Dynamics of Naive and Memory CD4+ T Lymphocytes in HIV-1 Disease Progression

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Summary: Understanding the dynamics of naive and memory CD4+ T cells in the immune response to HIV-1 infection can help elucidate typical disease progression patterns observed in HIV-1 patients. Although infection markers such as CD4+ T-cell count and viral load are monitored in patient blood, the lymphatic tissues (LT) have been shown to be an important viral reservoir. Here, we introduce the first comprehensive theoretical model of disease progression based on T-cell subsets and virus circulating between the two compartments of LT and blood. We use this model to predict several trademarks observed in adult HIV-1 disease progression such as the establishment of a setpoint in the asymptomatic stage. Our model predicts that both host and viral elements play a role in determining different disease progression patterns. Viral factors include viral infectivity and production rates, whereas host factors include elements of specific immunity. We also predict the effect of highly active antiretroviral therapy and treatment cessation on cellular and viral dynamics in both blood and LT. Key Words: HIV-1—Homing—Naive—Memory—HAART—Mathematical model.

To date, the mechanisms that lead to the depletion of an HIV-1–infected individual’s blood CD4+ T cells over a typical 10-year period of progression remain unclear. Further, the pathways that drive a relatively constant viral titer during much of the disease to significantly increase during end-stage disease are also unknown. Most clinical indications of progression are based on blood data, because these data are most easily obtained. Most infection occurs in lymphatic tissues (LT), however, where 98% of CD4+ T lymphocytes reside (1). Understanding the dynamics within this compartment is vital to uncovering information regarding cellular infection and viral production (2). Recent evidence indicates that lymphocyte circulation between blood and LT, which occurs as a normal process of immune surveillance in uninfected individuals, is also important when studying the effects of HIV-1 on blood lymphocytes (3–5).

Naive and memory CD4+ T-lymphocyte subsets have differing roles during an immune response, which are hypothesized to be important in HIV-1 disease progression and treatment. Our goal is to elucidate their roles and describe the pathogenesis of HIV-1 infection based on host cellular and viral factors in the blood and LT. We use mathematical modeling as our experimental method. This allows synthesis of the complex dynamics occurring during the host-pathogen interaction with HIV-1 and enables us to predict the mechanisms behind different outcomes observed in patients.

In previous work, we developed a model of lymphocyte circulation between blood and LT in which HIV-1 binding to lymphocytes causes accelerated homing to the lymph nodes and increased susceptibility to homing-induced apoptosis (3). This model, which tracked productively, latently, and abortively infected cells, supported the hypothesis that homing-induced apoptosis of
nonproductively infected cells is a key mechanism for CD4+ T-cell depletion (3). Comparison with data validated the model as a potential hypothesis for disease progression in both compartments. We did not monitor viral load directly, however. In this article, we introduce naive and memory subclasses of CD4+ T cells together with virus in both the blood and LT to determine more mechanistically the dynamics of HIV-1 disease progression. By validating our model with experimental data, we can use it to predict effects of highly active antiretroviral therapy (HAART) and to interpret clinical results observed with therapy and during treatment interruption.

**DYNAMICS OF A HEALTHY IMMUNE SYSTEM**

**Cell Phenotypes**

To understand the dynamics of cells during HIV-1 infection, we first describe them in a healthy uninfected host. Newly generated naive CD4+ T lymphocytes are input into the blood from the thymus. When naive cells are stimulated, those that survive their role as effector cells develop into memory cells. Combined expression of several phenotypic markers is necessary to identify a “pure” naive or memory population (1,6). Among several indicators, the CD45 protein is the most common marker for labeling a nonactivated cell as naive (CD45RA+) or memory (CD45RO+). For consistency between studies, we consider CD4+ T cells with the CD45RA+ and CD4+ markers and with no prior activation or exposure to antigen to be naive, whereas memory CD4+ T cells are those with the CD45RO+ and CD4+ markers that have previously undergone activation. We also accept that intermediates may exist based on differential expression of other proteins (7,8). Thus, when referring to data from the literature on naive and memory cells, this is the default status unless otherwise stated.

**Lymphocyte Circulation**

The lymph node (LN) is the area to which T cells, B cells, and antigen-presenting cells migrate to initiate and participate in the adaptive immune response to foreign pathogens (9). The adhesion molecule L-selectin (CD62L) expressed on naive CD4+ T cells is essential for entry of cells into the LN. The process of lymphocytes circulating to the LNs in this fashion is referred to as homing (7). Of the more than 10^11 immune system cells that are in constant circulation between the blood and LT, only a small proportion of memory cells (10%) travel to the LNs on a regular basis. The other 90% circulate to the spleen (50%), lung, liver, bone marrow, and other parts of the lymphoreticular system (1,10). The purpose of this trafficking is to maintain immune surveillance in all parts of the body so as to mobilize cells to sites of secondary antigen challenge (7). It is these mechanisms of homing that are altered during HIV-1 infection (see below).

Blood data can only provide a snapshot of immune and viral interaction taking place in LT. By developing a model of lymphocyte circulation between blood and LT, we can compare experimental data (from blood) while studying the dynamics occurring in LT. To this end, we first develop a virtual model of adult human lymphocyte circulation by incorporating the known circulation characteristics of naive and memory cells between the blood and LT. Our model system is depicted in Figure 1.

We develop a mathematical system to describe the interactions in Figure 1 and estimate associated param-

![FIG. 1. Healthy model. A two-compartment model of the dynamics of CD4+ naive (N) and memory (M) cells undergoing normal processes of lymphocyte circulation within blood (B) and lymphatic tissues (L), where e = circulation between blood and lymph tissues, λ = differentiation, S = proliferation, and μ = death. The α and β parameters are scaling terms for compartmental exchange.](image-url)
eters from the literature (Table 1 in Appendix). We then simulate the model by solving the differential equation system using appropriate numeric methods. The results of the computational experiments are shown in Figure 2 for a representative 10-year period.

