

# Two types of cytotoxic lymphocyte regulation explain kinetics of immune response to human immunodeficiency virus

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The organization of the cytotoxic T lymphocyte (CTL) response at organismal level is poorly understood. We propose a mathematical model describing the interaction between HIV and its host that explains 20 quantitative observations made in HIV-infected individuals and simian immunodeficiency virus-infected monkeys, including acute infection and response to various antiretroviral therapy regimens. The model is built on two modes of CTL activation: direct activation by infected cells and indirect activation by CD4 helper cells activated by small amounts of virus. Effective infection of helper cells by virus leads to a stable chronic infection at high virus load. We assume that CTLs control virus by killing infected cells. We explain the lack of correlation between the CTL number and the virus decay rate in therapy and predict that individuals with a high virus load can be switched to a low-viremia state that will maintain stability after therapy, but the switch requires fine adjustment of therapy regimen based on the model and individual parameters.

HIV | immunological | mathematical | model | helper

A wealth of information on molecular mechanisms of cytotoxic T lymphocyte (CTL) response controlling virus replication has been accumulated. Yet, the organization of the host–virus system at the level of the organism remains unknown. Open issues include the relevant cell phenotypes, the order of their differentiation, and control of their proliferation and death. It is also unknown why some notorious viruses (e.g., HIV, hepatitis B and C viruses) are not cleared but persist at high levels. Further progress in the area requires implementation of a system approach that views the host–virus system as a network of interacting elements and reverse-engineers it from the increasing flow of experimental data. Frequent quantitation of CTLs, helper cells, and virus by modern, accurate assays provides input information.

There has been substantial amount of mathematical work addressing important features of the CTL response (1–4). In the present work, we expand our previous model of CTL response during acute infection with simian immunodeficiency virus (SIV) and four strains of lymphocytic choriomeningitis virus (5) to explain properties of a chronic HIV infection. We present a series of 20 experimental facts that we analyze in three groups to reconstruct model blocks for dynamics of infected and infectible cells, CD4 helper cells, and CTLs. Then, we connect the three parts together and test the resulting model by fitting it to several kinetic experiments. We show that various observations can be understood from two key facts: that helper cells are infected with virus and that effector CTLs are activated either directly by antigen or by helper cells activated by antigen. In particular, the model explains the existence of two steady states in an HIV/SIV infection with strongly different virus loads and predicts that they are controlled by two distinct effector CTL subsets. Mathematical equations and additional simulations are given in *Supporting Text*, which is published as supporting information on the PNAS web site.

## Results and Discussion

**Constructing a Model from Separate Observations.** In the present subsection, we infer parts of a model from groups of experimental observations (Facts).

### *Cells permissive for virus replication.*

*Fact 1.* HIV is able to infect both resting and activated CD4 T cells, with resting cells being infected at a lower efficiency.

*Fact 2.* The turnover rate of CD4 T cells in HIV-infected individuals is increased severalfold as compared with uninfected controls (6, 7).

*Fact 3.* The virus load does not experience significant oscillations during its rebound after transient or transiently efficacious antiretroviral therapy (ART) (8, 9).

*Fact 4.* After transient ART, the virus load does not significantly overshoot its pretreatment level, although, before the virus rebound, the number of CD4 cells exceeds the predrug level (8, 9).

*Fact 5.* In monkeys acutely infected with a variant of SIV-mac251, the first peak of the virus load is followed by a second, smaller peak (10).

*Fact 6.* (a) In SIV-infected monkeys who received soluble BrdUrd, a compound that inserts into dividing chromosomes, the fraction of BrdUrd-labeled CD4 cells increases during labeling and decreases afterward at similar exponential rates (6, 7). (b) Experiments on labeling with deuterated glucose, which measure the total amount of label in cells, produced similar results (11).

