Chapter xx

CTL ACTION DURING HIV-1 IS DETERMINED VIA INTERACTIONS WITH MULTIPLE CELL TYPES

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During HIV-1 infection, interactions between immune cells and virus yield three distinct disease stages: high viral levels in acute infection, immune control in the chronic stage, and AIDS, when $CD4^+$ T cells fall to extremely low levels. The immune system consists of many players that have key roles during infection. In particular, $CD8^+$ T cells are important for killing of virally infected cells as well as inhibition of cellular infection and viral production. Activated $CD8^+$ T cells, or cytotoxic T cells (CTLs) have unique functions during HIV-1, most of which are thought to be compromised during HIV-1 disease progression. Controversy exists regarding priming of CTLs, and our work attempts to address the dynamics occurring during HIV-1 infection. To explore the influence of $CD8^+$ T cells as determinants in disease progression and issues relating to their priming and activation, we develop a two-compartment ordinary differential equation model describing cellular interactions that occur during HIV-1 infection. We track $CD4^+$ T cells, $CD8^+$ T cells, dendritic cells, infected cells, and virus, each circulating between blood and lymphatic tissues. Using parameter estimates from literature, we simulate commonly observed disease patterns. Our results indicate that $CD4^+$ T cells as well as dendritic cells likely play a significant role in successful activation of $CD8^+$ T cells into CTLs. Model simulations correlate with clinical data confirming a quantitative relationship between $CD4^+$ T cells and $CD8^+$ T-cell effectiveness.

Keywords: HIV-1, $CD8^+$ T cells, CTLs, T-cell help, dendritic cells, immune activation.

1. INTRODUCTION

HIV-1 infection leads to the gradual depletion of $CD4^+$ T cells over a typical adult disease course in the majority of patients. The mechanisms for this loss remains unclear, and can be attributed to several host and viral factors. Among them is a reduction in thymic production of uninfected cells or infection within the thymus (Douek, 1998; Ye et al., 2002), altered migration of circulation patterns (otherwise

known as enhanced homing) (Bajaria et al., 2002), a decrease in T-cell life span (enhanced apoptosis) (Hellerstein et al., 1999; McCune et al., 2000), and failure of $CD4^+$ T-cell help to $CD8^+$ T-cells, that normally assist them in performing their full cytolytic function (Shedlock and Shen, 2003; Janssen et al., 2003). We have studied altered homing and enhanced apoptosis in previous models (Bajaria et al., 2002; Ye et al., 2002). We now focus on mechanisms related directly to the function of $CD8^+$ T cells.

1.1. Clinical Progression of HIV-1

During HIV-1, immune cells and virus interact resulting in three distinct stages of infection (Pantaleo et al., 1993). Acute stage is characterized by high levels of virus, low $CD4^+$ T-cell counts, and a rapid increase in $CD8^+$ T-cell counts (Quinn, 1997). The asymptomatic or chronic stage is a function of the host immune system controlling viral growth and spread for an extended period of time, often resulting in undetectable levels of virus, even without the influence of drug therapy. The onset of the AIDS stage occurs when $CD4^+$ T-cell counts fall to extremely low levels, precluding the onset of a variety of opportunistic infections, and eventually death. While this pattern is observed in typical disease progressors, long-term non-progressors (LTNP) are a small percentage (5%-10%) of the infected population who, even without the help of antiretroviral therapy, do not progress to AIDS for over 15 years of infection (Zhang et al., 1997).

$CD8^+$ T cells have been shown to be influential in all three disease stages. $CD8^+$ T-cell numbers correlate with both viral load (Koup et al., 1994; Borrow, 1994; Ogg et al., 1998) and have a defined correlation with $CD4^+$ T-cell counts (Caruso et al., 1998). Due to the many known activities of $CD8^+$ T cells in antiviral immunity, we explore their specific roles during HIV-1 infection.

1.2. $CD8^+$ T-cell Mechanisms During HIV-1 Infection

$CD8^+$ T cells are important in the control of intracellular pathogens, including viruses such as CMV and influenza (McMichael et al., 1983; Webby et al., 2003; Ellefsen et al., 2002). These classically termed "killer" cells have various direct and indirect cytotoxic functions during infection. Upon successful differentiation to cytotoxic T cells (CTLs), $CD8^+$ T cells can perform cytolytic functions such as direct killing of infected cells expressing foreign proteins and MHC I (McMichael and Rowland-Jones, 2001; Yang et al., 1996; Berke, 1995), and noncytolytic functions such as inhibition of virus infection and production (Walker et al., 1986; Levy et al., 1996). The cytolytic pathway begins when CTLs secret perforin which forms pores in the target cell membrane. Granzymes are then released from CTLs and travel through these pores into target cells, resulting in cell death (McMichael and Rowland-Jones, 2001). A less common mechanism of CTL killing is via Fas

ligand on CTLs binding to the Fas receptor on target cells and initiating apoptosis (Katsikis et al., 1995).

In addition to the above mechanisms, CTLs prevent infection and virus production through indirect means during HIV-1 infection. CTLs inhibit viral replication through production of cytokines, such as INF-γ (Meylan et al., 1993; Emilie et al., 1992). CTLs also produce chemokines that compete with HIV-1 for binding with host cell coreceptors (Barker et al., 1998; Appay et al., 2000). HIV-1 entry into host cells requires binding of gp120 on the HIV-1 envelope to the CD4 receptor and a coreceptor on the surface of the host cell. By producing chemokines that are natural ligands for HIV-1 coreceptors, CTLs can outcompete HIV-1 by successfully preventing binding and subsequent entry of HIV-1 into host cells. Two of the most common coreceptors used by HIV-1 *in vivo* are CCR5 and CXCR4. Ligands for CCR5 are the CC-chemokines MIP-1α, MIP-1β, and RANTES (Cocchi, 1995; Wagner et al., 1998). High levels of these CC-chemokines are secreted from $CD8^+$ T cells, and have been shown to be inversely correlated with HIV-1 viral load (Ferbas et al., 2000). $CD8^+$ T cells also produce a soluble $CD8^+$ T-cell antiviral factor (CAF) distinct from the CC-chemokines (Barker et al., 1998; Moriuchi et al., 1996; Cocchi, 1995; Levy et al., 1996; McMichael and Rowland-Jones, 2001), which may suppress viral transcription through inhibiting the HIV promoter (Chang et al., 2003). There is evidence of colocalization of granzymes and CC-chemokines inside CTLs, implying that the direct killing and inhibitory pathways are linked such that the same CTL may exert both mechanisms during cytotoxic activities (Wagner et al., 1998).

1.3. Development of Successful CTLs

Differentiation of $CD8^+$ T cells into activated, HIV-1-specific CTLs usually results from the encounter of $CD8^+$ T cells with antigen, commonly found on antigen presenting cells (APCs). However, not all $CD8^+$ T cells that are activated by APCs will become fully differentiated CTLs capable of cytotoxic function (Auphan-Anezin et al., 2003). Differentiation of $CD8^+$ T cells into CTLs that are equipped to perform the functions described above is a result of many interactions involving multiple cell types. There is controversy in the literature as to the importance of $CD4^+$ T-cell help in the priming of the CTL response. Some have found $CD4^+$ T-cell help to be crucial for both priming and maintenence of the CTL response (Wang and Livingstone, 2003), while some have found $CD4^+$ T-cell help to be important only in the memory response (Shedlock and Shen, 2003; Janssen et al., 2003). Furthermore, if $CD4^+$ T-cell help is necessary, there is speculation about what specific interactions take place between $CD4^+$ and $CD8^+$ T cells or whether intermediates are required. We describe below the known functions of dendritic cells and $CD4^+$ T cells in the successful development of CTLs.

Activation by Dendritic Cells

During viral infection, antigen presentation occurs primarily via dendritic cells and macrophages transporting antigen to T-cell rich areas of LT (Buseyne et al., 2001). In recent years, dendritic cells (DCs) have been identified as the most efficient APCs during the course of HIV-1 disease progression (Bottomly, 1999). Thus, we focus here on the influence of dendritic cells in initiating the immune response and HIV-1-specific CTL activity.

Prior to antigen stimulation, DCs maintain an immature phenotype in the periphery and filter the environment for foreign antigen, but are inefficient at presenting antigen to T cells (Mellman and Steinman, 2001). Immature DCs internalize antigen upon initial encounter, and migrate to the LT undergoing maturation. Maturation is characterized by a decrease in antigen processing capability and an upregulation of costimulatory molecules CD80 and CD86 as well as increased expression of MHC Class II and B7.2 molecules (Sharpe and Freeman, 2002), all of which allows for proper activation of $CD4^+$ and $CD8^+$ T cells via DCs (Mellman and Steinman, 2001; Bousso and Robey, 2003; Banchereau and Steinman, 1998; Cella et al., 1997). Thus we track two classes of dendritic cells; the immature DCs (IDCs) that capture antigen in the peripheral blood and mature DCs (MDCs) that present antigen to T cells in the lymph tissues (LT).