Our simulation is comparable to experimental data on age-related decline of CD4+ naive T cells (11–13). The proliferation of memory CD4+ T cells allows the total CD4+ T-cell count to remain at a relative steady state over long periods, despite the continual decline in the number of new naive cells supplied by the thymus as a result of involution. The total number of CD4+ T cells in blood remains at approximately 1000 cells/mm$^3$ with some evidence for a gradual depletion over time (14,15), also shown in Figure 2. This supports the theory of Haase (16) and others who assert that homeostatic mechanisms maintain a quasi–steady-state population in which there is a balance of the growth of one compartment with death in the other. Our model system thus reflects the homeostasis that is observed in total CD4+ T-cell counts in blood and LT in an average healthy adult (17). We explored variations in parameters that do not have a direct estimate from the literature (see estimates in Table 1 in Appendix). Results indicate that if either the direct differentiation from naive to memory cells or the proliferation of memory cells is increased (Equation 6 in Appendix), there is an overall higher population of memory cells, but homeostasis is still maintained. If the parameters for memory cell proliferation are decreased by orders of magnitude, homeostasis is not achieved. Therefore, our model predicts that the processes of memory cell production are crucial to maintaining T-cell levels in steady state.

**HIV-1 INFECTION IN THE HUMAN HOST**

**Disease Progression**

Adult infection with HIV-1 results in three relatively common clinical periods of acute, asymptomatic, and end-stage disease, despite the patient-specific interaction of the pathogen with the human immune system. A viral setpoint is established in the acute stage and is thought to determine the speed of progression to AIDS (18,19). End-stage disease results in three common disease scenarios. Long-term nonprogressors (LTNPs) are a small percentage (5%–10%) of the infected population who evade the typical progression to AIDS without the help of antiretroviral therapy for as long as 20 years (18,20, 21). In contrast, individuals whose CD4+ T-cell levels decrease below 200 cells/μL after the 8- to 10-year asymptomatic period while virus levels in end-stage disease increase exponentially are characterized as typical progressors (22). Finally, some individuals develop AIDS within 3 to 5 years after initial infection and are known as rapid progressors. In this case, an unusually early decline in CD4+ T-cell count may be caused by infection with a particularly cytopathic strain or may occur in individuals who are more susceptible to AIDS based on factors such as immune status at the time of infection, age, and genetic profile. Our model predicts that a disruption in normal homeostasis of naive and memory cells is a crucial factor in determining an individual’s disease progression.

**HIV-1 Virus Effect on Lymphocyte Circulation and Cellular Infection**

Although conflicting hypotheses exist as to which CD4+ T-cell subset is preferentially infected by HIV-1, several studies support selective naive cell depletion early in disease progression (23,24). One reason for this may include enhanced homing-induced apoptosis of naive cells (5,25). There is also evidence for greater depletion of thymus-derived naive (CD45RA+ CD62L+) CD4+ T cells early in infection beyond involution (26–29).

A major change induced by HIV-1 binding is an alteration in lymphocyte circulation. HIV-1 can signal CD45RO+ CD4+ T cells to undergo homing to lymph nodes, a great deviation from their normal travel
throughout the lymphoreticular system (25). Steady-state levels of virus in the LT during the asymptomatic period of infection disguise effects to lymphocyte homing and circulation mechanisms. Throughout the course of the disease, naive cells continue to home but at an accelerated rate, whereas memory cells are recruited from their peripheral circulation to participate in LT homing (25). Memory T cells that circulate through the peripheral tissues are spared; however, memory and naive cells circulating through the LT are constant targets for viral infection or virus-induced signaling as a result of contact with virions or virus-producing cells (30). Given this HIV-1–induced memory cell circulation pattern, both memory (activated) and naive cells that come in contact with HIV-1 are equally susceptible to apoptosis after signaling through homing receptors (L-selectin or CD62L+) occurs (25). Signaling of CD62L+ and other homing receptors can induce apoptosis of HIV-1–signaled T lymphocytes in as much as 30% to 50% of abortively infected (HIV-1–signaled) resting or activated cells (5,25,31).

Activation-induced cell death has also been implicated as a major cause of CD4+ T-cell depletion. Several sources link activation-induced cell death with homing-induced apoptosis as described previously by showing that activation is a direct cause of apoptosis of the host cell or that activation is associated with apoptosis in virus-negative cells (32,33). When infected cells or free virions bind with neighboring uninfected cells, the uninfected cells become signaled, although they do not produce viral RNA (the “bystander effect”) (34,35).

We clarify for our purposes the different cell phenotypes in our model. Productively infected cells [approximately 1 in 100,000 T cells (36)] are activated cells; thus, viral DNA can enter the nucleus. These cells are able to produce viral progeny, and survive no more than 2 days (37). Provirus can survive for months or years, resulting in a latently infected cell. If HIV-1 DNA is unable to integrate into the nucleus and remains in the cytoplasm of resting (naive or memory) host cells, it is unstable and decays after a few days (25,31,36,38). These cells are referred to as abortively infected cells. Abortive infection can induce upregulation of L-selectin, causing most of these cells to home. They return to the naive class after viral DNA degrades and may participate in further homing or infection activities (25,31,36). Because 95% to 99% of T cells are resting at any given time, most infections are abortive (39,40).

The model developed here elucidates possible mechanisms describing differences in disease progression based on the differential interaction of the HIV-1 virus with the naive and memory subclasses of CD4+ T cells together with effects to lymphocyte circulation. Our goal is to build on our healthy model by including HIV-1 infection. We maintain varying circulation characteristics of memory and naive cells as in the baseline model and include their individual susceptibilities to infection. Infection directly produces abortive and productively infected classes of cells that are assumed to be present predominantly in the LT compartments owing to the assumption that infected cells in the blood (containing only 2% of all T cells) constitute a relatively small contribution to overall infection and that most HIV-1 replication occurs in the LT (39–42). Latently infected cells are derived only from productively infected cells that have deactivated (31,43). A cell that has become abortively infected is increased in its homing capability by upregulation of L-selectin. Therefore, it is believed that most of these cells either undergo apoptosis or revert to the naive class if they have not been signaled through their antigen receptors. Any cell that becomes productively infected (whether memory or naive) must have been activated and thus is counted as a memory cell until its death. Memory cells and productively infected cells have a higher death rate than naive cells (as a result of activation-induced cell death) (32). Figure 3 depicts the dynamics of naive, memory, and infected cells as well as virus within the two compartments of blood and LT.