To explain these observations, we will take into account two types of CD4 T cells permissive for virus replication (*Facts 1* and *2*). Resting CD4 T cells, *R* (Fig. 1), support virus replication in the beginning of infection (12, 13), whereas their activated dividing precursor cells, *A*, dominate at later times (12, 13). Because activated cells become resting cells in a short time, the number of activated cells *A* is small and responds rapidly to temporal changes in the virus load, damping its oscillations around a steady state (*Fact 3*). The assumption that activated cells come from a source other than division of preexisting resting cells, *R*, explains the lack of virus overshoot, the second virus peak in acute infection, and the rapid loss of label in the BrdUrd experiment (*Facts 4–6*). Expansion of CD4 T cells during transient drug therapy will not result in virus overshooting of the steady-state level, if we assume that dominant permissive cells, *A*, are supplied from a source independent on CD4 count at low CD4 counts (*Fact 4*). A constant source explains why virus is able to replicate and form a second peak in the face of strong CD4 T cell depletion in acute infection (*Fact 5*).

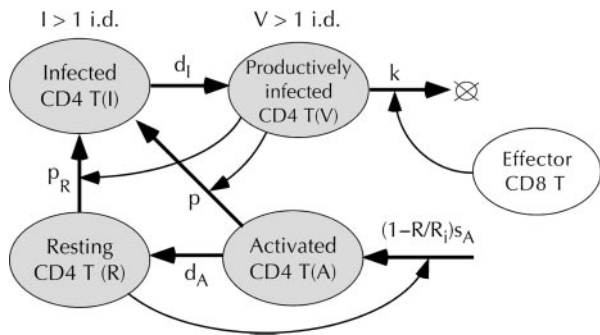
To explain *Fact 6a*, Mohri *et al.* (6) introduced a source and assumed that, for some reason, it supplies labeled cells in the presence of and unlabeled cells in the absence of soluble BrdUrd.

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Abbreviations: ART, antiretroviral therapy; CTL, cytotoxic T lymphocyte; i.d., infectious dose; SIV, simian immunodeficiency virus.

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**Fig. 1.** Partial model describing dynamics of cells infected with HIV/SIV and permissive for its replication. Ovals, cell compartments; uppercase letters, cell numbers in compartments (dynamic variables); thick arrows, flow of cells from one compartment to another because of change of phenotype or death; thin arrows, control (linear by default) of cell flow between two compartments by a third compartment; lowercase letters and  $R_i$ , constant proportionality coefficients (model parameters). Compartment  $V$  represents infected cells producing virus. Short-lived virus is cleared, if  $V$  and  $I$  are below 1 i.d. = 1 productively infected cell per animal =  $10^{-2}$  copies of RNA per ml (monkey) =  $3.4 \times 10^{-10}$  p27 ng/ml.

The biological origin of “source” is unclear; probably, it represents the mechanism of memory cell homeostasis. Grossman *et al.* (14) proposed that source originates from very long-lived precursors of memory cells that undergo rapid proliferation bursts, during which cells become labeled (only) in the presence of soluble label. For the source to stay constant, the long-lived precursors should be weakly infectable with HIV.

**Infected cells and their control by CTLs.**

**Fact 7.** (a) Depletion of CD8 T cells in rhesus macaques chronically infected with SIV results in an increase of the virus load by one to several orders of magnitude (15). (b) A strong inverse correlation between viremia and CTL response is observed in humans (16).

**Fact 8.** The infected cell lifetime inferred from the initial viremia decay rate under ART is  $\approx 1$  day (8, 17) and does not correlate with the CTL number (16, 18).

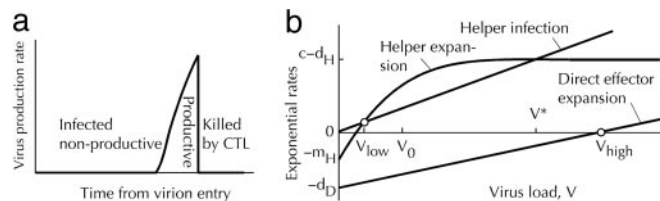
**Fact 9.** *In vitro*, most cells infected with HIV die within a few days.