HIV-1 has the capability to exploit this process of antigen presentation to its advantage (Teleshova et al., 2003). During infection, IDCs bind to gp120 on HIV-1 in the periphery via dendritic cell-specific, intracellular adhesion molecule-grabbing nonintegrin (DC-SIGN) (Curtis et al., 1992; Geijtenbeek et al., 2000). IDCs then internalize the virus through macropinocytosis (Sallusto et al., 1995; Baribaud et al., 2001), which extends the short half-life of the virus to several days and protects its infectious capability (Geijtenbeek et al., 2000; Kwon et al., 2002). IDCs then migrate into LT to present antigen to $CD4^+$ and $CD8^+$ T cells (Geijtenbeek et al., 2000). Among the most significant events that follow is DC activation of resting $CD4^+$ T cells into HIV-1-specific T helper cells. This occurs via interaction of CD40-CD40L or secretion of cytokines (Ridge et al., 1998).

Cell-associated virus carried on dendritic cells can infect both resting and activated $CD4^+$ T cells even more efficiently than free virus (McMichael and Rowland-Jones, 2001; Engering et al., 2002; Albert et al., 1998). Upon contact with a dendritic cell, HIV-1 receptors CD4, CCR5, and CXCR4 on T cells colocalize on the cell surface toward the junction of the dendritic cells with T cells, enhancing the opportunity for successful viral entry (McDonald et al., 2003). There is some evidence that DCs can also become infected, due to their surface expression of both CD4 molecules and the coreceptors CCR5 and CXCR4 (Fong et al., 2002; Patterson et al., 2001). However, this is not thought of as significant in disease progression because the total number of DCs in blood is much less than that of other cell types (Liu, 2001; Grabbe et al., 2000). Additionally, studies have found

HIV-1-infected DCs *in vivo* or *in vitro* are significantly less prevalent than infected $CD4^+$ T cells (McIlroy et al., 1995; Haase, 1986). Therefore, infection of DCs is not considered in this study.

Here we identify the infection route by which virus is carried from the periphery into LT via dendritic cells. We acknowledge the possibility that there are other mechanisms of virus entry into LT but focus on this as the major pathway.

$CD4^+$ T-cell Help to $CD8^+$ T Cells

$CD4^+$ T cells that become activated are termed helper T cells in that they play a significant role in aiding CTLs to perform their effector function. $CD4^+$ T cells have been implicated in both the initial priming (Wang and Livingstone, 2003) as well as long-term memory $CD8^+$ T-cell response (Janssen et al., 2003; Shedlock and Shen, 2003). Thus, since $CD4^+$ T cells decline significantly early in the infection process, the $CD8^+$ T-cell response is adversely affected during both short- and long-term immunity. HIV-1-specific helper T cells correlate strongly with HIV-1-specific CTLs that have antiviral activity (Kalams and Walker, 1998; Kalams et al., 1999).

The helper function of $CD4^+$ T cells can be attributed to both release of cytokines as well as direct cell-cell interactions. Activated $CD4^+$ T cells release IL-2, a cytokine that induces T-cell proliferation, thus expanding the populations of both $CD4^+$ and $CD8^+$ T cells. However, $CD4^+$ T cells that have encountered HIV-1 on dendritic cells express various activation markers that can further interact with either dendritic cells or $CD8^+$ T cells. This initiates the stages of $CD8^+$ T-cell activation that lead to fully differentiated CTLs. CD40L, expressed on the surface of activated $CD4^+$ T cells, is important for effector function in immunity (van Essen et al., 1995), particularly during HIV-1 infection (Bennett et al., 1998; Ridge et al., 1998; Schoenberger et al., 1998). This has been shown in experiments in which CD40L inhibition results in a population of unprimed $CD8^+$ T cells (Schoenberger et al., 1998). The interaction of CD40L on activated T cells with CD40 on DCs further stimulates virus-carrying DCs to activate $CD8^+$ T cells (Cella et al., 1996). Once activated, $CD4^+$ T cells can also have direct interaction with $CD8^+$ T cells (Bourgeois et al., 2002). Thus, $CD4^+$ T-cell help to CTLs is ultimately mediated by the interplay between $CD4^+$ T cells and DCs (Caux et al., 1994). The HIV-1 envelope protein Nef can also interfere with the antigen presentation pathway by intersecting CD40-CD40L activation (Pope, 2003; Andrieu et al., 2001).

HIV-1-specific CTL killing and inhibition is crucial even as early as the acute stage of infection (Zajac et al., 1998). Throughout chronic infection, $CD4^+$ T cells lose their ability to dictate functionality of $CD8^+$ T cells towards eliminating the virus, or ensuring specificity of adaptive immunity through a steady decline in $CD4^+$ T-cell numbers (McMichael and Rowland-Jones, 2001; Appay et al., 2000;

Lieberman et al., 2001). This effect would be even more pronounced however in the absence of CTLs specific for HIV-1 activated during acute infection. Even though there may be a significant proportion of $CD8^+$ T cells that are activated, virus can still persist without specific T-cell immunity due to loss of instructional $CD4^+$ T helper cells (Goulder et al., 2000). A higher frequency of HIV-1-specific $CD4^+$ T-cell responses is characteristic of LTNPs as compared to typical progressors (Pitcher et al., 1999). There exists both a quantitative and qualitative correlation between the specificity of the $CD4^+$ T-cell response during HIV-1 infection and the functionality of the $CD8^+$ T-cell response (McMichael and Rowland-Jones, 2001).

1.4. Failure of CTLs in Controlling Infection

There are a number of possibilities for the observed decline in CTL immunity in end-stage disease. In addition to the loss in the number of CTLs, CTL dysfunction could be due to the presence of a high number of activated cells without functionality (Kostense et al., 2002; Trimble and Lieberman, 1998), alterations in cell distributions at infected sites (Pantaleo et al., 1997b), or virus mutation to evade CTLs (Phillips et al., 1991).

CTLs may not necessarily traffick to sites of virus replication (Pantaleo et al., 1997b; Wherry et al., 2003). CCR7 receptors are generally found on molecules that travel to lymphoid tissue (Forster et al., 1999). HIV-1-specific CTLs are characterized as CCR7-, thus lacking the capability for travel to LT, the site of most viral infection and production (Chen et al., 2001). This finding is supplemented by evidence that perforin is not found in the lymph nodes of infected patients in both acute and chronic disease and perforin and granzymes are not colocalized in CTLs found in the LT (Andersson et al., 1999, 2002), both suggesting that CTLs are not in the anatomic location where they are most needed.

HIV-1-specific CTLs are lower in number than CTLs detected during other viral infections such as Epstein-Barr virus or cytomegalovirus (CMV) (Gillespie et al., 2000; Hislop et al., 2001). This may be a key reason for better immune control observed in other infections. Additionally, there are functional differences between HIV-1-specific CTLs and for example, CMV-specific CTLs. HIV-1-specific $CD8^+$ T cells express lower levels of perforin compared to CMV-specific cells (Papagno et al., 2002). HIV-1-specific CTLs are also in a less mature state as compared to other CTLs, and their killing and inhibitory capacities are reduced (van Baarle et al., 2002). In this work, we assume activated $CD4^+$ T cells are HIV-1-specific and correlate our simulation results with data on HIV-1-specific $CD4^+$ and $CD8^+$ T cells.

2. MODEL OF HIV-1 INFECTION

We develop a mathematical model to elucidate the role of $CD8^+$ T cells in various stages of HIV-1 infection as well as to explore priming and differentiation of HIV-1-specific CTLs. The most common clinical markers for HIV-1 disease progression are $CD4^+$ T-cell and viral load measurements from the blood. However, most lymphocytes (98%) reside in lymph tissues and circulate constantly between LT and blood (Haase, 1999). Therefore, we build a two-compartment model of HIV-1 infection in blood and lymph tissues (Figure 1), similar to our previous work (Bajaria et al., 2002). We account for the following populations in both the peripheral blood and lymph tissues: resting and activated $CD4^+$ T cells, resting and activated $CD8^+$ T cells, infected $CD4^+$ T cells, virus, and dendritic cells.

