parameter values. Values of β were chosen to be high enough to ensure that the virus was not eradicated.

$1/\sigma$	$1/\delta$	π (per day)	Γ (per day)	β*	Decline in V (n-fold)†	Time for decline (days)‡
1 hour	1 hour	10	1	0.1	3391	5.3
1 hour	1 hour	10	10	0.1	648	0.4
1 hour	1 hour	500	1	0.1	3499	0.5
1 hour	1 hour	500	10	0.1	744	0.4
1 hour	5 days	10	1	0.001	162	39
1 hour	5 days	10	10	0.001	12	13
1 hour	5 days	500	1	0.001	160	35
1 hour	5 days	500	10	0.001	16	14
5 days	5 days	10	1	0.001	53	35
5 days	5 days	10	10	0.001	7	17
5 days	5 days	500	1	0.0001	53	36
5 days	5 days	500	10	0.0001	7	17

*Expressed per 1 hour 20 min. †Given by the maximum value of V divided by the equilibrium value of V. ‡Defined as the time from the maximum value of V to first reaching a value of V less than two times the equilibrium value.

HIV RNA copies per milliliter (*10*).

It has been generally believed that the immune response to HIV is responsible for the decline of virus concentration from the high levels seen early in infection (*1*, *11*). In particular, appearance of a cytotoxic T lymphocyte (CTL) response specific to certain HIV epitopes close to the time of the peak of HIV virus level has been observed (*3*, *12*, *13*). The results from this model raise the possibility that the appearance of the HIV-specific immune response is a consequence of the high level of virus but is not a major cause of the decline from this high level. If this hypothesis is true, and the prevailing view that the HIV-specific immune response "controls" HIV replication is not, one might expect to find some patients in whom virus levels decline from a high peak even in the absence of specific immune responses. Suggestive evidence can be found in the literature. One patient (of five studied, patient AD11) experienced a 100-fold drop in virus concentration during primary HIV infection even though there was no detectable HIV-specific antibody or CTL response (*3*). Another patient (again of a total of five studied, HOBR) had no CTL response while viremia was being "controlled," albeit "less efficiently" than in patients with a response (*13*). Lastly, data from three children infected during childbirth showed no evidence of CTL responses or neutralizing antibodies, even though the increase in virus concentration consistent with primary viremia fell (*14*).

Estimates have suggested that infected cells and free virions have a very short half-life in HIV-infected persons (*15*, *16*), a mean of about 2 days for the sum of the lifetimes of virus-producing cells and free virions. We have chosen slightly slower rates of removal of free virions (average life span, 1/2 day) and virus-producing cells (average life span, 3 days) because for much of the period of infection being modeled (up to the peak in free-virus concentration), no HIV-specific immune response is thought to occur. No introduction of an immune response at the time of the peak (by increasing the values of σ and δ) has been modeled because it was felt to be important to study whether the virus could decline in the absence of any change in the rate of removal of free virions and virus-infected cells.

In conclusion, simple modeling of the population dynamics of acute HIV infection in the absence of any assumption of a growing immune response suggests the presence of a transient peak of virus concentration. These results, and the lack of a detectable HIV-specific immune response at the time of the decline from the initial virus peak in some patients, raise the possibility that the decline in virus level seen in acute HIV infection is not a reflection of the capacity of the HIV-specific immune response to control virus replication.