**Fact 10.** The amount of HIV RNA per infected (either activated or resting) CD4 cell at any one time does not change significantly during ART (<50%).

**Fact 11.** An infected cell starts to produce HIV proteins within  $\approx 24$  h after virus entry.

**Fact 12.** In an average chronic infection, the net turnover rate of productively infected cells inferred from the 1-day lifetime,  $10^7$  to  $10^8$  cells per day (19), is slower than the turnover rate of all CD4 T cells,  $\approx 10^9$  cells per day (6, 7).

**Fact 7** shows that CTLs control HIV replication *in vivo*. **Facts 8** and **9** induced Ogg *et al.* (16) to conclude that virus itself rather than CTLs kill cells and that CTLs control infection by either inhibiting virus production inside of cells or by blocking virus transmission between cells. **Fact 10** excludes the first, but not the second, scenario. The idea that the usual CTL function (killing infected cells) is not important for HIV and that, instead, CTLs exert control over HIV by using a mechanism specific for this virus, is intriguing. Yet, our task is to seek the simplest explanation of facts. We can interpret **Facts 8, 9, and 12** based on the well known delay of virus replication and assuming the usual, lytic mechanism of CTLs (**Fact 11**). We split the infection cycle of a cell into a nonproductive interval that is known to be  $\approx 1$  day (denoted  $I$  in Fig. 1) and a productive interval ( $V$ ) and neglect the effect of CTLs on virus production or transmission, i.e., on parameter  $p$  (Fig. 1). Suppose, at their steady-state concentration, CTLs kill a productively infected cell within a time much shorter than 1 day (Fig. 2a). Then, the average infected cell lifetime is determined mostly by the



**Fig. 2.** Virus production and two steady states (sketch). (a) Time dependence of virus production by an infected cell (sketch). (b) Net expansion and infection rates of activated helper cells,  $H_A$ , and the net expansion rate of direct effector cells,  $E_D$ , as functions of the virus load. Small circles, two stable steady states. Values at x-axis:  $V_{high} = d_D/a$ ,  $V_{low} = V_0 m_H / (c - d_H)$ ,  $V^* = (c - d_H) / p$ . The helper cell number in the low-virus steady state:  $H_2 = H_0 [(c - d_H) / m_H] \ln [(c - d + r) / (c - d)]$ .

nonproductive interval and depends only weakly on the CTL number (**Fact 8**). That a productively infected cell would die several days later because of viral pathogenicity (**Fact 9**) is irrelevant, unless CTLs are strongly depleted. The same idea shows that the turnover of productively infected cells is much more rapid than inferred previously (**Fact 12**), because it is determined by a short productive interval, rather than the infected cell lifetime. The specific time dependence of virus production after the delay is subsumed by parameters  $p$  and  $k$  used below as fitting parameters. Neglecting the delay in virus replication either requires invoking transmission-blocking and indirect killing of CD4 T cells by virus or predicts strong variation of virus decay rate during the first week of ART and across patients, in contrast to experiments.

We do not consider free virus particles as a separate compartment, because their lifetime is short (20, 21) and, probably, constant and not affected by antibodies because of their weak neutralization efficiency (22). Therefore, with few exceptions, the free virus load, at any time, should be proportional to the number of productively infected cells,  $V$ . A small fraction of infected cells that have unusually long nonproductive interval are termed latently infected cells. We do not consider them as a separate compartment but account for their effects, important at low virus loads, by injecting small amounts of virus (see below).

