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Role of Mathematical Modeling on the Optimal Control of HIV-1 Pathogenesis

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Mathematical modeling of HIV-1 infection has proven to be instrumental for the modern understanding basis of the AIDS pathogenesis, since it offers the unique means to adequately pose hypotheses concerning AIDS dynamics and treatment protocols. Focusing on the HIV-1 subtype-B epidemic, a comprehensive review and discussion of the state-of-the-art in the area is presented. Based on recent results, this multidisciplinary study is then extended to a more in-depth view at the cellular and molecular biology levels that address key issues concerned with the natural history of AIDS, as the basic human anatomic model, the host cell entry of HIV-1, the quantification the HIV-1 infectivity in terms of viral coreceptor specificity, as well as regulation and expression of CCR5 and CXCR4 molecules on the target cell, the T-lymphocyte generation and infection models, and the immune response model. In the sequence, modeling techniques for AIDS pathogenesis are revised and models concerned with either the general HIV-1 dynamics or specifically related to the HIV-1 primary infection are discussed. Ultimately, a general framework for the real-world problem of optimizing the highly active antiretroviral therapy (HAART) benefits is proposed regarding the important questions associated with the drug chemotherapy resistance, side effects and costs. © 2005 American Institute of Chemical Engineers *AIChE J,* 52: 856–884, 2006
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Introduction

A doctor confronted with a patient exposed to HIV-1 (human immunodeficiency virus type 1) faces the following fundamental questions[1]: "For how long must I treat, and which drug of what potency?" Hence, the need for studies that address the optimal treatment of HIV-1 infection that is currently the subject of intense research activity.[2–5]

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The current conventional antiretroviral treatment protocols deferred to HIV-1 infected patients are based on combinations of one or more of a nucleoside reverse transcriptase inhibitor (NRTI), non-nucleoside reverse transcriptase inhibitor (NNRTI), and protease inhibitor (PI). Various combinations are possible, with the following recommended **standard HAART** regimens[6,7]: (a) NRTI + NRTI + PI, (b) NRTI + NRTI + NNRTI, and (c) NRTI + NRTI + PI + PI.

Besides these standard protocols, modifications in the treatment regimen for HIV infection may be suggested for a variety of reasons. These may be prompted by increasing drug resistance,[8] as indicated by detectable HIV-1 RNA reappearing in

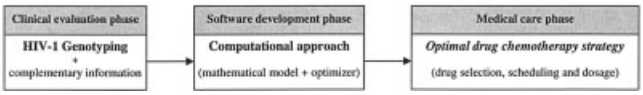


Figure 1. HIV-1 sequencing based optimal management of HIV-1 drug chemotherapy.

plasma after complete suppression or increasing HIV-1 RNA levels in plasma. In addition, failure of patient compliance may force therapy alteration, since drug intolerance and drug toxicity are significant problems for all drugs that treat HIV infection.

Strategies for dealing with drug resistance have been developed and theoretically evaluated.[1,2,9] Among these is the use of multidrug rescue therapy (MDRT), also known as mega-HAART therapy, which may combine up to 9 antiretroviral drugs.[6] The use of a "drug holiday" or planned interruption of treatment may transiently lead to reemergence of a "wild type" HIV that responds better to reinstitution of therapy, but reemergence of resistant HIV is inevitable. Testing for antiretroviral drug resistance can be performed in order to assess the potential for drug efficacy.[8] In primary HIV infection, such testing may detect the transmission of a drug-resistant strain of HIV. In established HIV infection, resistance testing may detect drug-resistant HIV for selection of therapeutic regimens. In patients receiving antiretroviral therapy who have had treatment failures, resistance testing may help to document resistance patterns to modify therapy.[6]

In short, the major potential benefits associated to the optimal management of acquired immunodeficiency syndrome (AIDS) chemotherapy, which includes the optimal drug selection, sequencing and dosage throughout a planning horizon, are as follows: (a) overestimation of the patient life by controlling the development of drug resistant isolates, and/or the increase/retention of the $CD4^+$ T-cell count,[2,10] (b) minimization of drug chemotherapy side-effects,[2] (c) minimization of the expensive treatment cost,[2] and (d) automation of the decision process (Figure 1). Here, superscript "+" in the *$CD4^+$ T-cell* term denotes the subset of T-lymphocytes that are positive to the CD4 marker, that is, those cells that present regulation and expression[134] of the CD4 molecule on the cell surface.

In this sense, the increased efficiency of modern computer resources and novel mathematical techniques allied to new drug developments,[11] accumulated experience on therapeutic regimes[12–17] and augmented knowledge on biological mechanisms that govern not only AIDS pathogenesis evolution,[18–27] but also cancer and several bacterial and other viral infections, have expanded the possibilities of mathematical modeling as a medical tool in an unprecedented way.[28] Mathematical models of HIV-1 pathogenesis and immunology, although providing a simplified description of an extremely complex dynamic system, offer the unique means to adequately pose hypotheses concerning treatment protocols, simulate alternative strategies and guide the qualitative understanding of AIDS dynamics and chemotherapy.[1,3,29–52]

Nevertheless, limited to the conventional approaches strongly based on prior experience, medical and biological research has not taken full advantage of these possibilities,[28] which result from multidisciplinary research directed to knowledge integration, specially in the areas of the *closed loop optimal control*[53–58] and large-scale dynamic optimization,[59] of long-term drug chemotherapy strategies applied to chronically and/or yet today incurable epidemic diseases.

The study of this multidisciplinary area, which is strongly characterized by its dynamic nature mainly because of new inputs related not only to drug chemotherapy research,[60–61] new cellular and molecular immunology discoveries,[62–74] but also to a better understanding of the pathogenic agent structure[14,75–82] and activity[24,25,83–87] are continuously produced. Moreover, these must necessarily encompass the hard task of mathematical translation of biology into mathematics. This is often performed by biologically-oriented researchers or vice-versa, and may involve the use of advanced optimization tools[88–94] if an objective, as the optimal control of the infectious agent propagation, is desired.

In fact, the current understanding of HIV-1 infection dynamics (as hepatitis-C virus infection, among others) relies largely on the mathematical analysis of changes in plasma virus levels after perturbation of the chronically infected (quasi-) steady-state with potent antiviral treatment or plasma apheresis,[33,34,95] due to the inability to infect animals with HIV-1, and to observe the natural course of HIV-1-mediated disease.[96] Moreover, the limited usefulness of an animal model for assessing the effectiveness of different treatments or the pathophysiology of HIV-1 infection may be at least partially circumvented by mathematical modeling and current optimization techniques, which offer the possibility of quickly testing different realistic scenarios.[28] Understanding of the several features of HIV-1-associated infection and its drug chemotherapy pharmacokinetics, as well as of the validated mathematical programming techniques may lead to new and more effective therapies for HIV-1 disease and AIDS driven by the: (a) optimal synergy between immune responses and drug chemotherapy activity, and (b) coordinated actions in order to drive HIV-1 mutations in an optimal way aiming to postpone occurrence of full-blown AIDS.