We develop a mathematical system to describe the terms in Figure 3 and estimate associated parameters from the literature (summarized in Appendix; see Table 2 in Appendix). We again simulate the model by solving the differential equation system using appropriate numeric methods. We discuss below the results of the computational experiments in two scenarios: long-term nonprogressive infection and typical progression.

Long-Term Nonprogressors

Our purpose in developing a virtual human LTNP model is to predict mechanisms responsible for the dynamics of lymphocyte circulation patterns of naive and memory cells in an HIV-1–infected patient during the primary, asymptomatic, and end stages of infection. Similar to others (44), we also consider the dynamics of memory and naive CD4+ T cells to play significant roles in the comprehensive picture of infection. We examine the behavior of these two cell populations in the blood and LT over the course of disease, monitor viral load, and compare model simulations with clinical data. Once this is achieved, we can use the model to ask questions about underlying mechanisms of LTNP progression. Figure 4 portrays our model simulation of LTNP progression. Panels A, B, and C encompass cellular and viral dynamics in the acute stage. Our simulations cor-
relate with findings that there is a dramatic drop in CD4+ T-cell counts 3 to 6 weeks after infection (48). Panel D depicts long-term dynamics in the blood from our model compared with representative data over the simulated 10-year disease course. Results were compared with those of six patients in a study of LTNP who showed a similar steady level of CD4+ T cells over the course of 10 years (45). Table 3 presents a comparison of the model simulations in LT with proportional data for each class of infected cells, uninfected cells, and free virus (measured as HIV-1 RNA). By validating the results of our model against clinical data, we then use this model to test further theories of the lymphocyte-viral interaction.

Key Parameter Dynamics

Because reaction rates likely vary among individuals and studies, parameter values used in the model are not exact. It is important to explore effects to the model through uncertainty and sensitivity analyses based on parameter variation (49). Such experiments can elucidate model elements that may most significantly contribute to the rapidity at which an individual progresses to AIDS as well as to the overall pattern of lymphocyte depletion and virus growth.

Our model predicts that several key parameters must lie within a specific range to exhibit the standard behavior of a typical HIV-1 LTNP. We explore five key parameters that are capable of dramatically changing the typical course of disease. The primary effects of their variation resulted in changes in onset of acute-stage disease and rapidity of progression to AIDS. These five parameters encompass both host factors and viral factors, indicating that all play significant roles in disease progression. Here, virulence factors of the virus include its...
ability to infect cells as well as to produce progeny, where an increase in either mechanism indicates an enhanced ability for sustained immune evasion. Host factors that play a role in HIV-1 infection likely involve production of antigen-specific CD4+ and CD8+ T cells against virus and virally infected cells as well as increased proliferation of uninfected cells. We elaborate these results as follows.

**Viral Factors**

**Infection rate of naive cells by virus** \((k_N)\). A low ability to infect naive cells results in a higher CD4+ T-cell steady state and a lower viral set point. Extremely low values of this parameter can result in clearance of virus and no effect to homeostasis of uninfected cells. A

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**TABLE 3. Long-term nonprogressor model simulation values compared with experimental data in lymphatic tissues**

<table>
<thead>
<tr>
<th>Cells by type in lymphatic tissues</th>
<th>Model value after 10 years</th>
<th>Experimental value(s)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Latently infected cells</td>
<td>(1.4 \times 10^7) cells</td>
<td>(1.0 \times 10^7)-(1.2 \times 10^7) cells</td>
<td>39, 67</td>
</tr>
<tr>
<td>Productively infected cells</td>
<td>(7.0 \times 10^7) cells</td>
<td>(1.4 \times 10^6)-(5.0 \times 10^8) cells</td>
<td>16, 25, 36</td>
</tr>
<tr>
<td>Abortively infected cells</td>
<td>(6.0 \times 10^5) cells</td>
<td>(10^5) cells</td>
<td>40</td>
</tr>
<tr>
<td>Viral production</td>
<td>(2.0 \times 10^{10}) virions/cell</td>
<td>(10^{10})-(10^{11}) virions/cell</td>
<td>99</td>
</tr>
<tr>
<td>Viral load</td>
<td>(5.0 \times 10^9) virions</td>
<td>(10^9)-(10^{11}) virions</td>
<td>40</td>
</tr>
</tbody>
</table>
large increase leads to a much higher viral setpoint with immediate depletion of naive cells.

**Infection rate of memory cells by virus** ($k_M$). A low ability to infect memory cells results in a lower viral setpoint and a higher CD4+ T-cell steady state. Extremely low values of $k_M$ produce a pronounced viral decline and absence of a viral setpoint rather than viral clearance, however. Large values of $k_M$ also lead to higher setpoints and low levels of CD4+ memory T cells but not depletion.

**Average number of virions produced by productively infected cells** ($N$). Experimental data indicate that the average number of virions produced from a productively infected cell is between 100 and 1000 virions per day per cell (25,50,51). Our model shows that values on extreme ends of this range maintained throughout infection can dramatically alter disease course; low values result in viral clearance, whereas high values result in an elevated setpoint and a low total CD4+ T-cell steady state.

**Host Factors**

**Clearance rate of productively infected cells from LT** ($\gamma_P$). A previous model examined the effects of an immune response to high viral levels produced during acute infection, affecting the rate of viral decrease to asymptomatic levels (52). Similarly, we consider the effects of a variable immune response in both blood and LT. Varying the strength of the immune response over six orders of magnitude reveals that the immune response against productively infected cells is of key importance. If the immune response is extremely efficient from the first day of infection ($\gamma_P$ on the order of $10^{-11}$ per day per cell), the individual may clear virus. Thus, our results imply that the strength of the immune response to infection plays a significant role in an individual’s eventual disease progression.

**Clearance rate of free virus from blood** ($\gamma_V$). Another example of the fine balance between host and viral factors can be shown by variation of immune clearance in the blood ($\gamma_V$). By varying this parameter over several orders of magnitude, our model captures varied ability of the immune response to combat infection. This experiment produces different viral set point levels (Fig. 5) (19).

Note that variation in any of the 5 parameters discussed can produce the different viral setpoints.