2.1. Model Equations

Using thirteen nonlinear ordinary differential equations, we tracked dynamics of $CD4^+$ (T_4) and $CD8^+$ (T_8) T cells circulating between blood ($\mathcal{B}$) and lymph tissue ($\mathcal{L}$) compartments. During infection, virus levels are monitored in both the blood (V_B) as well as lymph tissues (V), together with infected cells (I) and activated $CD4^+$ (T_H) and $CD8^+$ (T_C) T cells in the lymph tissues, MDCs in the lymph tissues (D) and IDCs in the blood (D_B). Equations for the model system are as follows:

$$\begin{aligned}
\frac{dT_4}{dt} &= S_4 - \mu_4 T_4 - \frac{rDT_4}{D+f_1}\left(\frac{f_5}{V+f_5}\right) - k_1\left(\frac{c_1}{T_C+c_1}\right)VT_4 \\
&\quad -k_2\left(\frac{c_2}{T_C+c_2}\right)DT_4 + \frac{p_4 T_H T_4}{T_H+f_2} + \alpha e_B T_{4B} - e_L T_4 \qquad (1)\\
\frac{dT_{4B}}{dt} &= \beta e_L T_4 - e_B T_{4B} \qquad (2)\\
\frac{dT_H}{dt} &= \frac{rDT_4}{D+f_1}\left(\frac{f_5}{V+f_5}\right) - \mu_H T_H - k_3\left(\frac{c_3}{T_C+c_3}\right)VT_H \\
&\quad -k_4\left(\frac{c_4}{T_C+c_4}\right)DT_H + \alpha e_B T_{HB} - e_L T_H \qquad (3)\\
\frac{dT_{HB}}{dt} &= \beta e_L T_H - e_B T_{HB} \qquad (4)\\
\frac{dI}{dt} &= k_1\left(\frac{c_1}{T_C+c_1}\right)VT_4 + k_2\left(\frac{c_2}{T_C+c_2}\right)DT_4 + k_3\left(\frac{c_3}{T_C+c_3}\right)VT_H \\
&\quad +k_4\left(\frac{c_4}{T_C+c_4}\right)DT_H - \mu_I I - \kappa T_C I \qquad (5)\\
\frac{dV}{dt} &= N\left(\frac{c_5}{T_C+c_5}\right)\mu_I I - \mu_V V \qquad (6)\\
\frac{dV_B}{dt} &= \beta e_V V - \mu_V V_B \qquad (7)
\end{aligned}$$

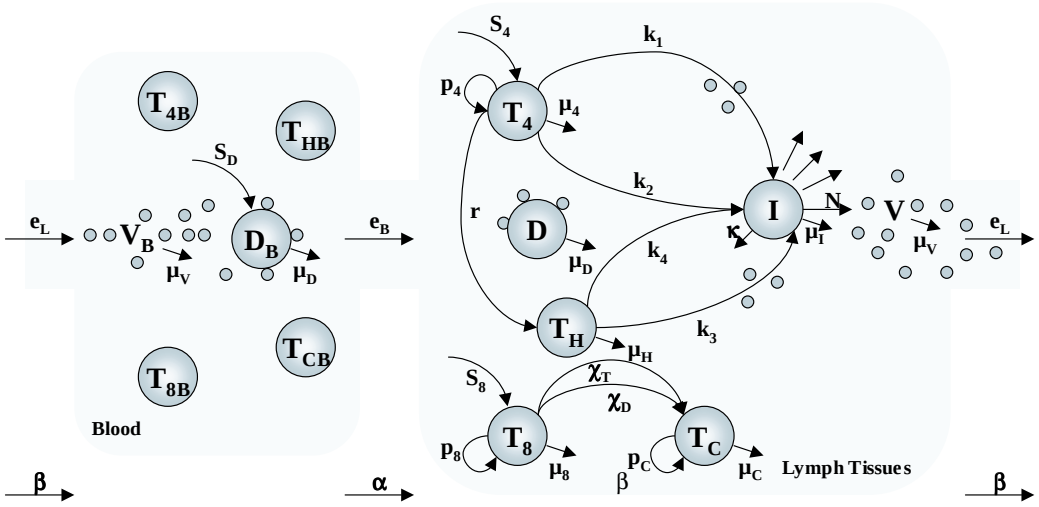

Fig. 1. Two-compartment HIV-1 infection model. The model developed here explores mechanisms of HIV-1 disease progression based on the interactions of a single viral strain ($V(t)$) of HIV-1 with resting ($T_4(t)$) and activated ($T_H(t)$) subclasses of CD4$^+$ T cells at rates k_1 and k_3, respectively. This leads to the development of infected cells ($I(t)$), which are also created by infection via virus ($V_B(t)$) on IDCs from the blood ($D_B(t)$) (at rates k_2 and k_4). Upon migration into the LT, IDCs mature into MDCs ($D(t)$) and activate T_4 into T_H at rate r. Infection directly produces actively infected ($I(t)$) 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 (Richman, 2000). CD8$^+$ T cells ($T_8(t)$) are activated into CTLs ($T_C(t)$) in the LT due to interaction with either $T_H(t)$ (at rate χ_T) or MDCs ($D(t)$) (at rate χ_D). CTLs can kill infected cells (at rate κ) and inhibit infection of T_4 cells (saturating at c_1 and c_2) and T_H cells (saturating at c_3 and c_4), and inhibit virus production from I (saturating at c_5). CTLs can proliferate in response to IL-2 secreted from T_H cells (at rate p_C). Resting and activated CD4$^+$ and CD8$^+$ T cells proliferate at rates p_4 and p_8 and circulate between blood ($\mathcal{B}$) and LT ($\mathcal{L}$) at rates e_B and e_L scaled by α and β. All initial conditions and parameters are described in Tables 1-3.

$$\frac{dT_8}{dt} = S_8 - \mu_8 T_8 - \chi_D \frac{T_H}{T_H + f_3} D T_8 - \chi_T T_H T_8 + \frac{p_8 T_H T_8}{T_H + f_4} + \alpha e_B T_{8B} - e_L T_8 \quad (8)$$

$$\frac{dT_{8B}}{dt} = \beta e_L T_8 - e_B T_{8B} \quad (9)$$

$$\frac{dT_C}{dt} = \chi_D \frac{T_H}{T_H + f_3} D T_8 + \chi_T T_H T_8 - \mu_C T_C + p_C \frac{T_H}{T_H + f_6} T_C + \omega \alpha e_B T_{CB} - e_L T_C \quad (10)$$

$$\frac{dT_{CB}}{dt} = \beta e_L T_C - \omega e_B T_{CB} \quad (11)$$

$$\frac{dD}{dt} = \alpha \delta_B \frac{V_B}{V_B + \psi} D_B + \lambda_L \frac{V}{V + \phi_L} D - \mu_D D \quad (12)$$

$$\frac{dD_B}{dt} = S_D - \mu_D D_B + \lambda_B \frac{V_B}{V_B + \phi_B} D_B - \delta_B \frac{V_B}{V_B + \psi} D_B \quad (13)$$

Equations (1) and (8) describe resting CD4$^+$ and CD8$^+$ T-cell populations in the lymph tissues. Their populations depend on influx from the blood at rate e_B (scaled by α for compartmental exchange) and loss due to emigration at rate e_L. Equations (2) and (9) describe resting CD4$^+$ and CD8$^+$ T-cell populations in the blood and have similar circulation terms (scaled by β for entry into the blood). The source of CD4$^+$ (S_4) and CD8$^+$ (S_8) T cells represents new constant input from the thymus while their half-lives are representated by the decay rates μ_4 and μ_8, respectively. Contact between a dendritic cell and a resting CD4$^+$ T-cell can result in either activation into CD4$^+$ T helper cells (T_H) at rate r (saturating at f_1) or infection at rate k_2. Infection can also result directly by meeting virus at rate k_1. Resting cells can also proliferate at rate p_4 dependent on the amount of IL-2 in the environment secreted from T helper cells (T_H), which we assume is a proportion relative to T_H cells. Activation can be hindered by Nef interference with the CD40-CD40L pathway of DC activation (saturating at f_5) (Pope, 2003). Activated CD4$^+$ T cells (T_H) have a death rate (μ_H), and become infected by directly meeting virus (k_3) or through presentation of virus by a dendritic cell (at rate k_4) (Douek et al., 2002). Similar circulation terms for T_H cells describe emigration from and to their counterparts in the blood (T_{HB} in Equation 4). Infected cells result from any of the pathways described above and are subject to a death rate μ_I. When dendritic cells come into contact with resting CD8$^+$ T cells at rate χ_D, new CTLs are created, an effect that is enhanced in the presence of T_H cells, saturating at rate f_3. CD8$^+$ T cells can also become activated through direct contact with a T_H at rate χ_T because CD8$^+$ T cells transiently express CD40 after activation (Bourgeois et al., 2002). The levels of resting CD8$^+$ T cells are supplemented by proliferation (at rate p_8), also in response to IL-2 secreted from T_H cells. There is evidence that pre-terminally differerentiated CTLs can proliferate (Champagne et al., 2001) in response to IL-2 (Jin et al., 1998) and the majority of HIV-1-specific CD8$^+$ T cells are at an intermediate stage of differentiation (Appay et al.,

2002; Champagne et al., 2001), thus we include proliferation of CTLs occurring at rate p_C. This effect saturates at rate f_6, since IL-2 expandable CTL responses are reduced in end-stage HIV-1 infection (Jin et al., 1998). Because there is evidence that HIV-1-specific CTLs lack the capacity to home to the LT during infection (Chen et al., 2001), we include a factor ω as the percentage of CTLs that circulate back into the LT from the blood. IDCs are initially present in homeostasis in blood with source S_D and death rate μ_D. They are recruited into LTs at rate λ_B in response to virus in the blood (V_B), a process which saturates at ϕ_B. Blood IDCs take up virus and migrate to lymph tissues at rate δ_B, which also saturates at ψ. There is also a recruitment rate (λ_L) of MDCs into the lymph tissues in response to viral stimulus or inflammation, an activity which saturates at ϕ_L. Virus (V) is produced in the LT from infected cells (at rate N) and has a short half-life (μ_V). Virus in the blood flows in from the LT (e_V), and is lost to natural death (μ_V).