It is the purpose of this article to delve into this complicated combination of mathematics and immunology in order to provide a comprehensive nevertheless unified overview of the main features directly involved in the development of mathematical models aimed at the optimal management of HIV-1 infection through highly active antiretroviral therapy (HAART) schemes. This work is presented in three sections. First, several features of the HIV-1 pathogenesis and human immunology are outlined. Reviews of HIV infection modeling (past and present), as well as optimal control techniques are then presented. Last, we discuss how current work has highlighted the importance of the optimal dosing strategy to control the emergence of resistant viruses.

Overview of the HIV-1 Infection

Infection by HIV-1 corresponds to a nonlinear[35] and highly dynamic[33,34] process characterized by many puzzling quantitative features.[97] When AIDS first appeared, its pathogenesis was frustratingly elusive because the disease does not appear immediately upon infection with HIV-1. Adult individuals infected with HIV-1 experience a progressive decline in $CD4^+$ T-cell number,[98,99] the primary target of HIV-1,[40] resulting in immunodeficiency and increased susceptibility to opportunistic

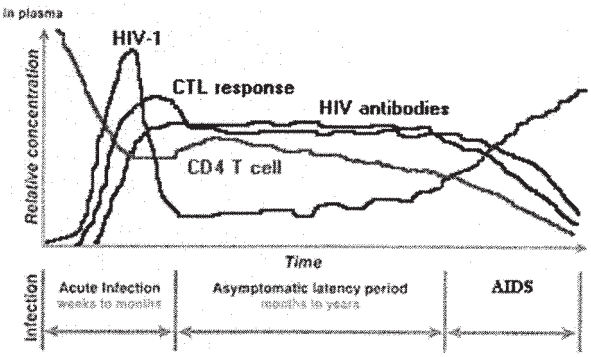


Figure 2. Typical course of HIV-1 infection.

infections and malignancies after an average lag of nearly ten years from infection with the virus.[47]

The HIV-1 infection course is typically characterized by three distinct phases[51,84]: primary infection, asymptomatic phase and AIDS (Figure 2). When HIV-1 enters a new host, there is typically a burst of viremia.[100] This event, known as primary (or acute) infection,[84] is generally annunciated by flu-like symptoms,[84–85] rash, diarrhea, among other symptoms,[101] during which plasma RNA levels are vertiginously incremented, approaching $2 \cdot 10^7$ copies/ml,[20,74] and the $CD4^+$ T-cell count abruptly reduced from its normal threshold of 1,000 cells/mm^3 in healthy individuals.[5,47] This is in turn followed by an accentuated reduction in the plasma viral load (from 10 to 200 times[20]) that is comparable to that provided by potent antiretroviral chemotherapy.[34] The viral load after seroconversion (appearance of antibodies to HIV-1 in the blood) has been mentioned as predictive of the long-term clinical outcome.[74,100,102] Despite impressive vigorous suppression, the immune system fails in eliminating the virus after acute infection[103] for reasons that are not fully understood.[104] The viral load then stabilizes in a pseudo-stationary condition, namely *set-point*[74,100] or *plateau concentration,*[14,51,76,104,105] which is generally maintained for years. Efforts directed to the understanding of the mechanisms responsible for this suppression, whose virologic and/or immunologic parameters may constitute an important predictor of the disease progression rate,[42,100,102,106] had provided several theories[105] which are intended to model the natural history of the HIV-1 primary infection.[20,42,51] After this stage, there is a prolonged period of clinical latency, namely *asymptomatic phase* or *chronic infection.*[4,6,84,85,101] During this period there is little, if any, detectable viremia, the number of infected cells in plasma is much reduced and it is difficult to demonstrate virus expression in these cells.[107] However, despite the slow progression of the disease that was responsible by the disseminated erroneous idea about the existence of true clinical latency,[40] this phase of the HIV-1 infection is characterized by a rapid turnover of virions and $CD4^+$ T-lymphocytes.[33–35]

As disease progresses, important changes in the distribution pattern of HIV-1 in the lymph nodes occur.[107] During the intermediate stage, within a given lymph node, virus is trapped in germinal centers but not in others. In this stage, HIV-1 accumulates in the lymphoid organs and replicates actively[95,99,103,108] despite a low viral burden and low-to-absent viral replication in peripheral blood mononuclear cells (PBMC).[107]

Therefore, there is truly no microbiological state of clinical latency during the course of HIV-1 infection, and the PBMC does not accurately reflect the actual state of HIV-1 disease, particularly early in the clinical course of HIV-1 pathogenesis.[99] In the late-stage disease, the architecture of the lymph node is disrupted and most of the germinal centers are involuted concomitantly with a loss of virus-trapping capability of the node. Degeneration and death of follicular dendritic cells (FDC) is generally associated with rapid disease progression,[107] since a high viral load is released to other anatomic compartments,[85] triggering decline of the PBMC $CD4^+$ T-cell count to values below 200 cells/mm^3 when the patient is clinically classified as having AIDS. For most AIDS patients, death is then caused by overwhelming infection by secondary pathogens that cause latent persistent infections (for example, cytomegalovirus, *Pneumocystis carinii* pneumonia, tuberculosis and toxoplasmosis), or that are easily cleared in immunocompetent individuals (for example, *Candida sp, Crypotosporidium sp* and *Isospora belli*). While some researchers argue that HIV-1 may not be the cause of AIDS,[109] the virus has been detected in all AIDS patients.[43]

Basic anatomic model

Some progress in the quantitative understanding of the HIV-1 dynamics has been made on the basis of relatively simple mathematical models that consider the body as a one-compartment system.[33,34,46,38,42,45,110] Despite their attractiveness due to the experimental and/or mathematical standpoints, the underlying simplifications neglect important viral/proviral concentration gradients, as well as peculiar kinetics linked to the infection and chemotherapy dynamics throughout the host.