**Typical Progressors**

Our LTNP virtual human model depicts steady-state viral levels (after the acute stage) throughout the simulated 10-year course of infection. If HIV-1 maintained constant virulence and the host immune response persisted (as in primary infection), the individual would exhibit the behavior of the LTNP model indefinitely. As we know through several decades of HIV-1 history, how-

![FIG. 5. Effect of varying the strength of the immune response ($\gamma_V$) in the blood on the viral set point. The increasing strength of the immune response is indicated by increasing line thickness (from top to bottom), with exact values for the parameter $\gamma_V$ varying from 0.001 to 100.0 as shown in the legend. Other parameters and initial values are identical to those given in Table 2 in the Appendix for the long-term nonprogressor model.](image)
ever, this is generally not the case. Clinical data indicate that for most individuals not receiving therapy, CD4+ T-cell levels in blood are constantly decreasing, with a dramatic increase in virus in the final stages of disease. To realize the viral increase observed in HIV-1 typical progressor patients during end-stage disease, there must likely be elements of the host-pathogen dynamic that change over the long-term course of infection. It is believed that HIV-1 uses one or more mechanisms to evolve and vary antigenically during infection. We consider these strategies as viral factors. Alternatively, the host may eventually lose mechanisms (host factors) that effectively contain viral growth. Likely candidates for host-disease elements that may evolve over time to produce AIDS in late disease are those that resulted in significant variation in simulations when tested over a large range in the baseline model (see previous section).

HOST AND VIRAL INFLUENCES

Virus Factors

HIV-1 is thought to become more virulent through various mechanisms during the course of disease. After sexual transmission, HIV-1 primarily uses the CCR5 coreceptor expressed on CD4+ T cells (53). In many cases, the virus may evolve to use both CCR5 and CXCR4 or CXCR4 exclusively (16,23,53–55). Because CXCR4 is thought to be present on most T cells (24), we represent CXCR4 exclusively (16,23,53–55). Because CXCR4 is the virus may evolve to use both CCR5 and CXCR4 or CXCR4 exclusively (16,23,53–55). Because CXCR4 is thought to be present on most T cells (24), we represent the increasing coreceptor repertoire by allowing the infection rates (kS and kMD) to increase linearly throughout disease progression in both cell subsets (variations in this linear increase were also tested). There is also the likelihood of greater replication ability over the course of disease. This evolution could result from an increase in the number of virions (N) produced by productively infected cells during the course of infection (56,57). We model the viral production number as also increasing linearly from our initial value of 500 virions per day per cell to 1000 virions per day per cell.

It is likely that in the rare rapid progressor scenario, patients begin with rates of infection or viral production so high that AIDS appears within 3 to 5 years. Increasing the initial infection rates or the viral production rate or accelerating their respective time-dependent increases (as in the typical progressor model) by much greater amounts than described previously results in behavior typical of rapid progressors (data not shown).

Other virus factors that may maintain an infected individual in the asymptomatic stage could be specific to viral strain. For example, deletions or mutations in the nef gene of the HIV-1 viral envelope have been shown to correlate with slower progression to AIDS (58).

Host Factors

In addition to an alteration in the numbers of CD4+ T cells, CD8+ T cells are also affected by HIV-1 infection. The loss of CD4+ cells is compensated for by a gain in the number of CD8+ cells (total T-cell homeostasis) (17). Although naive and memory CD4+ T-cell subsets decline, CD8+ naive cell numbers increase slightly and CD8+ memory cell numbers increase dramatically to compensate and maintain T-cell homeostasis (17). The normal ratio for CD4 to CD8 is approximately 2:1, and reverses early in HIV+ patients to approximately 1:2 in the blood and later in the LT (17,59,60). Our model presently includes only CD4+ lymphocytes, although recent evidence suggests that CD8+ T cells may also be directly infected by HIV-1 (61,62). We indirectly account for their role in eliminating virally infected cells in the immune clearance terms (Equations 11–13 and 15 in Appendix). Activated CD8+ T-cells, or cytotoxic T-lymphocytes (CTLs), are thought to mainly target infected cells; however, because we do not include the small proportion of infected cells in the blood, we indirectly model blood CTL activity. This captures greater clearance of infected cells from the body when the number of uninfected immune system cells is high and a decreased ability to clear virus when CD4+ T-cell levels fall (63). The function of this subset of cells is being directly modeled in ongoing work.

Memory CD4+ T-cell proliferation is impaired in the presence of HIV-1 (63). By late-stage disease, CD4+ T-cells encompass less than 10% of the total T-cell subsets, with CD8+ T cells, particularly memory CD8+ T cells, comprising the remainder (63). This is in contrast to an uninfected individual, whose CD4+ counts remain at approximately 66% of the total T-cell pool (63). Thus, in the progressor scenario, we model a gradual decline in the ability of CD4+ memory T cells to proliferate or clonally expand by considering a time-dependent source of memory cells.

A simulation of virtual human infection is given using the previously discussed time-dependent parameters (Fig. 6). Combinations of these factors result in a model outcome similar to that observed in the classic HIV-1 patient who progresses to AIDS (the stage at which the infected individual has less than 200 CD4+ cells/mm3 of blood) (65) in approximately 8 to 10 years.

The LTNP and typical progressor models are distinct in several dynamic aspects; however, viral load and CD4+ T-cell levels are the most obvious (see Fig. 4 and 6). Recall our indirect modeling of immune activity...
through the immune clearance terms. A high level of immune activity is observed in LTNP patients, whereas declining immune activity is associated with typical progression (66). The distinctions in immunity between a healthy individual, an LTNP, and a typical progressor are shown in Figure 7. The ability of CD4+ memory T cells to maintain homeostasis as well as to clear productively infected cells decreases significantly in the progressor compared with the sustained immune activity observed in the LTNP.

**HAART THERAPY**

We now use our model, which is representative of several hallmarks of HIV-1 disease progression, to describe the influence of treatment on an infected individual. HAART acts by either reducing viral production or blocking de novo infection or spread and typically results in an increase in CD4+ T-cell counts during the administration period. It is believed that therapy-induced suppression of viral replication reduces the number of cells that home to lymph nodes and undergo homing-induced apoptosis, thus resulting in the reappearance of blood lymphocytes (25). Additionally, some data indicate that treatment can augment thymic output (27,28). The output of new naive cells from the thymus has been hypothesized not only to recover but even to increase, counteracting the accelerated depletion during HIV-1 infection (27).
Clinical Response to HAART Treatment

Several clinical studies have been performed describing qualitatively and quantitatively the effects of HAART on the immune system. Most commonly, there is a biphasic or multiphasic decline in viral load (30). Virus in the blood decreases quickly for several weeks, followed by an indefinitely prolonged decline (67). Suppression of viral replication occurs such that virus levels fall to undetectable levels (defined by 20 to 500 copies/mL of blood, depending on the assay) (39, 67, 68).