Direct cell-to-cell contact is necessary for CTLs to eliminate infected cells (Folkvord et al., 2003). We incorporate clearance of infected cells due to CTLs (at rate κ) acting through the perforin/granzyme pathway and/or Fas pathway. As of yet there is little evidence as to what proportion of cells are eliminated by Fas-mediated apoptosis and which by perforin and granzyme killing; only that both result in target cell death. We include these cytolytic activities occurring in the lymph tissues only, concurrent with previous data that most viral replication and production processes occur in the LT (Haase, 1999). Free virus is not a common target for clearance by CTLs and thus the virus equation only includes a loss term due to half-life of the virus. CTLs inhibit both viral infection and virus production. Low CTL counts allow full infectivity and virus production, whereas increased CTL levels impair these infection processes. Thus, the inhibitory effect of CC-chemokines is captured by CTLs decreasing infection rates of $CD4^+$ T cells (k_1 through k_4) and the rate of virus production from infected cells (N), an effect that saturates at a maximum number of CTLs (c_1 through c_5).

2.2. Parameter Estimation

Table 1 presents initial conditions for the thirteen variables in our model system. In the absence of infection, $CD4^+$ T cells, $CD8^+$ T cells, and dendritic cells exhibit homeostasis. Initial values for infected cell populations and virus are all zero to ensure that initially there is no infection before virus is introduced into the system. We account only for recently emigrated virus-carrying IDCs from the blood, without considering any background level of MDCs in the lymph tissues. Thus the initial condition for MDCs in the LT is zero.

Tables 2 and 3 present parameter values used in our simulations. Infection rates (k_1 through k_4) are estimated using LHS analysis (discussed below) as no data exist on these processes. We estimate, however, that infection of $CD4^+$ T

helper cells (at rates k_3 and k_4) highly exceeds that of resting cells (at rates k_1 and k_2) due to high susceptibility of activated cells to infection (Gougeon and Montagnier, 1993). During contact with MDCs, HIV-1 receptors colocalize on the surface of T cells (Douek et al., 2002; McDonald et al., 2003). Additionally, Tat, a gene expressed during HIV-1 infection, exploits immature dendritic cell activities. Tat mediates chemokine upregulation in immature dendritic cells, causing enhanced recruitment of activated T cells (Izmailova et al., 2003). Thirdly, virus that has been taken up by IDCs has a longer half-life than free virus. Lastly, the kinetics of viral transfer from dendritic cells to T cells is much greater than that of free virus to T cells (Gummuluru et al., 2003). This last feature is crucial to infection due to the short half-life of activated cells (Gougeon and Montagnier, 1993). These processes make trans-infection of $CD4^+$ T cells by DCs very efficient, and the infection rate of cell-associated virus is estimated to be much higher than that by free virus (i.e. $k_4 > k_3 > k_2 > k_1$). Similarly, death rates of activated cells are estimated as greater than that of resting cells, with free virus having the shortest half-life (i.e. $\mu_V > \mu_H$ and $\mu_C > \mu_4$ and μ_8). Infected cells are assumed to be productively infected and able to actively produce virus over their lifespan (Haase, 1999). Productively infected cells produce anywhere from 100-1000 virions per cell (Haase, 1999), and we use 800 virions/cell as the baseline value in our model simulations. This parameter has been shown to have a significant impact on the dynamics of infection (Bajaria et al., 2002). Half-saturation constants for the effect of T-cell help to $CD8^+$ T cells (f_3), the strength of CTL inhibition (c_1 through c_5), the impact of dendritic cells on $CD4^+$ T-cell activation (f_1), inhibition of $CD4^+$ T-cell activation by Nef-induced interference (f_5) and the influence of IL-2 on T-cell proliferation (f_2, f_4, f_6) have also been estimated. α and β are included as compartmental parameters scaling exchange between LT and blood compartments, since blood cells are measured per mm^3 of blood and the LT in total cells.

Uncertainty and Sensitivity Analysis

There are several parameters in our model for which no *in vivo* nor human data exists. By comparison with non-human data, *in vitro* experiments, and studies of other pathogens, we can estimate a wide range of possible values for each unknown parameter. Using Latin hypercube sampling (LHS), we find values for those parameters for which reported estimates do not exist (Blower and Dowlatabadi, 1994). This process generates a hypercube through a random combination of parameter values from each estimated range; simulations are then performed for each sample. Output is compared with clinical data on T cells and viral load, and we ultimately choose those parameters for which model simulations exhibit the best fit with known output variables. We further extend this LHS method to test system output sensitivity to various key parameters. By examining the effect each

key parameter has on the outcome variable (in this case viral load) using a partial rank correlation (PRC), we can assess the relative influence of each parameter on system dynamics (e.g. (Blower and Dowlatabadi, 1994)). In fact, those parameters that have significant PRC values have been shown to be bifurcation parameters. Using the LHS/PRC method to study sensitivity and identify bifurcations is a tool to study differences in disease outcomes.

Table 1. Initial conditions for HIV-1 model

Variable	Definition	Value	Units	Reference
Populations in LT				
$T_4(0)$	Resting $CD4^+$ T cells	2 x 10^{11}	cells	1
$T_H(0)$	Activated $CD4^+$ T cells	0	cells	
$I(0)$	Infected $CD4^+$ T cells	0	cells	
$V(0)$	Virus	0	viral RNA	
$T_8(0)$	Resting $CD8^+$ T cells	1 x 10^{11}	cells	1
$T_C(0)$	Activated $CD8^+$ T cells	0	cells	
$D(0)$	Dendritic cells	0	cells	
Populations in blood				
$T_{4B}(0)$	Resting $CD4^+$ T cells	1000	cells/mm^3	1
$T_{8B}(0)$	Activated $CD4^+$ T cells	0	cells/mm^3	
$V_B(0)$	Virus concentration	10	viral RNA/ml	
$T_8(0)$	Resting $CD8^+$ T cells	500	cells/mm^3	1
$T_C(0)$	Activated $CD8^+$ T cells	0	cells/mm^3	
$D_B(0)$	Dendritic cells	20	cells/mm^3	2

1 (Haase, 1999)
2 (Barron et al., 2003)

3. RESULTS

Using the equations and parameters described above, we simulate the three distinct stages of typical disease progression. We then use this positive control to vary parameters related to $CD8^+$ T-cell dynamics and examine effects to the typical HIV-1 disease course.

3.1. Healthy Control

We simulate total $CD4^+$ and $CD8^+$ T cells in healthy, uninfected individuals as a negative control. In healthy individuals, total $CD4^+$ T-cell counts in the LT

Table 2. Parameter values for homeostasis

Parameter	Value	Units	Reference
Scaling between compartments			
Blood to LT (α)	5 x 10^6	mm^3 or μl	
LT to blood (β)	2 x 10^{-7}	mm^3 or μl	
Circulation of T cells			
LT to blood (e_L)	0.01	/day	1
Blood to LT (e_B)	0.4	/day	1
Source terms			
$CD4^+$ T cells into LT (S_4)	4 x 10^8	cells/day	2
$CD8^+$ T cells into LT (S_8)	2 x 10^8	cells/day	2
Dendritic cells into blood (S_D)	0.1	cells/day	estimated
Death rates			
Resting $CD4^+$ T cells (μ_4)	0.002	/day	3
Resting $CD8^+$ T cells (μ_8)	0.002	/day	3
Dendritic cells (μ_D)	0.005	/day	estimated

1 (Sprent, 1973; Sprent and Basten, 1973)
2 (Haase, 1999)
3 (Richman, 2000)

are about 2x10^{11} cells and 1000 cells/μl in the blood (Haase, 1999). The ratio of $CD4^+$ T cells to $CD8^+$ T cells is approximately 2:1 in healthy individuals in both blood and LT (Haase, 1999; Rosenberg et al., 1998). Our model simulations correlate with estimates of 10^{11} $CD8^+$ T-cells in the LT and 500 cells/μl of blood from (Haase, 1999) (data not shown). There is a median 14-20 dendritic cells per milliliter of blood in healthy individuals (Barron et al., 2003). All infected cell populations and dendritic cells in the lymph tissues are set to zero prior to infection.