The lymphoid tissue concentrates 98% of overall lymphocyte pool,[5] and has been shown to be the major site of viral replication,[99] whereas the central nervous system (CNS) has been cited as a sanctuary compartment, since the movement of immune cells, as well as drug chemotherapy flow is thought to be restricted through the blood-brain barrier.[47,85,111] Once inside the CNS, the main targets of HIV-1 are brain macrophages, or *microglia,* whose expression and regulation of CCR5 and CXCR4 coreceptors may, for mathematical modeling purposes, be approximated to the one observed for the PB monocyte-macrophage cell lineage for which a larger amount of information is available,[112] although differences have been documented.[111] Since NSI M-tropic (R5) HIV-1 strains predominate during the early stages of the infection, high CCR5 levels in microglia may explain the high infection rate of the CNS during HIV-1 primary infection and, partially, the elevated degree of virus genotypic homogeneity throughout disease evolution.[108]

The role of sanctuaries in the evolution of drug resistance is likely to be even more crucial for multidrug therapies, since many mutations typically offer resistance to the therapy as a whole.[113] In this case, the process of generating mutants in one compartment and selecting them in another, where drug concentration is large enough to provide mutants a selective advantage, may be repeated several times in the stepwise accumulation of resistant mutations.[113] In this sense, a more realistic model for HIV-1 dynamics might incorporate three connected compartments, namely the peripheral blood (PB) for clinical evaluation, the lymphoid tissue (LT), where most

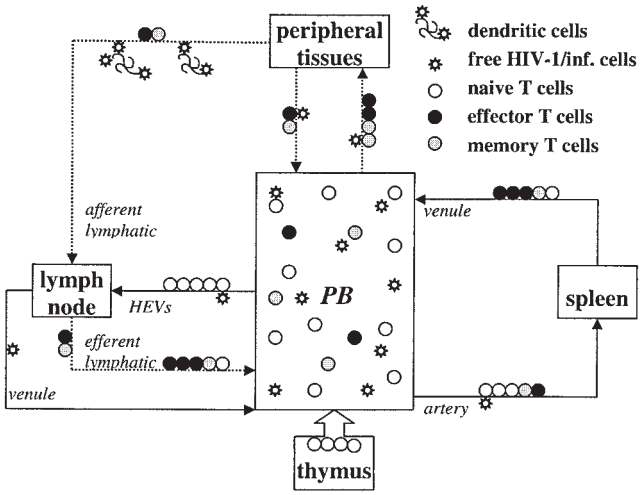


Figure 3. T-cell homing and recirculation scheme.

HIV-1 replication occurs,[107] and the central nervous system (CNS), where mutants may be generated.[113]

Similarly, early events in HIV-1 infection may be importantly related to lymphocyte homing and recirculation,[84] as in the model illustrated in Figure 3. Following selection and thymic education, T lymphocytes migrate to the periphery to populate lymphoid organs. These newly exported cells are considered to be immunologically naive and are preferentially recruited to the peripheral LT, where the encounter with a foreign antigen promotes triggering of the T-cell activation and differentiation into effector and memory T-cells (section entitled "Host cell entry of HIV-1"). On the other hand, effector and memory T-cells display a broad range of migratory capacity and are assumed to be released by the LT to the PB, and, thus, to other peripheral compartments.[84] Differently, after monocytes emerge into the PB from the bone marrow, they are assumed to migrate directly to any peripheral tissue, where cell maturation into macrophages occurs.[84]

The degeneration of the FDC network is associated with disease progression.[107] By assuming that the cytopathic action of HIV-1, as well as to the apoptotic (details are given in the section entitled "$CD4^+$ T-Cell Model") and cytotoxic processes triggered by its presence in the LT correspond to the driving forces of the FDC network disruption, one can admit that this process is accelerated (or reverted, since HAART-mediated regeneration of the FDC network has been documented,[107] according to the rate of viral load increment, as in Eq. 1

$$\frac{dI_{FDC}}{dt} = -k_V^{deg} I_{FDC} \frac{d(V^{LT} + \bar{V}^{LT})}{dt} \tag{1}$$

where $I_{FDC} = 1$ denotes full integrality of the FDC network, as typically observed early in the course of HIV-1 infection, and $I_{FDC} = 0$ denotes total collapse of the FDC architecture, failure of HIV-1 filtering the LT, extravasation of its viral load to the periphery and AIDS; k_V^{deg} is the degeneration rate of the FDC network caused by HIV-1 infection of the LT, and V^{LT}/$\bar{V}^{LT}$ represent the non-viable/viable virus mean concentration in the LT at time t, respectively.

Host cell entry of HIV-1

Epidemiological and experimental evidence indicates that the CD4 molecule and chemokine receptor levels affect the efficiency of viral entry,[62] and that this may have consequences for the pathogenesis of the HIV-1 disease.[114,115] Chemokine receptors are seven transmembrane spanning G protein-coupled receptors that mediate several functions on leukocyte subsets, particularly selective cell migration to inflammation sites, and to specific niches in lymphoid organs.[62] Chemokine signaling through these receptors is vital for the positioning of cells within tissues, and possibly also for integrin activation during the multistep process of leukocyte extravasation.[116] Chemokine receptors are expressed differentially on leukocyte subsets,[112] which account for chemotactic patterns *in vitro,* and are divided into two main classes[21,117–123]: C-X-C (α) chemokines that are primarily active on neutrophils, and C-C (β) chemokines that act on several leukocyte populations (monocytes, basophils, eosinophils and natural killer cells).[124] Host cell entry of HIV-1 and other primate lentiviruses is mediated by sequential interactions that induce the formation of a trimolecular complex between viral envelope glycoproteins (gp120 and gp41), the CD4 receptor and a coreceptor.[84,85,123] After attachment of a viable virion on the cell surface, conversion of a newly infected cell into a productively infected cell is a multistep process[41,84] that requires viral entry into the host cell, reverse transcription of viral RNA into DNA, transport of the newly synthesized DNA into the nucleus, integration of the viral DNA into the chromosome, production of viral RNA and protein, and the creation of new viruses from these newly made RNA molecules and proteins (Figure 4).

The identification of CCR5 and CXCR4 as the major coreceptors for the HIV-1 infection[122,123] has provided better understanding of viral tropism, disease pathogenesis and disease susceptibility.[62,112,117,125] Recognition of the aforementioned patterns of HIV-1 evolution on the basis of quantitative knowledge of CD4, CCR5, and CXCR4 dynamic levels on T lymphocyte subsets[21,62,120,122,126–129] and monocyte-macrophage cell lineage[112,124,130–132] in terms of mathematical modeling is clearly an important element to be considered in the formulation of methodologies for HIV-1 drug chemotherapy.[62,133] Towards this end, Joly and Pinto[134] proposed a mathematical model for the regulation and expression of the principal coreceptors for HIV-1 entry into T cells, and cells of the monocyte-macrophage lineage. The main results, which are outlined in the next subsection, provide insight into the types of cells that are more susceptible to infection by distinct HIV-1 strains, and an explanation of the discrepancies in the literature regarding