REFERENCES AND NOTES

1. S. J. Clark *et al.*, *N. Engl. J. Med.* **324**, 954 (1991).
2. E. S. Daar, T. Mougdil, R. D. Meyer, D. D. Ho, *ibid.*, p. 961.
3. R. A. Koup *et al.*, *J. Virol.* **68**, 4650 (1994).
4. A. R. McLean, V. C. Emery, A. Webster, P. D. Griffiths, *AIDS* **5**, 485 (1991).
5. D. Shenzle, *Stat. Med.* **13**, 2067 (1994); A. S. Perelson, in *Mathematical and Statistical Approaches to AIDS Epidemiology: Lecture Notes in Biomathematics*, C. Castillo-Chavez, Ed. (Springer-Verlag, New York, 1989), vol. 82, pp. 350–370; A. S. Perelson, D. E. Kirschner, R. J. De Boer, *Math. Biosci.* **114**, 81 (1993).
6. M. Piatak *et al.*, *Science* **259**, 1749 (1993).
7. S. D. Gowda *et al.*, *J. Immunol.* **142**, 773 (1989).
8. M. Peakman *et al.*, in *Cell Activation and Apoptosis in HIV Infection*, J. M. Andrieu and W. Lu, Eds. (Plenum, New York, 1995).
9. T. L. Katzenstein *et al.*, in preparation.
10. S. Kinloch-de Loes *et al.*, *N. Engl. J. Med.* **333**, 408 (1995).
11. R. A. Koup, D. D. Ho, *Nature* **370**, 416 (1994).
12. J. T. Safrit, C. A. Andrews, T. Zhu, D. D. Ho, R. A. Koup, *J. Exp. Med.* **179**, 463 (1994); J. T. Safrit, A. Y. Lee, C. A. Andrews, R. A. Koup, *J. Immunol.* **153**, 3822 (1994).
13. P. Borrow, H. Lewicki, B. H. Hahn, G. M. Shaw, M. B. A. Oldstone, *J. Virol.* **68**, 6103 (1994).
14. K. Luzuriaga *et al.*, *J. Infect. Dis.* **167**, 1008 (1993).
15. X. Wei *et al.*, *Nature* **373**, 117 (1995).
16. D. D. Ho *et al.*, *ibid.*, p. 123.
17. W. E. Paul, *Fundamental Immunology* (Raven, New York, 1985).
18. C. A. Michie, A. McLean, C. Alcock, P. C. L. Beverly, *Nature* **360**, 264 (1992).
19. D. S. Dimitrov *et al.*, *J. Virol.* **67**, 2182 (1993).

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Dependence of Cyclin E–CDK2 Kinase Activity on Cell Anchorage

Fang Fang,*† Gertraud Orend,* Nobumoto Watanabe, Tony Hunter, Erkki Ruoslahti‡

Most nonmalignant cells are anchorage-dependent; they require substrate attachment for growth and, in some instances, survival. This requirement is lost on oncogenic transformation. The cyclin E–CDK2 complex, which is required for the G_1-S transition of the cell cycle, was activated in late G_1 phase in attached human fibroblasts, but not in fibroblasts maintained in suspension. In transformed fibroblasts the complex was active regardless of attachment. The lack of cyclin E–CDK2 activity in suspended cells appeared to result from increased expression of CDK2 inhibitors and a concomitant decrease in phosphorylation of CDK2 on threonine-160. Suppression of cyclin E–CDK2 activity may thus underlie the anchorage dependence of cell growth.

Most untransformed mammalian cells fail to proliferate when they are prevented from attaching to a solid substrate, a phenomenon known as anchorage dependence of growth (*1*). Loss of anchorage dependence is the property of transformed cells that correlates best with tumorigenicity in vivo (*2*). Anchorage dependence has been attributed to the arrest of cell cycle progression in late G_1 phase resulting from a lack of substrate adhesion (*3*). Because the G_1 cyclins—cyclin D and cyclin E—together with their associated cyclin-dependent kinases (CDKs) are key regulators in the G_1 phase (*4*), we investigated whether the activation of CDK complexes with cyclin D or cyclin E is controlled by cell anchorage.

F. Fang, G. Orend, E. Ruoslahti, La Jolla Cancer Research Foundation, Cancer Center, 10901 North Torrey Pines Road, La Jolla, CA 92037, USA.
N. Watanabe and T. Hunter, Molecular Biology and Virology Laboratory, Salk Institute for Biological Studies, La Jolla, CA 92037, USA.

*These authors contributed equally to this work.
†Present address: Department of Pathology, Massachusetts General Hospital, Boston, MA 02114, USA.
‡To whom correspondence should be addressed.

Both KD and IMR90 untransformed human diploid fibroblasts were synchronized in G_0 by serum deprivation, split into attached and suspended cultures (*5*), and exposed to fetal bovine serum. Both cell lines failed to initiate DNA synthesis when cultured in suspension (*6*), confirming that nonadherent cells are arrested before entering S phase. Although attached and suspended cells differ in size and shape, the protein contents per cell were similar (*6*). Immunoblot analysis of KD cell extracts showed that the expression of cyclin D1 and cyclin D3 (cyclin D2 is absent from KD cells) did not differ between attached and suspended cells during the G_1 phase (Fig. 1A). The amounts of CDK4, CDK5, and CDK6, all of which can associate with cyclin D, were also similar under both culture conditions. Moreover, in vitro retinoblastoma protein (Rb) kinase assays (*7*) showed that cyclin D1 and CDK6 immunoprecipitates from G_1 phase cells were equally active under the two culture conditions (Fig. 1B). These observations confirm previous data (*3*) showing that cell cycle progression through mid G_1 phase,