**HIV-specific CTL response.** Facts relevant for CTL regulation are as follows.

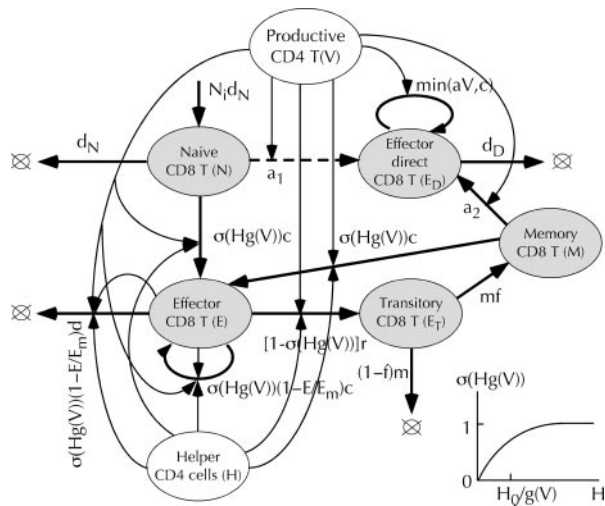
**Fact 13.** The chronic stage of a HIV/SIV infection is a stable quasi-steady state: the numbers of relevant cell compartments stay almost constant, because their replenishment compensates for their death.

**Fact 14.** There are two types of steady state characterized by drastically different virus loads (23–26). Approximately 75% and 25% of monkeys infected with SIVmac251 develop moderately high ( $10^5$  to  $10^6$  copies per ml) and very low virus loads (< $10^3$  copies per ml), respectively (27). The second group is smaller in humans infected with HIV-1. The CTL response levels (in humans) are 0.1–0.2% and 0.2–2% CD8 T cell count, respectively (16).

**Fact 15.** (a) Potent ART administered to high-viremia individuals depletes virus to very low (often undetectable) levels and brings the CTL number below the low-viremia state (16, 23, 26). (b) Interruption of ART after many months has two possible outcomes: virus either rebounds to the predrug level or stays low (27, 28). In either case, CTLs rebound to a level similar to the predrug level (28).

**Fact 16.** The helper-cell response to HIV measured with a lymphocyte proliferation assay correlates negatively with the virus load (29, 30).

**Fact 17.** CTLs isolated from infected individuals can be induced to divide either by antigen-presenting cells or by helper cells activated with soluble antigen (or by cytokines secreted by helper cells).



**Fig. 3.** Partial model of CTL dynamics. Loops with arrows, cell division. Dashed line, a variant model where direct effector cells are produced from naïve cells, not memory cells. The rest of the notation is as in Fig. 1. (Inset)  $\sigma(Hg(V)) = 1 - \exp[-Hg(V)/H_0]$ , nonlinear control of CTL by helper cells and virus.

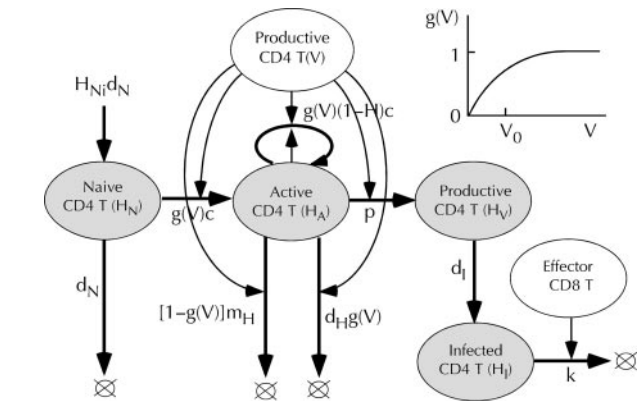
**Fact 18.** CTL kinetics in the beginning of ART in infected humans consists of three phases (25): the CTL number decreases severalfold or more, increases, and decreases to a constant level represented by memory cells (23).

**Fact 19.** Multiphase CTL kinetics also is observed in monkeys acutely infected with a passaged isolate of SIVmac251 (10). The initial CTL expansion, soon after a viremia peak, is followed by a drop in the CTL number concurrent with a decrease in the CTL death rate. Another increase of viremia is followed by a slower expansion of CTLs with a small death rate. Finally, viremia falls again and reaches a steady-state level.

**Fact 20.** The second viremia peak is not pronounced in acute infection with SIVsmE660 (9), HIV (31), SHIV (32), and some other isolates of SIVmac251.