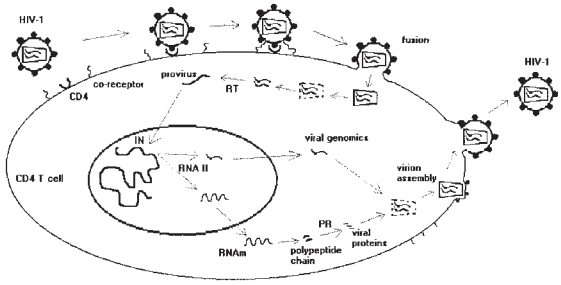


Figure 4. HIV-1 intracellular cycle life.

receptor expression in cultured cell lines. Recognition of this pattern may help to explain previous conflicting data on the relationship between viral evolution and disease progression,[18,19,22–24,26] and may provide a useful framework for evaluating immune damage and recovery in untreated and treated HIV-1 infections, since the degree and rapidity of immune reconstitution that occurs under HAART may differ according to the phase at which it is initiated.[2,33] The designations of R5 and X4 for M- and T-tropic *env* and R5X4 for dual-tropic isolates will be used thereafter in this paper and reflect this functional relationship.[121,135]

Quantifying the HIV-1 infectivity

Infectivity of R5 HIV-1 Viruses. It has been observed that the CCR5 concentration on the cell surface affects the efficiency of R5 HIV-1 infections.[62,114,136] Actually, as demonstrated by Platt et al.,[114] the infection mechanism is more complex, and also depends on the CD4 expression level on the cell surface. In particular, for low CCR5 concentrations the R5 HIV-1 infectivity becomes highly dependent on CD4 expression. On the other hand, if the CCR5 concentration is above a limiting threshold, R5 strains can efficiently infect cells, regardless of CD4 levels. As a result, since the efficiency of this pathway depends on CCR5 and CD4 concentrations, but not on the precise amounts of either component, it may be assumed that it behaves as expected for a mass action process. Extending the work of Platt et al.,[114] Lee et al.[115] demonstrated that the efficiency of the virus-cell fusion is influenced not only by the presence of CD4 and an appropriate coreceptor, but also by the expression level of the other coreceptors expressed on the same target cell, suggesting the existence of competition among coreceptors for molecular association with CD4, which presumably may contribute to cell susceptibility to HIV-1 infection.[115] According to this mechanism, and especially when CD4 expression is limiting, the presence of both CCR5 and CXCR4 (to restrict the discussion to the major HIV-1 coreceptors) on the cell surface implies in less successful R5 (X4) HIV-1 infection events than the case in which only CCR5 (CXCR4) is present at the same concentration.[115] Since most of the HIV-1 target cells express multiple coreceptors for HIV-1, limiting amount of CD4 surface expression and, principally, differential expression and regulation of these coreceptors on activating cells, this may become an important issue to be considered.

Dynamics of Cell Susceptibility to HIV-1 Infection. HIV-1 infection is not dormant at any time and many cells are continuously infected, leading to a chronic immunological stimulus that spans several years. Hence, the evaluation of the dynamic behavior of cell susceptibility to HIV-1 during cell activation may be of singular importance in defining strategies against AIDS. In fact, the discovery that chemokine receptors act as HIV-1 coreceptors has been a major advance in delineating tropism, and the role of these receptors in HIV-1 cell entry and replication. In this sense, Joly and Pinto[134] proposed a mathematical modeling approach that quantifies the potential susceptibility degree of a target cell to HIV-1 infection after a collision between a viable viral envelope and the target cell, or equivalently, the *HIV-1 infectivity* as function of the superficial characterization of the cell in terms of CD4 marker, CCR5 and CXCR4 coreceptor expressions (see Appendix A). Furthermore, the chemokines RANTES, MIP-1α and MIP-1β have been characterized as $CD8^+$ T cell-derived soluble inhibitors of R5 variants of HIV-1.[63,73] Here, the antiviral effect is assumed simply to result from the competition between virus and ligand for association with CCR5 that addresses the role of autologous $CD8^+$ T-cell in inhibition of R5 viral infectivity. We next proposed a modeling approach that addresses this issue, which has been contemplated in an integrated way with levels of CC chemokine production by the $CD8^+$ T-cell pool. Although soluble factor-mediated inhibition of T-tropic HIV-1 strains has been also documented at post-entry level,[63,73] the responsible suppressive factors remain unidentified and subject of future research.

Coreceptor specificity

While the NSI $\rightarrow$ SI phenotypic evolution throughout the disease progression is well documented,[22–24,26,87,108,126,137–139] only in recent years comprehensive works have addressed the relationships among R5, R5X4 and X4 HIV-1 strains.[24,26] Here, the following questions are in the core of the discussion: (A) although viruses originated in the late stages are characterized by the ability of using both CCR5 and CXCR4 coreceptors, does this fact reflect the prevalence of R5X4 or R5 + X4 strains? B) similarly, while the presence of SI R5X4 viruses at the final stages is well documented,[26] their position within the viral phenotypic evolution is not clear; does it represent the last stage of the viral evolutionary change indeed corresponding to the fittest viruses,[26] or a simple intermediate during the R5 $\rightarrow$ X4 transition,[23] which may be not completed due to lack of available time (that is, the death of the HIV-1 infected individual would occur first)?

Although the relevant genotypic similarity among *env* genes from several variants does not allow a formal analysis of the relationships among R5, R5X4 and X4 strains from the temporal viewpoint,[26] one can conclude that[26]:

- R5 strains persist to the emergence of CXCR4 using viruses[25,108,121];
- R5X4 viruses arise from the R5 lineage and,
- according to Hu et al.[24] X4 viruses can ultimately emerge from the R5X4 lineage, indeed indicating the intermediate condition of this phenotype.

Therefore, the phenotypic evolution of the envelope specificity may, in principle, follow a unidirectional model that involves necessarily two transition stages, as in M1

$$R5 \overset{I}{\rightarrow} R5X4 \overset{II}{\rightarrow} X4 \qquad \text{(M1)}$$

However, model M1 may be unrealistic because it implies an irreversible increase in the X4 phenotype without verifying the typical peak in SI population.[22]

The CXCR4-using population evolution after initial infection can be estimated by neglecting potential influences of the NSI R5 isolates on the SI (R5X4 and X4) strain population evolution (and vice-versa), as Darwinian competition among distinct HIV-1 phenotypes, as well as the time window between the initial infection and seroconversion. Let t_{sc} be the time after seroconversion (t_{sc} in years). From Shankarappa et al.,[22] the emergence and the peak of representation of SI strains are typically detected at approximately ($t_{sc} - 0.3$) and ($t_{sc} +$