3.2. Typical Progression of HIV-1

$CD8^+$ T cells in Acute Infection

The acute stage of HIV-1 disease is characterized by extremely low $CD4^+$ T-cell counts and high levels of virus, often higher than 10^6 copies/ml of blood. Consequently, activation and differentiation of large numbers of $CD8^+$ T cells into CTLs results in a significant number that provide killing and inhibitory activities towards viral infection and production. A strong CTL response coincides with resolution of high viremia in the acute stage whereby virus levels decrease approximately 100-fold (McMichael and Rowland-Jones, 2001; Koup et al., 1994;

Table 3. Parameter values for infection

Parameter	Value	Units
Death rates		
Activated $CD4^+$ T cells (LT) (μ_H)	0.5	/day
Activated $CD8^+$ T cells (LT) (μ_C)	1.5	/day
Infected $CD4^+$ T cells (μ_I)	0.1	/day
Free virus (μ_V)	3.0	/day
Infection rates		
Resting $CD4^+$ T cells by virus (k_1)	2.0×10^{-14}	/day-virion
Resting $CD4^+$ T cells by dendritic cells (k_2)	4.0×10^{-13}	/day-cell
Activated $CD4^+$ T cells by virus (k_3)	4.0×10^{-11}	/day-virion
Activated $CD4^+$ T cells by dendritic cells (k_4)	2.0×10^{-7}	/day-cell
Number of virions produced from infected cells (N)	800	virions/cell
Activation/Differentiation/Proliferation rates		
Activation of $CD4^+$ T cells by dendritic cells (r)	0.01	/day
Differentiation of $CD8^+$ T cells by dendritic cells (χ_D)	1.0×10^{-10}	/day-cell
Differentiation of $CD8^+$ T cells by $CD4^+$ T helper cells (χ_T)	8.0×10^{-11}	/day-cell
Proliferation of $CD4^+$ T cells (p_4)	0.02	/day
Proliferation of $CD8^+$ T cells (p_8)	0.02	/day
Proliferation of CTLs (p_C)	0.5	/day
Half-saturation constants		
$CD4^+$ T cells on activation (f_1)	10^{11}	cells
$CD4^+$ T cells on proliferation (f_2)	10^7	cells
$CD4^+$ T-cell help to $CD8^+$ differentiation (f_3)	10^4	cells
$CD8^+$ T cells on proliferation (f_4)	8.0×10^6	cells
Virus on interference with activation (f_5)	8.0×10^6	cells
Migration of dendritic cells to LT (ψ)	1.0	virus
Recruitment of dendritic cells to LT (ϕ_L)	100.0	virus
Recruitment of dendritic cells to blood (ϕ_B)	10.0	virus
Inhibition constants		
$CD4^+$ T-cell infection by virus (c_1)	10^8	cells
$CD4^+$ T-cell infection by dendritic cells (c_2)	10^6	cells
$CD4^+$ T helper cell infection by virus (c_3)	10^8	cells
$CD4^+$ T helper cell infection by dendritic cells (c_4)	10^7	cells
Virus production from infected cells (c_5)	10^8	cells
Clearance rate of infected cells (κ)	9.0×10^{-10}	/day-cell
Circulation/Migration/Recruitment		
Proportion of CTLs that travel to LT from blood (ω)	0.002	scalar
Circulation of virus (LT to blood) (e_V)	0.8	/day
Migration of blood DC to LT (δ_B)	0.0448	/day
Recruitment of dendritic cells to LT (λ_L)	0.007	/day
Recruitment of dendritic cells to blood (λ_B)	0.04	/day

All parameters were estimated except μ_I (Cavert et al., 1997), μ_V (Perelson et al., 1996; Stafford et al., 2000), and N (Haase, 1999).

Safrit and Koup, 1995). Additionally, the CD4:CD8 ratio is inverted from 2:1 to 1:2 after seroconversion (Rosenberg et al., 1998; Schacker et al., 1996).

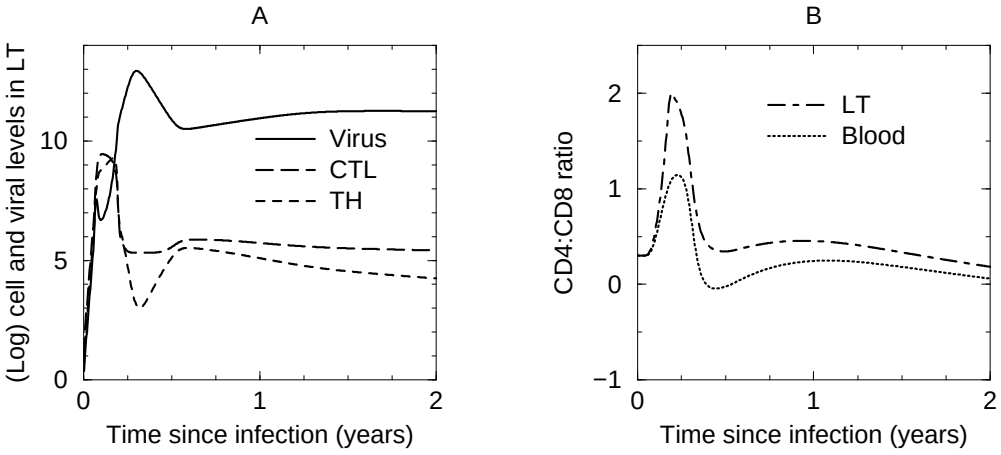

Fig. 2. Acute stage of HIV-1 infection. Panel A: Virus (solid line), CTL (long dashed), and TH (short dashed) dynamics over 2 years post-infection in the LT. Panel B: CD4/CD8 ratio in the blood (dotted) and LT (dot-dashed) over 2 years post-infection.

Our model simulations reflect a rise in CTL number concurrent with a rapid increase in viremia during acute stage of disease (Figure 2, Panel A). $CD4^+$ T-cell help and CTL levels initially follow this increase in virus, while for the remainder of disease progression they correlate inversely with viral load (Ogg et al., 1998). We simulate the ratio inversion of CD4 to CD8 from 2 to 0.5 during acute infection; however our results show that there is a slightly lower CD4:CD8 ratio in the blood with a higher ratio in the LT (Figure 2, Panel B). This indicates either a higher number of $CD8^+$ T cells or lower numbers of $CD4^+$ T cells in the blood as compared to LT, reflecting the skewed distribution of lymphocytes during massive viral infection.

$CD8^+$ T cells in Chronic and End-Stage Infection

After the acute stage of infection, which occurs over the first few months of infection, resting $CD4^+$ T-cell, activated $CD4^+$ T helper cell, and CTL levels increase and virus decreases to a quasi-steady-state level. This resolution of virus to a quasi-steady-state level, referred to as the viral setpoint, occurs for several years and is characteristic of the chronic stage of disease (Ho et al., 1995). Our simulation shows a quasi-steady-state level of virus in the blood and LT following the acute stage of disease that lasts for several years (Figure 3, Panel A). We also show the typical rebound of resting $CD4^+$ and $CD8^+$ T cells to high levels fol-

lowed by the gradual $CD4^+$ T-cells decline throughout a typical ten-year disease progression (Figure 3, Panel B).

Resting $CD8^+$ T cells decline initially due to massive expansion of the CTL population, but recover to normal levels rapidly and only decrease slightly throughout disease progression (Figure 3, Panel B and (McCune et al., 2000; Hellerstein et al., 1999; Kovacs et al., 2001)). Because there is little to no helper activity in the blood after the acute stage of infection in data (Betts et al., 2001), we are able to examine dynamics of resting and activated $CD4^+$ and $CD8^+$ T cells only in LT (Figure 3, Panels C and D).

For comparison, we present clinical data for cell populations and virus in the chronic phase of disease with results from our model simulations (Table 4).

Table 4. Comparison of simulation at year 3 post-infection with clinical data

Population	Model Value	Clinical value	Reference
Resting $CD4^+$ T cells in LT	7×10^{10}	10^{10} cells	1
Resting $CD8^+$ T cells in LT	8×10^{10}	10^{10} cells	1
Infected cells in LT	4×10^{9}	10^{10} cells	1
Virions in LT	9×10^{10}	5×10^{10} virions	1
Activated $CD4^+$ T cells in blood	0.0000007%	0.12% of $CD4^+$ T cells	2
Activated $CD8^+$ T cells in blood	8.8%	0.1% - 10% of $CD8^+$ T cells	3
Median DC in blood	10 cells/ml	7 cells/ml	4

1 (Haase, 1999)
2 (Pitcher et al., 1999)
3 (Ogg et al., 1998; Scott-Algara et al., 2001)
4 (Barron et al., 2003)

Progression to AIDS occurs for a number of reasons, and one key factor is likely the ultimate failure of the CTL response in controlling viral growth and spread. This may occur due to any of the reasons outlined in section 1.4. With our model, we can observe decreased CTL levels with ongoing disease progressions, alterations in cell distributions, and a high number of activated cells with limited function.