There are distinct dynamics of memory and naive cells during treatment. An earlier rapid rise in the memory cell class occurs at the initiation of treatment, whereas naive cells return later, with a more sustained response (1,6,16,30,69–72). Several studies indicate a biphasic re-population of CD4\(^+\) T cells: an initial increase in memory cells over the first few months of treatment, followed by a less dramatic rise lasting up to 1 year (71,73,74). One study proposed that the early return of memory cells is an interruption in HIV-1–induced circulation and virus production, whereas the slower second phase represents immune generation (69).

After cessation of drug therapy, the persistence of resting CD4\(^+\) T cells carrying replication-competent HIV-1 suggests that latently infected cells are likely to be a major viral reservoir even if active virus replication is suppressed by HAART for as long as 2 years (16,54,75). The long period during which virus decays at a much slower rate can be attributed to a low level of viral transcription as well as the long half-life of latently infected cells (up to 44 months) (76,77). This effect is seen as HIV-1 rebounds promptly after cessation of drug therapy in most patients (75,78,79).

Incorporating Treatment in the Infected Model

In an actual clinical setting, there is debate as to whether HAART actually reduces viral infectivity and production to zero, despite undetectable levels of plasma virus and productively infected cells (80–82). Although many models assume that infection is completely eliminated at the onset of therapy, evidence suggests there is residual viral replication occurring below the detection limit (83). The biphasic decline also indicates that there is more than one reservoir producing infectious particles with varying lifespans even during the treatment window (67). To include HAART in our model of HIV-1 disease progression, we alter the system Equations 9, 10, and 12 through 14 (see Appendix) by reducing viral infectivity and production by 90% on the day treatment begins. The increase in thymic output during HAART is modeled by a slight increase in thymic output (1% per year; see Appendix) (28).

Our model shows the effect of administering HAART over a 108-week duration beginning 9 years after the initial infection (Fig. 8). The model predicts that there is a rapid decrease in all types of infected cells after the onset of therapy. Another relevant model outcome is the preferential elimination of infected classes. The produc-
tively infected cells decline first as a result of their relatively short half-life (37). Abortively infected cells are the next to die out, whereas the latent reservoir declines the slowest. The characteristic biphasic decline in viral load is also observed. The sharp reduction of virus in both blood and LT within the first 4 to 12 weeks of treatment can be attributed to the relatively short half-lives of productively infected cells and free virus (78). Repopulation of the naive class is dependent on an individual’s capacity for regeneration (72). We vary the rate of increase in thymic output during treatment and observe a similar result (data not shown). Cells also return to the blood compartment as a result of cessation of HIV-1–induced homing and homing-induced apoptosis; thus, an increase in the number of memory cells occurs almost immediately at the onset of therapy (31). Our simulation also predicts that the latent reservoir in resting memory CD4+ T cells fails to be eradicated over a long period (approximately 4 years; data not shown). The persistence of latently infected cells, even in undetectable amounts, causes the re-emergence of virus to more than 50 copies/mL (RNA) within weeks after discontinuing HAART (75, 84).

Panel D of Figure 8 shows the model simulation compared with data for a 2-year study in which naive and memory cells were at similar levels at the onset of the triple therapy and all patients were previously naïve to treatment.

Optimal Treatment Strategies
Administration of HAART is dependent on the individual, and need is assessed on a case-by-case basis. The
ideal treatment schedule takes account of the host’s ability to respond to therapy as well as the level of viral suppression. Scheduled treatment interruptions that allow the patient a temporary break from continuous treatment regimens are becoming more common and successful as suggested by mathematical modeling (86). The effectiveness of such strategies is largely based on the individual’s recovery after cessation of therapy, however. Individuals with impaired immune responses, even after 2 years of therapy, may return within weeks to viral levels as high as those present at the onset of HAART. Others may be able to suppress viral replication successfully enough to experience a delay in re-emergence of virus between treatments (87). In all of our HAART simulations, treatment is administered between years 9 and 11, and stopped at year 11. Panels A and B of Figure 9 show the effects of suppressing viral production and infection during treatment between 0% and 40%, with 90% as a comparison (from Figure 8). It can be seen that when viral infectivity and production are not effectively suppressed during HAART, the viral load remains above the detection limit or even rebounds within the 2-year treatment window. Upon cessation of therapy at year 11, the level to which CD4+ T-cell numbers decline and virus rebounds is dependent upon an individual’s responsiveness to therapy. In Panels C and D we show the effect of varying viral rebound to between 50% and 90% of pretreatment levels. Patients whose virus returns only after a significant delay would be ideal candidates for treatment interruptions, as temporary cessation of therapy would not cause immediate viral rebound.

![Graphs showing effect of varying treatment efficacy on HAART](https://example.com/graphs.png)

**FIG. 9.** Effect of varying treatment efficacy during and after a 2-year period on HAART. (A and B) Effect of the total CD4+ T cells and virus in blood from variations in viral suppression during treatment. Percentages shown are differing levels of drug efficacy. (C and D) Dynamics of T-cell decline and viral rebound after cessation of therapy based on viral rebound immediately after treatment cessation (to between 50 and 90% of pretreatment levels). All other parameters are the same as for Figure 8.
Our model simulations with HAART support the experimental findings that there exists a large variation in the return of CD4+ T cells after the onset of therapy. Our model predicts that both host and viral factors play a significant role in recovery and maintenance of treatment effects.

DISCUSSION

There is a significant amount of clinical and experimental data describing HIV-1 disease progression in adult humans. The mechanisms explaining the dynamics of the host-viral interaction remain unclear, however. We believe that including virus and infected cells in our model as well as the nonlinear interaction of cells with virus provides a more definitive picture of CD4+ T cells and virus in the in vivo context of blood and LT.