1.2), respectively. By assuming (a) linear rate for the increase of SI population between the initial infection and the peak of SI representation, when the SI population corresponds to $\Omega\%$ ($\Omega \in \Re | 80\% < \Omega < 100\%$) of the overall HIV-1 population in the host,[22] and (b) following the peak of SI representation, the SI phenotype is linearly reduced at a rate of $\psi\%$ by year ($\psi \in \Re | \psi \geq 0$). Hence, the fraction of SI population within the host, that is, $F_{SI}(t)$, $0 < F_{SI}(t) < 1$ with t in years after initial infection, may be estimated as in M2

$$F_{SI}(t) = 0 \qquad \text{if } t \leq (t_{sc} - 0.3)$$

$$\frac{dF_{SI}(t)}{dt} = \left(\frac{\Omega}{1.5}\right) \qquad \text{if } (t_{sc} - 0.3) < t \leq (t_{sc} + 1.2)$$

$$F_{SI}(t) = \Omega - \Psi[t - (t_{sc} + 1.2)] \qquad \text{if } (t_{sc} + 1.2) < t \leq \left(\frac{\Omega}{\Psi}\right) + (t_{sc} + 1.2)$$

$$F_{SI}(t) = 0 \qquad \text{if } t > \left(\frac{\Omega}{\Psi}\right) + (t_{sc} + 1.2) \tag{M2}$$

where $F_{SI}(t) = V_{R5X4}(t) + V_{X4}(t)$. On the other hand, regarding the presumed random nature associated to HIV-1 genomic mutation, a more realistic scenario would consider reversibility among phenotypes, as in (M3)

$$R5 \overset{I}{\leftrightarrow} R5X4 \overset{II}{\leftrightarrow} X4 \tag{M3}$$

The relaxation of M1 into M3 is associated with the following assumptions: (1) HIV-1 half-life ($t_{1/2}$) as well as viral intracellular half-life periods are negligible (that is, viral generation and death occur instantaneously), (2) all viral particles generate a single viable virion (that is, viral load at steady-state). Hence, it allows us to write M4

$$\frac{dV_{R5}(t)}{dt} = V_{R5}(t) \cdot \gamma_{R5\to R5} + V_{R5X4}(t) \cdot \gamma_{R5X4\to R5} - V_{R5}(t) \cdot \delta_{R5}$$

$$\frac{dV_{R5X4}(t)}{dt} = V_{R5}(t) \cdot \gamma_{R5\to R5X4} + V_{R5X4}(t) \cdot \gamma_{R5X4\to R5X4} + V_{X4}(t) \cdot \gamma_{X4\to R5X4} - V_{R5X4}(t) \cdot \delta_{R5X4}$$

$$\frac{dV_{X4}(t)}{dt} = V_{R5X4}(t) \cdot \gamma_{R5X4\to X4} + V_{X4}(t) \cdot \gamma_{X4\to X4} - V_{X4}(t) \cdot \delta_{X4} \tag{M4}$$

Here, $V_a(t)$ (a = R5, R5X4, X4) denotes the normalized fraction of population a at time t, $\gamma_{a\to b}$ $(a, b) \in E | E = \{$(R5, R5), (R5, R5X4), (R5X4, R5X4), (R5X4, X4), (X4, X4)$\}$ denotes the normalized fraction of generation of descendent strains b from a (mutation $a \to b$, if $a \neq b$). From assumption 1), we have unitary δ (viral death rate) and

$$\gamma_{R5\to R5} + \gamma_{R5\to R5X4} - \delta = 0$$

$$\gamma_{R5X4\to R5} + \gamma_{R5X4\to R5X4} + \gamma_{R5X4\to X4} - \delta = 0$$

$$\gamma_{X4\to R5X4} + \gamma_{X4\to X4} - \delta = 0$$

with the following bounds

$$V_a(t) \geq 0 \quad a = \{R5, R5X4, X4\}$$

$$0 \leq \gamma_{a\to b} \leq 1 \quad (a, b) \in E^R | E^R = \{(R5, R5), (R5, R5X4), (R5X4, R5), (R5X4, R5X4), (R5X4, X4), (X4, R5X4), (X4, X4)\}$$

and the following initial conditions: $V_{R5}(0) = 1$, $V_{R5X4}(0) = 0$ and $V_{X4}(0) = 0$ which, for the non-trivial case (that is, $\gamma_{R5\to R5X4} = 0$), is characterized by the steady-state conditions defined in Eq. (2).

$$V_{R5}^{ss} = \frac{\gamma_{R5X4\to R5} \cdot \gamma_{X4\to R5X4}}{\gamma_{R5\to R5X4} \cdot \gamma_{X4\to R5X4} + \gamma_{R5X4\to R5} \cdot \gamma_{X4\to R5X4} + \gamma_{R5X4\to X4} \cdot \gamma_{R5\to R5X4}}$$

$$V_{R5X4}^{ss} = \frac{\gamma_{R5\to R5X4} \cdot \gamma_{X4\to R5X4}}{\gamma_{R5\to R5X4} \cdot \gamma_{X4\to R5X4} + \gamma_{R5X4\to R5} \cdot \gamma_{X4\to R5X4} + \gamma_{R5X4\to X4} \cdot \gamma_{R5\to R5X4}}$$

$$V_{X4}^{ss} = \frac{\gamma_{R5\to R5X4} \cdot \gamma_{R5X4\to X4}}{\gamma_{R5\to R5X4} \cdot \gamma_{X4\to R5X4} + \gamma_{R5X4\to R5} \cdot \gamma_{X4\to R5X4} + \gamma_{R5X4\to X4} \cdot \gamma_{R5\to R5X4}} \tag{2}$$

The relaxation of M1 into M3 allied to the reformulation of $\gamma_{a\to b}$, $(a, b) \in E^R$ into a time-dependent function $\gamma_{a\to b}(t)$, $(a, b) \in E^R t \geq 0$, in M4 allows the derivation of a model that is able to predict a peak of SI population. Hence, dynamic mutation rates in M4 result in

$$\frac{dF_{SI}(t)}{dt} = V_{R5}(t) \cdot \gamma_{R5\to R5X4}(t) - V_{R5X4}(t) \cdot \gamma_{R5X4\to R5}(t) \tag{3}$$

Clearly, since $\gamma_{R5\to R5X4}(0) > 0$, it yields: $dF_{SI}(0)/dt > 0$ because $V_{R5}(0) = 1$ and $V_{R5X4}(0) = V_{X4}(0) = 0$. Hence, $\exists t > 0 | F_{SI}(t) > 0$. Let $\gamma_{R5\to R5X4}(t)$ be a decreasing asymptotic function (asymptote $\gamma_{R5\to R5X4}^{\infty} = 0$) with image Im $\gamma_{R5\to R5X4}(t) \subset]0, 1]$, $\forall t$). Then, the function $\gamma_{R5\to R5}(t)$ is an increasing monotonic asymptotic function (asymptote $\gamma_{R5\to R5}^{\infty} = 1$) and Im $\gamma_{R5\to R5}(t) \subset [0, 1[$, $\forall t$. For these functions, (Eq. 2) becomes