We observe $CD4^+$ T-cell decline to AIDS-defining levels (< 200 cells/mm^3 of blood) in the latter stage of infection. However, we do not observe an exponential rise in viral load in the blood in the end stage of disease. This could be due to several factors, among them the lack of target cells for infection once resting and activated $CD4^+$ T cells have declined to extremely low levels (Phillips, 1996). Homeostatic mechanisms may bring more uninfected target $CD4^+$ and $CD8^+$ T cells from the thymus during infection; however we have not included this effect

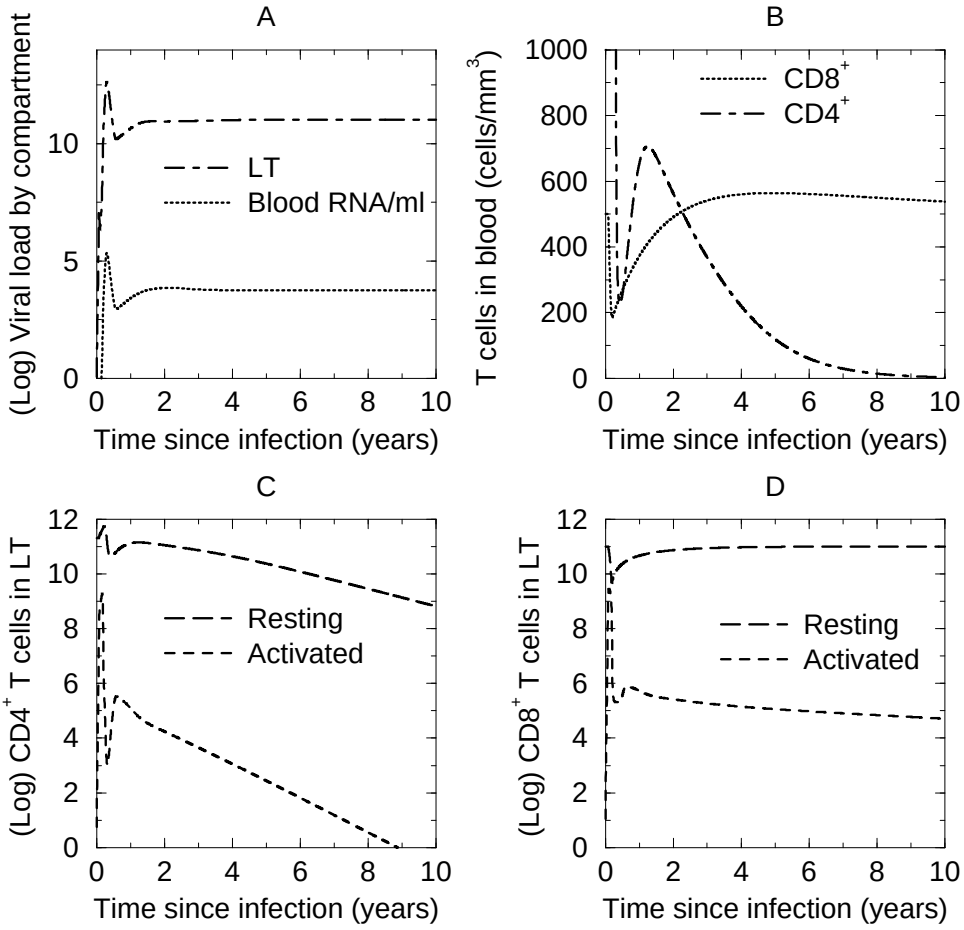

Fig. 3. Three stages of typical HIV-1 disease progression. Shown are simulations of early, chronic, and AIDS stages of disease progression over a 10-year course of infection. Panel A: Virus levels in blood (dotted line) and LT (dot-dashed). Panel B: Total $CD4^+$ (dot-dashed) and $CD8^+$ (dotted) T cells in blood. Panel C: Resting (long dashed) and activated (short dashed) $CD4^+$ T cells in LT. Panel D: Resting (long dashed) and activated (short dashed) $CD8^+$ T cells in LT.

as we are solely examining CTL dynamics in the two compartments of blood and lymph tissues.

3.3. HIV-1-Specific CTL Levels

Measurement of HIV-1-specific $CD8^+$ T-cell responses varies between studies. Specificity for a single peptide is not indicative of the breadth of the CTL response.

One study that measures specificity by flow cytometric detection of intracellular IFN-γ, found 1.4-22% of circulating $CD8^+$ T cells to be HIV-1-specific (Migueles and Connors, 2001). Gag, an HIV-1 protein, is most predominantly recognized by CTLs during asymptomatic HIV-1 infection (Johnson et al., 1991; van Baalen et al., 1993). A study that examined the frequency of CTL precursors in response to Gag found them to be in the range of 0.005% - 0.3% (Klein et al., 1995). In another study, the gag-specific $CD4^+$ T-cell proportion ranged from 0.2% to 2% of total $CD4^+$ T cells with a mean of 1%. HIV-1-specific $CD8^+$ T cells ranged from 0.1% to 5% with a mean of 3% (Sester et al., 2000). In that study, it was found that the frequency of HIV-1-specific $CD8^+$ T cells was typically higher than that of HIV-1-specific $CD4^+$ T cells. In general, as much as 10% of the $CD8^+$ T-cell population could be activated against HIV-1 antigens (Pantaleo et al., 1997a). In Table 5 we present variations in specificity of $CD4^+$ and $CD8^+$ T cells concomitant with disease progression based on our model simulations.

Table 5. HIV-1-specific T cells in three stages in our model simulations

Cell type	Acute (50 days)	Chronic (3 years)	AIDS (10 years)
$CD4^+$ helper T cells in blood	0.009%	0.0000007%	0.000000003%
$CD8^+$ cytotoxic T cells in blood	35%	8.8%	1.1%

We see that there are extremely low percentages of activated $CD4^+$ T cells in blood. We need to examine LT dynamics (Figure 3, Panel C) to observe the existence of $CD4^+$ T-helper cell activity, though it is undetectable in the blood compartment. CTLs however are greater in number than $CD4^+$ T helper cells, but also diminish throughout disease progression (Figure 3, Panel D).

3.4. HIV-1-Specific CTL Action

It is not known whether CTL killing or inhibition is more influential in disease progression, or whether or not a significant reduction in one or more of these mechanisms would have an impact on cell or viral dynamics. We vary those parameters that impact CTL action on viral infection and production (half-saturation constants on k_1 through k_4 and N). From these results (Table 6), we can see that infection via cell-cell interactions mediated by DCs (c_2 and c_4) has a larger impact than infection by free virus (c_1 and c_3) for both resting and activated $CD4^+$ T-cell infection. Our results show that a 100-fold change in inhibition of cell-mediated infection yields significant changes in disease progression, as compared to a 10000-fold change on inhibition of virus infection necessary to significantly affect the results. Interestingly, changing the infection rate of activated $CD4^+$ T cells by virus is not affected significantly by CTLs. Activated cells may not contribute significantly to the infected cell class due to their extremely short half-life.

Additionally, the interaction between T helper cells and DCs could be far more influential in the infection process than the effect of free virus to T helper cells.

Table 6. Effects of variations in CTL activity on disease dynamics

Parameter	Fold change	Results
Inhibition		
Viral infection of resting $CD4^+$ T cells (c_1)	$\downarrow$ 10000	delayed acute stage
DC infection of resting $CD4^+$ T cells (c_2)	$\downarrow$ 100	$\uparrow$ resting $CD4^+$ T cells
Viral infection of activated $CD4^+$ T cells (c_3)	$\downarrow$ 10000	no significant change
DC infection of activated $CD4^+$ T cells (c_4)	$\downarrow$ 100	delayed acute stage
Virus production by infected cells (c_5)	$\downarrow$ 10	delayed acute stage
Killing		
Infected cells by CTLs (κ)	$\uparrow$ 100	lower viral setpoint

It is important to note that CTLs do not prevent infection, but only alter disease dynamics, i.e. the establishment of the acute stage of infection or the viral setpoint. Likely other cell types (such as those involved in innate immunity) or mutation in coreceptors (such as the CCR5Δ32 deletion) may play a role in the possible elimination of or resistance to initial virus introduced into the host.

3.5. $CD4^+$ T Helper Cells and Dendritic Cells Work Together to Yield a Successful CTL Response

One key hypothesis for the failing CTL response is the declining role of $CD4^+$ T-cell help to $CD8^+$ T-cell action. $CD4^+$ T cells both activate and give instructional signals to $CD8^+$ T cells during priming of CTLs. Low levels of help early in infection are attributed to a highly non-specific $CD8^+$ T-cell response, which is ineffective for long-term immune control (McMichael and Rowland-Jones, 2001; Brodie et al., 1999; Kalams and Walker, 1998). We explore the effects of both an increase or decrease in activation of $CD4^+$ T cells into $CD4^+$ T helper cells (parameter r in Equation 1). Our simulations show that higher $CD4^+$ T-cell numbers correlate with higher CTL levels (Figure 4). Additionally, increased activation, although decreasing numbers of resting $CD4^+$ and $CD8^+$ T cells, results in a higher total number of $CD4^+$ T cells in the blood (Figure 4, Panel A) and a higher level of CTLs in the LT (Figure 4, Panel B). This follows from our assumption that increased $CD4^+$ T helper cells activate DCs and $CD8^+$ T cells early enough to prevent further $CD4^+$ T-cell loss, as is observed during acute infection.