Long-term chronic infection represents a stable equilibrium between death and proliferation of all relevant cell types. A broad class of dynamic models predicts such behavior (Fact 13). Other observations, including a second steady state and various kinetic features, are more specific and more difficult to explain. The two modes of CTL activation (Fact 17) provide the clue. We postulate that two distinct subtypes of effector CTLs, those activated directly by antigen (termed “direct effector”;  $E_D$  in Fig. 3) and those regulated by helper cells ( $E$  in Fig. 3), are responsible for two different steady states (Fact 14). In the high-viremia steady state, helper cells are infected and depleted to very low levels (Fact 16), and direct effector CTLs control the virus. In the low-viremia-load state, the virus load is controlled by CTLs that depend on CD4 helper cells that are activated by small amounts of virus. The virus load is too low to maintain proliferation of direct CTLs. As we demonstrate below, interplay between the two modes of CTL activation naturally explains the nonmonotonous dynamics of CTLs and virus observed after infection and under ART (Facts 18–20). Intermittent or moderately efficacious ART can switch the system from the high-viremia to the low-viremia state that, once established, maintains stability on its own (Fact 15).

The model of CTL regulation is shown in Fig. 3. The part that describes helper-dependent CTLs is similar to a model we developed earlier (5) for acute infections with SIVmac251 (10) and lymphocytic choriomeningitis virus (33). Naïve cells are activated by helper cells to become effector cells, which keep dividing as long as they receive signals from helper cells detecting virus. Once signals become low, CTLs progress to a short-term memory (“transitory”) phenotype and then either die or become long-term memory cells.