In this work, we first develop a mathematical model to capture the dynamics of naive and memory CD4+ T cells as they undergo normal growth, death, and circulation between the blood and LT in the absence of HIV-1 infection. We then introduce HIV-1 in the model to study how the known and hypothesized interactions of virus and CD4+ T cells lead to different disease outcomes as seen in clinical settings. Our simulations yield the characteristic depletions of CD4+ T cells in the blood together with a relatively constant number in LT. In our model, subtle mechanisms of circulation, alteration, and enhanced apoptosis of naive and memory (activated) CD4+ T cells can account for cell and viral measurements commonly observed during the three typical stages of HIV-1 disease. The model provides a means for organizing these elements into a comprehensive quantitative understanding of disease progression. It also elaborates optimal routes of therapy accounting for differences in disease progression and response to treatment between patients based on individual-specific host and viral factors.

The model yields both LTNP and typical progressor outcomes. The differences seen between these patients, as predicted by the model, are dependent on both viral and host factors. Viral infectivity and production rates strongly determine the progression rate of disease; this is corroborated by studies of R5 and X4 viral strains, where known differences in virulence are correlated with progression. The recognition and clearance of virus and infected cells by the immune response also strongly influences disease progression. In the simulations, increasing viral tropism and decreasing host response factors strongly influence the rate of disease progression.

We also incorporate HAART in the model by reducing viral infection and production rates and by increasing the source of CD4+ T cells during the treatment phase. Model simulations of HAART show a rapid return of both lymphocyte subclasses, with memory preceding the naive class. The simulations also show a biphasic decline of virus in both blood and LT, indicating the presence of more than one class of viral production with differential dynamics. These phenomena are well documented in patients undergoing HAART. Consistent with the findings of clinical studies, the model also predicts a rapid rebound of virus when treatment is stopped. Ideally, treatment would contain viral production and infectivity such that treatment interruptions would be possible. Our model predicts that variations in both host and viral factors play a significant role in the development of an optimal treatment strategy.

APPENDIX

Model of Lymphocyte Circulation

Equations for naive (N) and memory (M) cells with the B and L subscripts denoting populations in the blood and lymphatic tissues (LT), respectively, are as follows:

\[ \frac{dN_B}{dt} = \beta N_L N_B - \epsilon_{NB} N_B + S_N(t) \]
\[ \frac{dM_B}{dt} = \beta e_{MB} M_B - \epsilon_{MB} M_B \]
\[ \frac{dN_L}{dt} = S_N(t) + \alpha \epsilon_{NB} N_B - \epsilon_{NL} N_L - \mu_N N_L \]
\[ \frac{dM_L}{dt} = \lambda \mu_N N_L + S_M(t) + \alpha \epsilon_{MB} M_B - \epsilon_{ML} M_L - \mu_M M_L \]

The first equation describes the rate of change of the uninfected naive cells in the blood, \( N_B(t) \). An influx of uninfected cells (\( S_N(t) \)) circulating in from the LT at the rate \( \epsilon_{NB} \) is multiplied by \( \beta \) to scale* for exchange of units between compartments (from the LT to blood). Uninfected lymphocytes (\( N_B(t) \)) emigrate from blood at rate \( \epsilon_{NB} \) to the LT. Equation 2 encompasses the same interactions for the uninfected memory cells in the blood. Exchange rates of memory cells to and from blood and the LT are \( \epsilon_{MB} \) and \( \beta e_{MB} \), respectively. Equation 3 represents the change in the number of naive cells in the LT \( N_L(t) \) per day. Naive cells (\( N_L(t) \)) arrive from the blood compartment at rate \( \epsilon_{NL} \) with scaling factor \( \alpha \) and are lost to circulation (at rate \( \epsilon_{NL} \)) or to natural death (\( \mu_N \)). Similarly, memory cells die naturally (at rate \( \mu_M \)), efflux from the LT at rate \( \epsilon_{ML} \), or influx from the blood (\( \epsilon_{MB} \)).

We also allow for a small percentage of naive cells to directly differentiate from naive to memory during activation through division or differentiation (\( \lambda \)).

We include a source of naive cells in the LT \( S_N(t) \) similar to our assumptions of most dynamics occurring in the LT system as opposed to the source of CD4+ T cells in the blood. Model simulations of HAART show a rapid return of both lymphocyte subclasses, with memory preceding the naive class. The simulations also show a biphasic decline of virus in both blood and LT, indicating the presence of more than one class of viral production with differential dynamics. These phenomena are well documented in patients undergoing HAART. Consistent with the findings of clinical studies, the model also predicts a rapid rebound of virus when treatment is stopped. Ideally, treatment would contain viral production and infectivity such that treatment interruptions would be possible. Our model predicts that variations in both host and viral factors play a significant role in the development of an optimal treatment strategy.

* To convert between the representation of cells and virus in blood, measured in cubic millimeters and milliliters, respectively, and that of LT measured in total cell/virus numbers, we use scaling factors (\( \alpha \) and \( \beta \)) in the equations when describing exchange between the two compartments. Because there is 5 L of blood in the average human adult, when describing movement of CD4+ T cells from blood to LT, we scale the terms by 5 L, or 5 \times 10^6 mL. Describing circulation in the opposite direction, we scale by the inverse, or 2 \times 10^{-7} mL of blood for CD4+ T-cells and 4 \times 10^{-7} mL for virus.
to the blood. It is believed that recent thymic emigrants of CD4+ T cells are significantly lower in adults compared with children (27,28). To account for the influence of a slowly decaying source during aging, we include a decreasing input over our 10-year model time frame. The number of thymocytes entering blood is high during the first year of life and then decreases because of thymic involution at a rate of 5% per year between the ages of 25 and 40 years and at a rate of 0.1% thereafter (16,88). Thus, we assume that the source of naive cells decreases approximately 3% per year in an uninfected adult. Other ongoing work in our laboratory explores age-dependent decline in the lymphocyte population (89). This relation is represented by:

\[ S_{M}(t) = \mu_{B} \cdot N_{B}(0) = 0.975 \times \frac{t}{365} \]  

Similarly, the rate of change in memory cells in the LT is enhanced by a source \((S_{M}(t))\) as a result of proliferation and direct differentiation from the naive state. To maintain homeostasis of total CD4+ T cells in both LT and blood, we estimate a memory cell population that remains inversely proportional to the number of naive cells. Instead of a mechanistic term for non-antigen-specific proliferation, we model the rate of change in source cells of memory class as a combination of proliferation and nonspecific antigen stimulation. This depends on the size of the naive cell class, thus reflecting a carrying capacity–dependent growth. Our model accounts for this mechanism with the following relation, where \(R\) and \(K_{1}\) are constants (6.5 × 10^10 and 1 × 10^11, respectively):

\[ S_{M}(t) = R \frac{N_{L}(t)}{N_{L}(t) + K_{1}} \]  

### Parameter Estimation for the Healthy Model

To fully derive the model systems (Equations 1–4), values for the parameters must be estimated. Often, parameter values can be obtained from literature; however, discrepancies in estimates from different sources result in a range of possible values. In this case, or when data are not available, we employ uncertainty and sensitivity analyses to study the effect of each parameter and explore the sensitivity of our model to their variations (49).