The interaction between resting $CD4^+$ T cells and MDCs in the LT can result in the development of $CD4^+$ T helper cells and dendritic cells with $CD8^+$ T-cell priming capability. Thus, the differentiation of $CD8^+$ T cells to HIV-1-specific CTLs can occur either through $CD8^+$ T cells coming into contact directly with

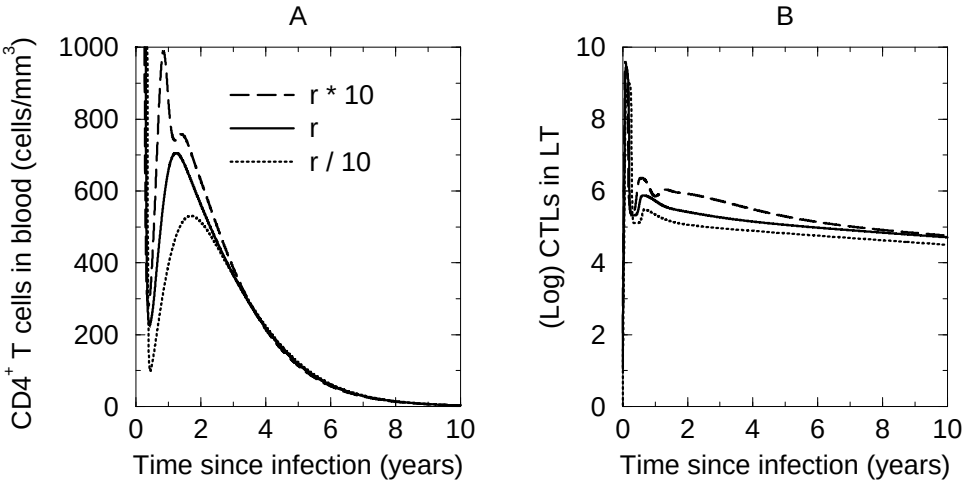

Fig. 4. CD4$^+$ and CD8$^+$ T cell levels vary with activation rate, r. Shown is a simulation of CD4$^+$ T cells in the blood (Panel A) and CTLs in the LT (Panel B) with respect to the rate of activation of resting CD4$^+$ T cells by MDCs in the LT (parameter r in Equation 1).

CD4$^+$ T helper cells or through contact with dendritic cells. However, to distinguish between contact with any MDCs and those that have received activation signals from a CD4$^+$ T cells, the equation for differentiation via dendritic cells includes augmentation by CD4$^+$ T-cell help (which saturates at f_3). To examine whether the dendritic cell-differentiation pathway or the direct CD4$^+$-CD8$^+$ T cell-activation pathway is more significant, we vary the differentiation parameters χ_D and χ_T, respectively, and examine effects to the total production of CTLs in the LT (Figure 5). We find that differentiation of CD8$^+$ T cells by meeting CD4$^+$ T cells directly is more significant than by meeting MDCs (augmented by help), and speculate that dendritic cells may be more crucial in the activation of HIV-1-specific CD4$^+$ T helper cells than the activation of HIV-1-specific CTLs.

There is conflicting information as to numbers or proportions of blood dendritic cells in HIV-1 infected individuals. There is evidence that DCs are depleted in the blood early in HIV-1 infection, corresponding with an increase in blood viral levels (Donaghy et al., 2001; Feldman et al., 2001; Pacanowski et al., 2001; Grassi et al., 1999). This loss could be attributed to a down-regulation of DC markers in the blood, a decrease in new cells input from the bone marrow (Pacanowski et al., 2001), enhanced DC deletion or death, or a rapid export of DCs into the lymph tissues (Cyster, 1999; Lore et al., 2002). Support for migration of mature DCs to T-cell rich areas in the LT comes from studies of selective recruitment of DCs by chemokines to sites of infection (Dieu et al., 1998; Foti et al., 1999).

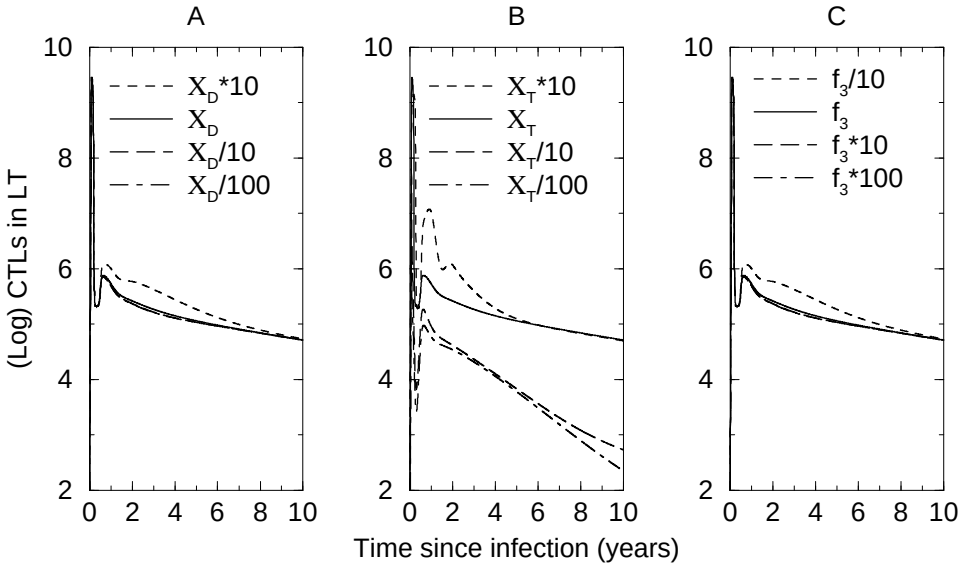

Fig. 5. Success of CTL priming by MDCs and T helper cells. Shown is a simulation of CTL levels in the LT based on changes in the differentiation rate of resting $CD8^+$ T cells by dendritic cells, χ_D, (Panel A), the differentiation rate of resting $CD8^+$ T cells by $CD4^+$ T helper cells, χ_T, (Panel B), and half-saturation for $CD4^+$ T-cell help to $CD8^+$ T-cell differentiation, f_3 (Panel C) from Eqns. 8 and 10). We vary these parameters over an order of magnitude range encompassing baseline values and observe effects to the time progression of CTLs.

Our model does not account for differences between the plasmacytoid DC class and the myeloid DCs. These subsets are distinguished based on their phenotype and function during HIV-1 infection and these will be explored in future work. However, both subsets are depleted in the blood during infection to approximately half of their original values in therapy-naive patients (Barron et al., 2003) and our model reflects that deficiency in the blood compartment (see Figure 6, Panel A). This decrease in blood DCs has also been shown to have a strong correlation with viral load (Donaghy et al., 2001). A rapid influx of DCs into LT is observed in acute infection, as shown in Figure 6, Panel B and described in (Lore et al., 2002). The specific mechanisms of DC trafficking into and out of the LT are considered in other work (in preparation).

In this work, we assume that the heightened state of immune activation during infection reported in several studies (Hazenberg et al., 2003b,a; Leng et al., 2001; Papagno et al., 2004) can be captured by enhanced recruitment of DCs into the LT due to virus. Because dendritic cells are the primary activators of both resting $CD4^+$ and $CD8^+$ T cells in our model, this assumption mimics in-

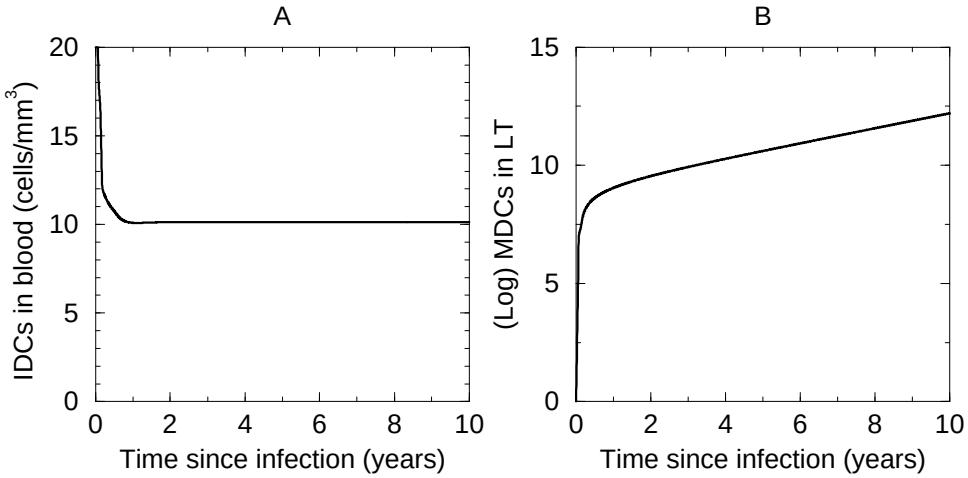

Fig. 6. Dendritic cell dynamics. Shown is a simulation of immature DCs (IDCs) in the blood (Panel A) and mature DCs (MDCs) in the LT (Panel B) over the course of a typical 10-year disease progression.

creased immune activation during the course of infection. To explore the role of priming, we study immune activation and activated T-cell levels in Figure 7. We observe that for less activation (due to no recruitment of MDCs into LT with increasing viral levels), $CD4^+$ T cells in blood remain elevated similar to that of a long-term non-progressor (Zhang et al., 1997). With higher than baseline immune activation (excessive recruitment of MDCs into LT), there is a rapid decline in the $CD4^+$ T-cell count (Figure 7, Panel B). Finally, even though activated T-cell levels fall greatly after the acute stage, our simulation shows their numbers remain steady in the LT when immune activation is low and fall more rapidly when immune activation is excessive (Figure 7, Panel C). Some groups have suggested that HIV-specific CTL might become exhausted through telomere shortening during constant divison resulting from chronic immune activation (Wodarz et al., 1998; Effros et al., 1996). Many studies have suggested that chronic immune activation may be a better predictor of disease progression than viral load (Simmonds et al., 1991; Leng et al., 2001; Roussanov et al., 2000; Giorgi et al., 1999) and our simulations concur (based on more rapid $CD4^+$ T-cell decline with increased immune activation).