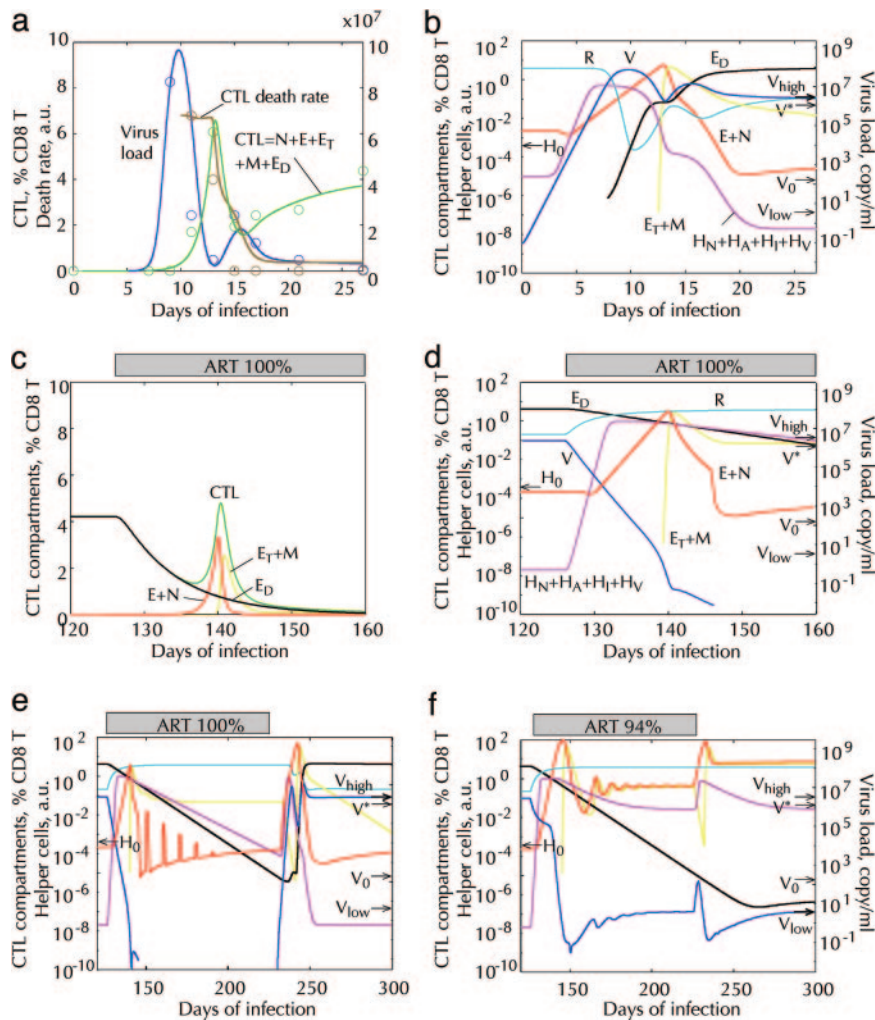


**Fig. 4.** Partial model of dynamics of virus-specific CD4 T helper cells. (Inset) Nonlinear control of helper cells by virus,  $g(V) = 1 - \exp(-V/V_0)$ . The remaining notation is as in Figs. 1 and 3.

memory cells. In the presence of virus and helper cells, memory cells are reactivated into secondary helper-dependent effector cells. An important addition is the direct effector cells that represent progenitors of memory cells activated by the virus independently of helper cells. To explain the dynamics of some SIV strains, we also considered a variant of the model where direct effector cells are progenitors of naïve cells. We do not consider anergic cells here, although anergy is important for lymphocytic choriomeningitis virus infection (3, 33). We also do not include the death and homeostatic replenishment of virus-specific memory cells, because these processes are too slow to affect any of experiments we discuss. To prevent effector cells from disappearing altogether when the helper cell count or the virus load are low, we include in the model a weak constant source of naïve cells (Fig. 3). Introduction of transitory CTLs is necessary to fit kinetics during acute infection and ART (see below).

**Virus-specific helper cells.** Correlation between the level of HIV-specific CD4 helper cell response and viremia (Fact 16), the fact that virus infects CD4 cells much more effectively than other cell types, and anomalies of the CTL response in an HIV/SIV infection (Fact 19) demonstrate a key role for CD4 helper cells in anti-HIV response. Unlike professional antigen-presenting cells featured as regulatory cells in our previous model (5), CD4 T cells can rapidly divide and are antigen-specific. Few data are available on kinetics of virus-specific CD4 cells. Homann *et al.* (34) used the tetramer assay to measure kinetic response to one epitope of the Armstrong strain of lymphocytic choriomeningitis virus. They observed rapid expansion and, after virus clearance, rapid decay of CD4 cells, followed by slower decay and, then, by a low-level long-term memory phase. Because we are not aware of any detailed data on HIV that could support a more complex model, we consider a single compartment of uninfected activated CD4 T cells,  $H_A$  (Fig. 4). The fraction of helper cells recognizing virus  $[g(V)]$ , dividing, and dying because of activation-induced death is assumed to be proportional to the virus load,  $V$ , at very low loads,  $V \ll V_0$ , and reach 1 at higher loads,  $V \gg V_0$ . A low activation threshold  $V_0$ , which implies collective signaling between activated helper cells, explains the existence of the low-viremia steady state under an active helper cell response. After the virus is cleared or strongly depleted, helper cells die out very slowly, which accounts for the slow phase of decay (34). Memory helper cells are not included in this part of the model. A constant weak source of naïve cells prevents helper cells from disappearing. Similar to nonspecific CD4 cells, virus-specific CD4 cells can become infected,  $H_I$ , then productively infected,  $H_V$ , and then killed by CTLs (compare Figs. 1 and 4).