Values for blood CD4+ T lymphocytes are typically measured in cells/mm^3 of blood and in total cell numbers for the lymphatic system. To estimate the number of CD4+ lymphocytes in the blood, we begin with the value of 2 × 10^11 to 1 × 10^12 total lymphocytes in the body (4,16). Approximately 2% of these cells are present in the blood at any given time (41), totaling approximately 5 × 10^9 blood lymphocytes. Dividing this value by a blood volume of 5 L (for the average 70-kg adult human) yields 1000 cells/mm^3, the initial condition for total CD4+ T cells \((N_{B}(0) + M_{B}(0))\). The ratio of naive to memory CD4+ T cells is approximately 45% to 55% in an individual by the age of 20 years, respectively, in both blood and LT (16,90–92).

The input of cells to the naive and memory classes described previously is balanced by their respective lifespans. Some data indicate that the lifespan of a naive cell is approximately 7 weeks (7), although other data indicate that CD45RA+ CD62L+ lymphocytes have a half-life of 4 to 12 months (41,54). This yields a range of naive cell death between 0.002 and 0.02 per day. Memory cells have been shown to have a half-life ranging from 1 to 2 months (41,54) up to almost 4 months, giving a death rate of 0.006 to 0.02 per day. Generally, it is thought that memory cells die one to two times as fast as naive cells because of their enhanced potential for activation, and thereby death (6,93). Thus, we assume a death rate for memory cells that is 1.5 times that of their naive counterparts.

Circulation rates were estimated based on the average time of 24 hours that a lymphocyte spends traveling through the LT (94,95). Lymphocytes exchange through the blood 48 times per day; thus, only 2% of the entire lymphocyte pool is present at any time in the blood (1,4,41).

In Table 1, we summarize the values used in the circulation model, which serve as a baseline for development of the subsequent models.

### Infected Model Equations

Nine nonlinear ordinary differential equations describe the dynamics of the schematic outlined in Figure 3: four equations for rates of change

<table>
<thead>
<tr>
<th>Variable</th>
<th>Definition</th>
<th>Initial value (reference)</th>
<th>Units</th>
</tr>
</thead>
<tbody>
<tr>
<td>(N_{B}(0))</td>
<td>Uninfected naive CD4+ T cells (blood)</td>
<td>450 (16)</td>
<td>Cells/mm^3 of blood</td>
</tr>
<tr>
<td>(M_{B}(0))</td>
<td>Uninfected memory CD4+ T cells (blood)</td>
<td>550 (16)</td>
<td>Cells/mm^3 of blood</td>
</tr>
<tr>
<td>(N_{M}(0))</td>
<td>Uninfected naive CD4+ T cells (LT)</td>
<td>9 × 10^10 (16)</td>
<td>Cells</td>
</tr>
<tr>
<td>(M_{M}(0))</td>
<td>Uninfected memory CD4+ T cell (LT)</td>
<td>1.1 × 10^11 (16)</td>
<td>Cells</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Definition</th>
<th>Value (reference)</th>
<th>Units</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\alpha)</td>
<td>Scaling term (blood → LT)</td>
<td>5 × 10^6</td>
<td>mm^3 or μL of blood</td>
</tr>
<tr>
<td>(\beta)</td>
<td>Scaling term (LT → blood)</td>
<td>2 × 10^-7</td>
<td>mm^3 or μL of blood</td>
</tr>
<tr>
<td>(v_{NL})</td>
<td>Circulation of naive cells (LT to blood)</td>
<td>1.0 (94, 95)</td>
<td>Day</td>
</tr>
<tr>
<td>(v_{NB})</td>
<td>Circulation of naive cells (blood to LT)</td>
<td>40.0 (94, 95)</td>
<td>Day</td>
</tr>
<tr>
<td>(v_{ML})</td>
<td>Circulation of memory cells (LT to blood)</td>
<td>0.25 (94, 95)</td>
<td>Day</td>
</tr>
<tr>
<td>(v_{MB})</td>
<td>Circulation of memory cells (blood to LT)</td>
<td>10.0 (94)</td>
<td>Day</td>
</tr>
<tr>
<td>(\mu_{N})</td>
<td>Death rate of uninfected naive cells (LT)</td>
<td>0.002 (41, 54)</td>
<td>Day</td>
</tr>
<tr>
<td>(\mu_{M})</td>
<td>Death rate of uninfected memory cells (LT)</td>
<td>0.003 (41, 54)</td>
<td>Day</td>
</tr>
<tr>
<td>(S_{NL})</td>
<td>Source of naive cells into LT</td>
<td>See Equation 5 (16, 88)</td>
<td>Cells/day</td>
</tr>
<tr>
<td>(S_{ML})</td>
<td>Proliferation of memory cells (LT)</td>
<td>See Equation 6 (63)</td>
<td>Cells/day</td>
</tr>
<tr>
<td>(\lambda)</td>
<td>Direct differentiation from naive to memory cells</td>
<td>0.1 (estimated)</td>
<td>Scalar</td>
</tr>
</tbody>
</table>

LT, lymphatic tissues.
of uninfected cells in the blood and LT (naive and memory cells), one equation for the rate of change of virus in each compartment, and equations for rates of change of the three infected classes in the LT:

\[ N_i'(t) = \beta e N_i N_j(t) - e N_i N_j(t) \]

\[ M_i'(t) = \beta e N_i M_j(t) - e M_i M_j(t) \]

\[ V_i'(t) = S_i(t) + \alpha e N_i N_j(t) - e N_i N_j(t) - k_v V_i(t) N_j(t) - \mu_s N_i(t) + \mu_b M_i(t) \]

where \( N_i, M_i, V_i \) represent the naive, memory, and virus in the LT, respectively.