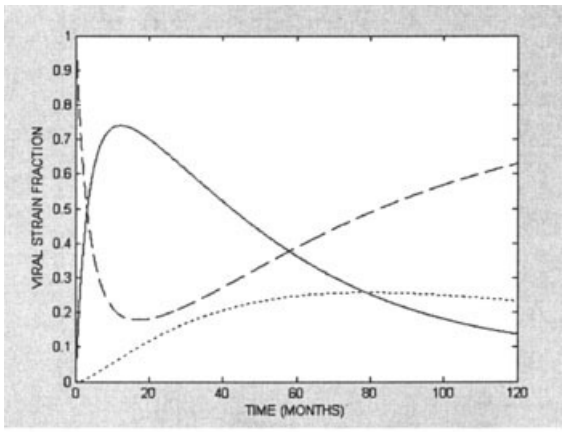
$$V_{R5}^{ss} = \frac{\gamma_{R5X4\to R5}^{\infty} \cdot \gamma_{X4\to R5X4}^{\infty}}{\underbrace{\gamma_{R5\to R5X4}^{\infty}}_{=0} \cdot \gamma_{X4\to R5X4}^{\infty} + \gamma_{R5X4\to R5}^{\infty} + \gamma_{X4\to R5X4}^{\infty} + \gamma_{R5X4\to X4}^{\infty} \cdot \underbrace{\gamma_{R5\to R5X4}^{\infty}}_{=0}} = 1$$

$$V^{ss}_{R5X4} = \frac{\overbrace{\gamma^{\infty}_{R5\to R5X4}}^{=0} \cdot \gamma^{\infty}_{X4\to R5X4}}{\gamma^{\infty}_{R5\to R5X4} \cdot \gamma^{\infty}_{X4\to R5X4} + \gamma^{\infty}_{R5X4\to R5} \cdot \gamma^{\infty}_{X4\to R5X4} + \gamma^{\infty}_{R5X4\to X4} \cdot \gamma_{R5\to R5X4}} = 0$$

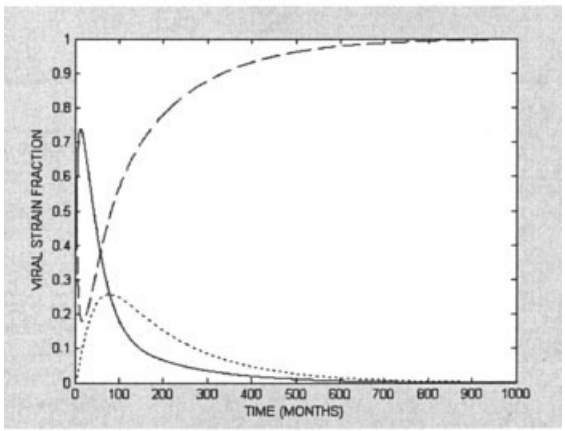
$$V^{ss}_{X4} = \frac{\overbrace{\gamma^{\infty}_{R5\to R5X4}}^{=0} \cdot \gamma^{\infty}_{R5X4\to X4}}{\gamma^{\infty}_{R5\to R5X4} \cdot \gamma^{\infty}_{X4\to R5X4} + \gamma^{\infty}_{R5X4\to R5} \cdot \gamma^{\infty}_{X4\to R5X4} + \gamma^{\infty}_{R5X4\to X4} \cdot \gamma^{\infty}_{R5\to R5X4}} = 0 \quad (4)$$

that is, at the stationary condition the system returns to its initial condition, that is, $F_{SI}(0) = F^{ss}_{SI} = 0$. In (Eq. 4), "$\infty$" denotes the asymptotic condition. Given that $\exists t > 0 | F_{SI}(t) > 0$, the occurrence of (at least) one peak for the SI population is guaranteed. Note that the aforementioned relaxation $\gamma_{a\to b}$, $(a, b) \in E^R = \gamma_{a\to b}(t)$, $(a, b) \in E^R$ may be restricted to generation of dynamic functions $\gamma_{a\to b}(t)$, $(a, b) \in E^R | a =$ R5, since the results presented in (Eq. 4) do not depend upon mutation rates $\gamma_{a\to b}(t)$, $(a, b) \in E^R | a =$ R5X4, X4.

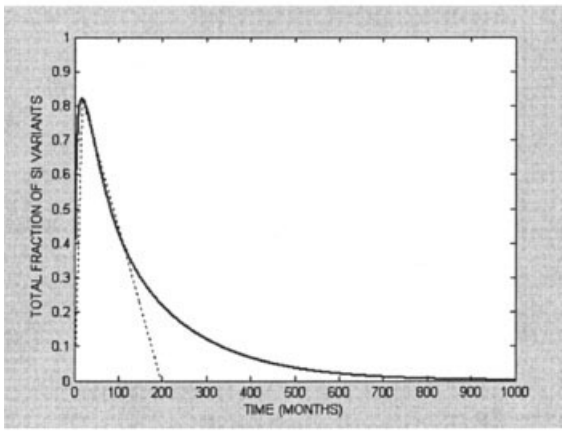
Finally, Figure 5 shows some results provided by enforcing $\gamma_{a\to b}$, $(a, b) \in E^R = \gamma_{a\to b}(t)$, $(a, b) \in E^R$. The agreement between these results and the predominance of R5X4 isolates typically observed in the late stages of the infection[123] should be pointed out.



(a)



(b)



(c)

CD4$^+$ T-cell model

Susceptibility to HIV-1 infection has been mentioned as a cause of $CD4^+CD8^+$ thymocyte depletion in the thymus,[140] which has been exclusively associated with the presence of SI-isolates.[96] Phenotypically, double positive thymocytes disappear first, followed by the reduction of the matured $CD4^+CD8^-$ thymocyte population. Even (future pre-CTLs) $CD4^-CD8^+$ matured thymocytes are also lost as a (probable) consequence of the rarefaction of their $CD4^+CD8^+$ precursors. As a first estimate, the reduction amplitude of the double-positive population may be directly correlated with the extension of the thymic infection by free HIV-1, since potent antiretroviral schemes have been mentioned to allow thymopoiesis reestablishment.[140] In particular, one can model the referred population contraction as due to the reduction of the precursor cell generation by the thymus.[141] The HIV-1 infection has also been shown to induce reversion in the ratio of CD4:CD8 mono-positive thymocyte production.[140] A simpler model may assume that the intensity of the reversion is proportional to the HIV-1 thymic infection and that it may be reverted when HAART is in course.[140] By assuming that the proportion of $CD4^+$ T-lymphocytes (future helper T cells), and $CD8^+$ T-lymphocytes (pre-CTLs) that leave the thymus follows, respectively, the proportion of CD4 and CD8 monopositive thymocytes that live in the thymus, one can assume the ratio CD4:CD8 as 2:1 for healthy individuals, as determined by flow cytometry.[84] Once extravasated to the peripheral blood compartment, these cells are subject to activation events that render them to perform their immunologic functions.