4. CONCLUSIONS AND DISCUSSION

Several groups have considered the role of CTLs in HIV-1 infection using mathematical models (Wodarz et al., 1998; Ribeiro et al., 2002; Antia et al., 2003; Wick

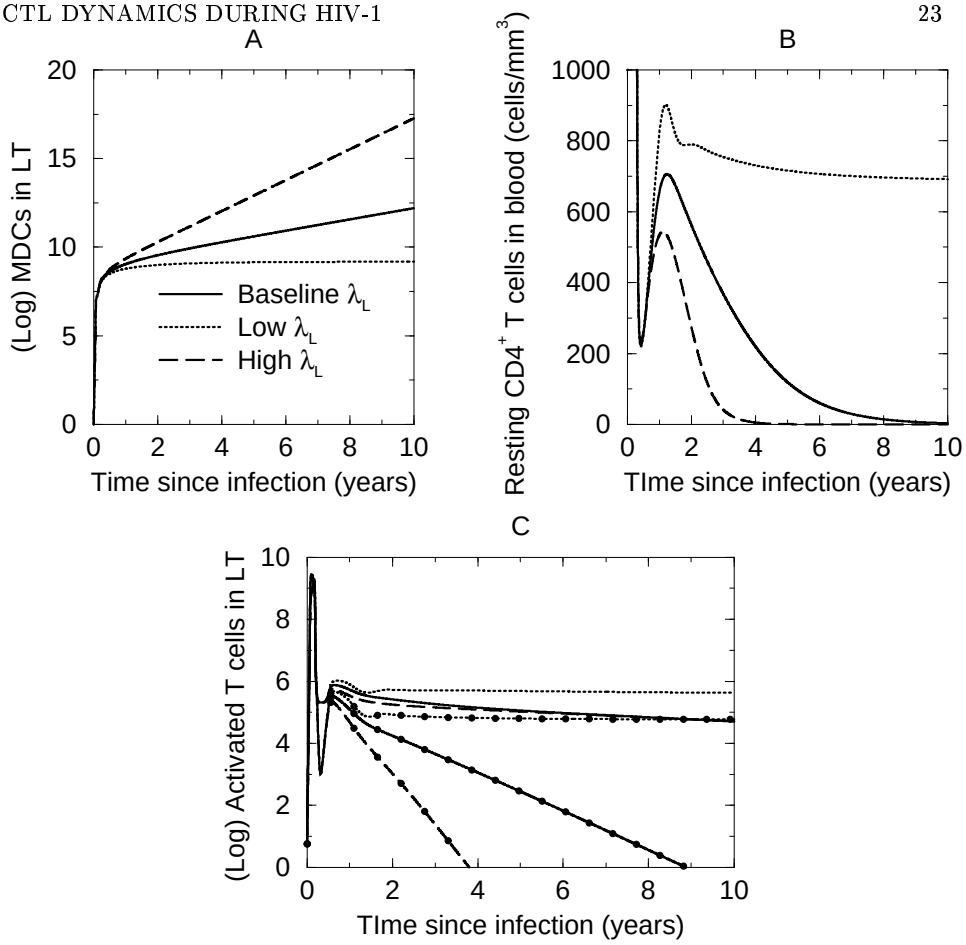

Fig. 7. Changes in time to AIDS based on immune activation status. Here, immune activation is captured by the rate of recruitment of DCs into the LT (Parameter λ_L in Eqn. 12). We vary this parameter above (increased activation, $\lambda_L = .0105$) and below (decreased activation, $\lambda_L = .0035$) the baseline value ($\lambda_L = .007$) to observe effects. Panel A shows changes to levels of DCs in LT and Panel B shows changes to blood CD4$^+$ T cell counts for these three scenarios. In Panel C, we show levels of activated T cells in the LT (i.e., CD4$^+$ (filled circles) and CD8$^+$ T cells (lines only), respectively). We observe a more rapid decline in HIV-1-specific T helper cells and CTLs with increasing immune activation.

and Self, 2002; Arnaout et al., 2000; Fraser et al., 2002), and others the role of CD4$^+$ T-cell help to CTL responses (Wodarz and Jansen, 2001; Wodarz, 2001). Our work expands on these ideas in many ways. First, we study lymphocyte circulation in health and disease with the use of a two-compartment model and are able to incorporate important dynamics of lymphocyte circulation as well as defects

imposed on circulation by HIV-1 infection. Additionally, we include the specific roles of dendritic cells as antigen-presenting cells and their numbers and functional properties in antigen uptake as well as presentation to $CD4^+$ and $CD8^+$ T cells in blood and LT. We consider the total lymph system and use biologically relevant parameter estimations derived from literature, and our simulations compare with clinical and experimental data during various stages of disease progression.

Our simulations reflect the three-stage disease course in a typical HIV-1 progressor with gradually declining $CD4^+$ T-cell levels throughout the chronic phase of disease ((Collaboration, 2003) and Figure 3, Panel B). We use this positive control to test variations in $CD4^+$ T-cell help and DC activation to the successful differentiation of $CD8^+$ T cells to CTLs. In this context, we find that $CD4^+$ T-cell help is necessary for the priming of CTLs, and that interaction of $CD8^+$ T cells with both $CD4^+$ T cells and DCs is crucial for successful CTL development and actions during HIV-1 infection.

There is evidence that inadequately developed $CD8^+$ T cells lack the capacity to effectively control HIV-1 infection, especially as disease progresses (Champagne et al., 2001). It is thought that $CD4^+$ T-cell help may function in the memory $CD8^+$ T-cell response during recurring episodes of virus growth throughout the chronic phase of infection (Kaech and Ahmed, 2003; Janssen et al., 2003; Sun and Bevan, 2003; Shedlock and Shen, 2003; Bourgeois et al., 2002). However, in some experimental systems, $CD4^+$ T-cell help is also essential for the primary response (Wang and Livingstone, 2003). This model does not examine the development of a memory response. However, the development of activated CTLs does decline concommitant with the decline in $CD4^+$ T-cell help, and so we expect that there would also be a proportional decrease in memory $CD8^+$ T cells available for a recall response.

It has been suggested that the activity of IL-2 is necessary for perforin expression due to high, early perforin expression in infection that declines with disease progression (Zhang et al., 2003). However, we tested the effect of reducing CTL killing of infected cells based on levels of $CD4^+$ T helper cells in the system (which are the main producers of IL-2) and found little to no variation in outcome (data not shown). This could be due to our assumption accounting only for HIV-1-specific CTLs, which have been shown to be generally deficient in perforin, and only a percentage successfully migrate to the LT for cytotoxic activities (Chen et al., 2001). Therefore, as our model simulations show, it is important to consider the unique properties of HIV-1-specific CTLs to be able to study their dysfunctions during disease progression.

HIV-1 has evolved several properties to intervene in normal immune system functioning. For example, the HIV-1 envelope protein Nef interferes with the antigen-presentation pathway by intersecting the CD40-CD40L pathway of dendritic cell activation (Pope, 2003; Andrieu et al., 2001). This may result in impaired activation of dendritic cells that would render them inefficient at antigen presenta-

tion. However, dendritic cells and T cells are in close contact during infection, and the proximity of dendritic cell-associated virus to T cells will render T cells highly susceptible to cell-associated infection. Thus, activation levels of dendritic cells become important when determining under which circumstances dendritic cells result in either activation or in $CD4^+$ T-cell infection (Lutz and Schuler, 2002; McDonald et al., 2003). Inadequate signaling may be deleterious to the system, and the role of dendritic cells in HIV-1 infection requires further study.

This work has examined the dynamics of CTLs in HIV-1 infection. Our simulations indicate that activation of $CD4^+$ T cells by DCs and activation of $CD8^+$ T cells by both $CD4^+$ T cells and DCs is crucial in infection. Thus, although CTLs cannot be the sole determinant in disease outcome, they are significant contributors to dynamics influencing disease progression.

ACKNOWLEDGEMENTS

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