\[ M_j'(t) = \mu_b N_j(t) + S_j(t) + \alpha e N_j N_i(t) + \frac{\rho M_j V_i(t)}{V_i(t) + K_2} + \alpha e M_j M_i(t) - e M_j M_i(t) - k_v M_j(t) M_i(t) - \mu_b M_j(t) \]

\[ L_i'(t) = s_i P(t) - s_j L(t) - \gamma_i(N_j(t) + M_j(t)) * L(t) - \mu_j L(t) \]

\[ P_i'(t) = \rho M_j V_i(t)(N_j(t) + k_v M_j V_i(t)(M_j(t)) - \gamma_i(N_j(t) + M_j(t)) * P(t) - \mu_j P(t) + s_j L(t) - s_i P(t) \]

\[ A_i'(t) = \rho A_i + (k_v V_i(t)(N_j(t) + k_v M_j(t)M_j(t)) - \gamma_i(N_j(t) + M_j(t)) * A(t) - \mu_j A(t) \]

\[ V_j'(t) = \beta e N_j V_j(t) - e V_j(t) - \mu_b V_j(t) \]

Equations 7 and 8 remain as Equations 1 and 2 in the lymphocyte circulation model, because inflow and outflow from the blood compartment are the same in the absence and presence of virus. Equations 9 and 10 are altered with the addition of two mass action terms at rates \( k_s \) and \( k_m \) that model infection of naive and memory cells, respectively. The naive class also includes the addition of cells from the abortively infected class that revert to uninfected cells at conversion rate of \( m_a \). T-cell production is also slightly altered during the course of HIV-1 infection (96). It has been estimated that the rate of new virus-producing cells is proportional to the immune response term in the LT for the three classes of infected cells at rates \( \gamma_i, \gamma_m \), and \( \gamma_v \). Because infected classes are not present in the blood, the immune response term of the form \( \gamma_i(M_i + N_i) \) is represented as a function of the immune cells present at any given time point. In accordance with certain theories, that DNA in latently infected cells remains inactive, we investigate removal of the immune response to latently infected cells. We find that this makes no qualitative difference in disease progression.

**Parameter Estimation for the Infected Model**

The values used for the infection parameters presented in the LTNP model are detailed in Table 2: Virus titer in blood is typically measured as RNA copies/mL, whereas the lymphatic system is measured in total viral numbers. We outline below how we estimated their values. Our model steady states are robust to small changes in these parameter values as our sensitivity analyses confirm (see below).

Because the model is initiated at inoculum, other than the small amount of virus introduced in the LT, the initial conditions reflect that the other infected classes begin at zero.

Lifespans of productively infected cells are orders of magnitude shorter than those of uninfected cells. It is estimated that productively infected cells live for only 1 to 2 days (37,90,99), producing an adequate number of virions to infect new cells and sustain infection for several years. One or 2 days after infection, most of the abortively infected cells have circulated back to the blood, undergoing rapid homing to the lymph nodes, where approximately 50% undergo apoptosis (5,25). The remainder of abortively infected cells can revert to the uninfected memory class after a period of 5 days based on the half-life of viral DNA (25), becoming potential candidates for future infection. Latently infected cells harbor integrated proviruses. Sources claim this viral reservoir has a half-life of anywhere from 6 to 44 months (67,76,77). Of all the model components in our system, free virus has the shortest half-life of approximately 6 hours (19).

Infection rates of naive (\( k_n \)) and memory (\( k_m \)) cells account for infection of each cell subset meeting virus within the LT. These rates have been estimated per day per virion. We estimate an infection rate for naive cells greater than that for memory cells based on the discussion in the previous section.

The proportion of infected cells of each type is a subject of debate, mainly because of differences in classification from one study to the next. Productively infected cells are easiest to characterize because they are actively producing virus. This proportion is approximately 5 to 10.
cells per million or 10^5 per gram of lymphoid tissue (~1 in 100,000 cells) (16). The number of latently infected cells is similarly small, ranging from about 0.1% to 1% of the total number of infected cells or approximately 5 to 10 latently infected cells per million (40). Latently infected cells do not result from direct infection but from deactivation of productively infected cells (31,36). Abortively infected cells, resting cells that have come in contact with virus, exist at a ratio of about 7500 to 10^5 virions (25,50,51). As a starting estimate, we use a baseline value of viral production as 500 virions per day per cell and explore the full variation of this parameter within the text.

A single productively infected cell can produce anywhere from 10^2 to 10^5 virions (25,50,51). As a starting estimate, we use a baseline value of viral production as 500 virions per day per cell and explore the full variation of this parameter within the text.

Because the CD4/CD8 ratio is observed to switch early in infection from 2:1 to 1:2 (17), our model indirectly accounts for this in our representation of the immune response. We model the CD8^+ T-cell response as proportional to the number of CD4^+ T cells present at any given point in disease progression.

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REFERENCES


**TABLE 2. Parameter values used in models with HIV-1 infection**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Definition</th>
<th>Initial value (reference)</th>
<th>Units</th>
</tr>
</thead>
<tbody>
<tr>
<td>L(0)</td>
<td>Latently infected CD4^+ T cells (LT)</td>
<td>0</td>
<td>Cells</td>
</tr>
<tr>
<td>P(0)</td>
<td>Productively infected CD4^+ T cells (LT)</td>
<td>0</td>
<td>Cells</td>
</tr>
<tr>
<td>A(0)</td>
<td>Abortively infected CD4^+ T cells (LT)</td>
<td>0</td>
<td>Cells</td>
</tr>
<tr>
<td>V_a(0)</td>
<td>Virus concentration in blood</td>
<td>0</td>
<td>Virions/µL of blood</td>
</tr>
<tr>
<td>V_v(0)</td>
<td>Virus (total) in LT</td>
<td>10 (can vary)</td>
<td>Virions</td>
</tr>
</tbody>
</table>

LT, lymphatic tissues.
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42. Li XD. Gradual shutdown of virus production resulting in latency is the norm during the chronic phase of human immunodeficiency virus replication and differential rates and mechanisms of shutdown are determined by viral sequences. Virology 1996;225:196–212.


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