Activation of naive $CD4^+$ T-cells requires two signals, which correspond to multiple engagements between cellular receptors and class II major histocompatibility complex/pep-

Figure 5. Dynamic population profiles for HIV-1 isolates.

(a–b) Profiles for R5 (dashed), R5X4 (line) and X4 (dotted) after the emergence of the SI phenotype in the host according to model M4, modified for dynamic mutation rates. Here, the simulation results were generated for two distinct time horizons by assuming $\gamma_{R5\to R5}(t) = 1 - 1.07^{t-20}$; $\gamma_{R5\to R5X4}(t) = 1.07^{t-20}$; $\gamma_{R5X4\to R5}(t) = 0.02$; $\gamma_{R5X4\to R5X4}(t) = 0.97$; $\gamma_{R5X4\to X4}(t) = 0.01$; $\gamma_{X4\to R5X4}(t) = 0.01$; $\gamma_{X4\to X4}(t) = 0.99$. (c) Evolution of the SI population for a hypothetical horizon of 1000 months after its emergence in the host. Line: SI population simulated profile that corresponds to a); Dotted line: hypothetical profile generated by simulation of M2 for $\Omega = 82.10\%$ and $\Psi = 0.46\%$. Here, the peak in SI population (18 months after SI emergence in the host) agrees with the simulated profiles presented in *a-b*.

tide complexes (signal I) and between the first and B7 molecules (signal II) expressed on the surface of APCs.[84] Among the first detectable cellular responses after antigen recognition is the IL-2 secretion, an autoctone factor for enhanced stimulation of naive $CD4^+$ T-cells.[84,85] Previous studies have demonstrated improved $CD4^+$ T-cell numbers and function following IL-2 treatment, posing the question whether IL-2 may enhance virus suppression, promoting immune control or even eradication.[47,85,142] In Stelbrink et al.[17] it was shown that IL-2 accelerates the normalization of $CD4^+$ T-cell counts, but does not impact on virus production or latency. In order to examine this question on the basis of computational analysis, IL-2 modeling may be also considered.

Once T-cells are activated, they undergo major phenotypic and functional changes as they proliferate, ultimately resulting in cell differentiation.[62,112] Previous models for $CD4^+$ T-cell differentiation for uninfected individuals fall in two major classes,[69] which constitute the basis for derivation of more sophisticated models.[68,69] The first class is composed by linear models, which neglect the heterogeneity of the $CD4^+$ T-cell population, by assuming that memory $CD4^+$ T-cells emerge from reversion of effector $CD4^+$ T-cells to the quiescent state. Divergent models fall into a second class. Here, memory and effector $CD4^+$ T-cells are generated directly (and exclusively) from the clonal expansion of antigen-stimulated naive $CD4^+$ T-cells.[69,84] In this case, the mechanisms that determine whether a cell differentiates into memory or effector phenotype remains unknown.

However, the hypothesis that a memory cell derives from an effector one, as proposed by linear models, cannot be discarded, as in divergent models.[69] Moreover, it has been reported that memory $CD4^+$ T-cells upon specific stimulation can proliferate and differentiate into effector cells. In this sense, differences in the rate of reactivation of memory cells may occur because the HIV-1 specific clone is not patient-specific, or because the virus has mutated since memory cell production, or even because the virus directly inhibits reactivation.[42]

$CD4^+$ T-lymphocytes respond to the antigen presence through cellular activation and proliferation. However, if this proliferative response is not regulated, each encounter with a foreign antigen would lead to unending $CD4^+$ T-cell expansion. Down-regulation of $CD4^+$ T-cell proliferation occurs by a highly regulated and coordinated cellular death process that is known as apoptosis[143] that is essential for cellular homeostasis.[84]

Major apoptotic mechanisms proposed for the uninfected state are as follows[84]: (a) activation induced cell death (AICD), which occurs only in cells that have been previously activated due to development of apoptosis susceptibility that is induced by the Fas-FasL system; (b) programmed (or passive) cell death that results from the survival stimuli privation and (c) suppression of the IL-2 stimuli when elevated $CD4^+$ T-cell concentration levels are present.

Besides the chronic immunologic stimulus in HIV-1 patients, HIV-1 infection may be unique in its ability to induce lymphocyte apoptosis through a vast repertory of other mechanisms.[143] Moreover, since a minor fraction of the $CD4^+$ T-cell pool is infected by HIV-1, enhanced apoptosis of $CD4^+$ T-lymphocytes results from mechanisms other than direct infection. Indeed, HIV-1-encoded proteins have been shown to induce apoptosis of infected, as well as of uninfected cells. In short, proposed mechanisms of HIV-1-associated lymphocyte apoptosis involve[143]: (a) gp120-induced apoptosis. Gp120 is an HIV-1 viral envelope glycoprotein that can bind and cross-link the CD4 receptor and coreceptors. Crosslinking of $CD4^+$ T-cells by gp120 causes enhanced susceptibility to Fas-mediated killing (from viable and replication-incompetent HIV-1, as well as circulating immune complexes); (b) Tat induced apoptosis[144]; its clinical relevance comes from observations that Tat is readily secreted by infected cells; (c) AICD, which is now aggravated by the chronic immune stimulation of the host, and (d) autologous infected cell-mediated killing. It appears to be selective to uninfected cells and may involve the Fas-FasL system. Here, the mediating cells are assumed to be infected macrophages. Figure 6 summarizes the activation, proliferation, differentiation and death/apoptosis model for $CD4^+$ T-cells.