**Fig. 6.** Fitting of three experiments consecutive in time, acute infection (10), onset of ART (25), long-term and interrupted ART (28), based on the model (Fig. 5, without the dashed arrow). (a and b) Acute infection with 20 infectious doses ( $0.2$  RNA copy per ml) of virus. (a) Circle, data for animal p88 infected with SIVmac251 (10). Solid lines, best-fit predicted dependencies. (b) Predicted cell numbers in six important cell compartments (on the curves). The values of  $V$  and  $R$  are shown in virus units on the right y-axis. Arrows show the values of  $V_{low}$ ,  $V_{high}$ , and  $V^*$  (Fig. 2 and legend) and  $H_0$ . (c and d) First weeks of 100% efficacious ART ( $p = p_R = 0$ ) simulating experiment in ref. 25. Predicted cell compartments are shown in linear (c) and logarithmic (d) scale. (e and f) Simulated kinetics of interrupted ART similar to experiment in ref. 28. Drug efficacy: 100% (e) and 94% (f):  $p(126 < t < 236) = 0.06p(t < 126)$ . One-day pulses of 5 i.d. of virus ( $V$ ) each 10th day simulate bursts from latent reservoirs. (a–f) Fitting parameters (Fig. 5):  $p = 100$  per day per virus unit (vu);  $R_i = 3.8$ vu;  $d_A = 0.94$ /day;  $d_I = 1.26$ /day;  $H_{N_i} = 10^{-5}$ ;  $N_i = 2.2 \times 10^{-3}$  %CD8 cells;  $f = 0.14$ ;  $c = 3.41$ /day;  $d = 2.42$ /day;  $k = 1.06$ /day per cell;  $r = 6.5$ /day;  $V_0 = 4.3 \times 10^{-6}$ vu;  $H_0 = 4 \times 10^{-4}$ ;  $a = 1.4$ /day per vu;  $d_D = 0.13$ /day;  $m = 1.18$ /day;  $d_H = 0.085$ /day;  $p_R = 1.98$ /day per vu;  $s_A = 0.44$ vu/day. Here,  $vu = 1$  p27 ng/ml =  $3 \times 10^7$  RNA copies per ml. Fixed parameters:  $d_N = 10^{-3}$  per day;  $m_H = 0.1$ /day;  $E_m = 100\%$ .

dominant permissive compartment from resting,  $R$ , to activated cells,  $A$ , and a deep depletion of resting cells near the time of the (first) virus peak (Fig. 7) agree with recent studies (12, 13).

The second virus peak observed for SIVmac251 isolate used in ref. 10 is not pronounced for other SIV strains (*Fact 20*). Our simulation of SIVsmE660 kinetics (see Fig. 8, which is published as supporting information on the PNAS web site) shows a better fit for the model variant in which direct effector cells are descendants of naïve rather than memory cells (dashed arrow in Figs. 3 and 5). Further, even for secondary direct effectors, small changes in parameter values can destroy the second peak (results not shown). The SIV isolate used in ref. 10 is useful, because it reveals both types of CTLs, important for interpreting other experiments on our list.

**Highly potent ART: First weeks.** The next simulation shows changes in cell compartments after the onset of highly potent ART started when in the high-virus steady state (Fig. 6c and d). We continue our simulation from the previous experiment using the same parameter set. We introduce ART by setting infectivity parameters  $p$  and  $p_R$

to zero. The results are similar to the three-phase CTL kinetics observed in human patients (compare Fig. 6c with figure 1A–D in ref. 25). In the steady state, most CTLs are direct effector cells,  $E_D$ , that are depleted under ART because of rapid decay of the virus load. When the virus load,  $V$ , falls below level  $V^*$  (Fig. 2b), helper cells start to expand, causing, when their level exceeds  $H_0$ , subsequent proliferation of helper-dependent CTLs,  $E$ . When the virus load decreases below the level  $\approx V_0 H_0 / H$ , helper-dependent effector cells,  $E$ , cease proliferation and become transitory cells that either die or become long-lived memory cells,  $M$ . Finally, once the virus load is below the low-steady-state point  $V_{low}$  (Fig. 2b), the helper cell number starts to decline.

Thus, for a sufficiently slow virus decay, a transitory CTL expansion is predicted, as has actually been observed in several patients (25). The prediction that the right slope of the CTL peak is dominated by cells with memory phenotype,  $E_T$ , also agrees with the experiment (24).

**Interrupted and long-term ART.** Now we consider kinetics during ART on longer time scales (Fig. 6e). After short-lived infected cells are