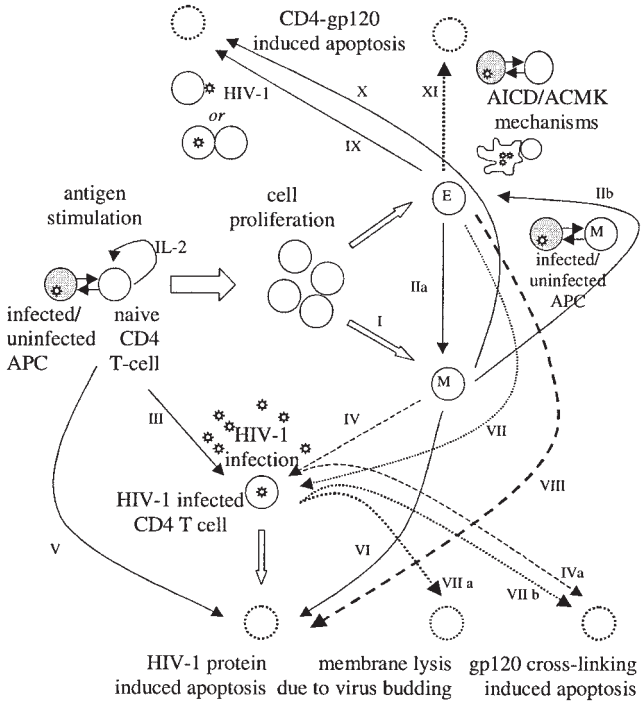


Figure 6. $CD4^+$ T-cell activation, proliferation, differentiation and death/apoptosis model.

E = effector $CD4^+$ T-cell; **M** = memory $CD4^+$ T-cell.

There is an expressive amount of literature that addresses mathematical models of HIV-1 pathogenesis and immunology in which the $CD4^+$ T-cell population is discretized into uninfected and infected clones.[1,3,39,47,49,51] However, more comprehensive approaches may be based on an extended cell categorization regarding distinct cellular activation states, namely naive, effector and memory phenotypes under three cellular infection states: *uninfected, eclipse phase* (intracellular viral life cycle for the first cell infection event), which evolves to the *productive infection* state.

$CD4^+$ T-cell infection model

Although $CD4^+$ T-cells have been identified as major HIV-1 targets[47] and replication habitats,[85,97,145] the monocyte-mac-

rophage cell-lineage,[66,132,146,147] immature and matured dendritic cells,[22,146,148] brain macrophages[85,111,145,146] or even $CD4^-$ cells[145] have also been shown to undergo HIV-1 infection. However, the vast majority of mathematical developments have considered only $CD4^+$ T-lymphocytes.[1–3,30,37,38,42,49,50,52,75,149–151] This simplification allows:

(1) the determination of fundamental estimates on the viral replication kinetics *in vivo*[34,35];

(2) the obtainment of analytical laws of the infection process[47] and,

(3) a thorough stability analysis.[43,47]

Nevertheless, more sophisticated models are expected to be more robust and to allow the mathematical evaluation of the dynamic behavior of the infection process through sensitivity analysis that is embedded into a more realistic scenario. In this sense, a natural extension of basic models should comprise $CD8^+$ T-cells as a component of an immune response model (section entitled "Early HIV-1 dynamics modeling") and long-lived cell populations, in particular cells of the monocyte-macrophage lineage, which are susceptible to chronic HIV-1 infection since macrophages can resist to subsequent activation events,[105] differently from $CD4^+$ T-cells.

Activated and memory $CD4^+$ T-cells are susceptible to infection at post-integration level with HIV-1. One determining factor of such infection is the concentration of infectious particles in the neighborhood of a target cell.[66] This HIV-1 could emerge from two main sources.[105] Firstly, it could be produced at a distance by T-cells recently infected. This long-range transmission may well be dominant when viral burdens are high; a continuous and expressive concentration of HIV-1 should exist in the extracellular medium to promote a pseudo-steady-state between $CD4^+$ T-cell infection and death rates, in order to sustain the infection process. Alternatively, the infectious agent may emerge locally, coupled with an immunologic interaction with an APC that results in T-cell activation. Because resting T-cells are reported not to support productive HIV-1 replication,[145] it is possible that contact with infected macrophages, even of autologous origin, may produce sufficient activation stimuli for recipient T lymphocytes to support HIV-1 replication.[66] This proximal activation and transmission form may be particularly important when viral burdens are low. Current models of HIV-1 pathogenesis *in vivo* are primarily based on measurements of cell-free virus loads in the patients' plasma.[33,34,110] These models attribute less than 1% of cell-free virus as derived from infected macrophages and related long-lived cells.[66] However, due to their location and antigen presenting cell function, macrophages may be of central importance for cell-to-cell transmission of virus, especially in the LT compartment that is the major site of HIV-1 replication *in vivo*.[99] The predominance of M-tropic strains of HIV-1 early in infection *in vivo* also suggests that cells of the macrophage lineage may play a major role in the initiation and early spread of the HIV-1 infection. Furthermore, while chronically and latently infected cells may not contribute significantly to the viral load, they may be instrumental in sustaining the infection.[105,142] In this sense, two models may be developed in order to consider virus-to-cell (long-range transmission), cell-to-cell (proximal activation and transmission) and mitotic infection routes (Figure 7).

According to Figure 7, after maturation in the thymus, naïve $CD4^+$ T-cells are susceptible to infection.[145] However, there is

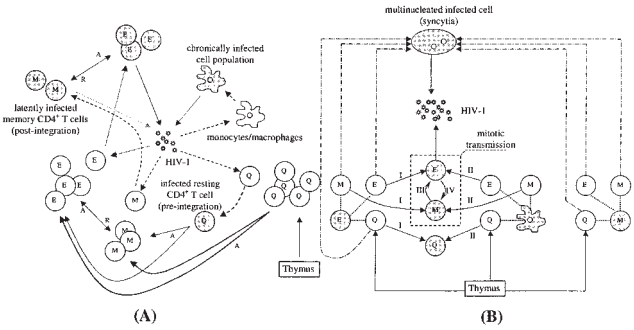


Figure 7. HIV-1 infection models. (A) virus-cell route; (B) mitotic and cell-cell routes.

E = effector $CD4^+$ T-cell; **M** = memory $CD4^+$ T-cell; **Q** = quiescent/naive $CD4^+$ T-cell; unfilled symbols denote uninfected cell; filled symbols denote HIV-1 infected cell.

no provirus formation and, even when these cells are activated, productive infection is not considered. Hence, we assume that activation of infected naïve $CD4^+$ T-cells produces uninfected effector and memory $CD4^+$ T-cells. Conversely, as proposed by Essunger and Perelson,[31] infection of memory $CD4^+$ T-cells enables incorporation of viral DNA to cell genome and, consequently, viral replication, albeit in a lower level than that observed for productive infection of activated $CD4^+$ T-cells. Furthermore, these infected cells can revert to the memory phenotype after a single cell division,[38] resulting in the increment of the latent reservoir through mitotic transmission. Mitotic and cell-to-cell infection models are illustrated in Figure 7. Here, cell-to-cell transmission (dotted line) is assumed to occur from productively (left), and latently (right) infected $CD4^+$ T-cells, as well as from chronically infected macrophages (center), and has susceptible $CD4^+$ T-cells as target. Regarding the viral homogeneity in R5 strains during the acute phase, cell-cell fusion and syncytia formation among $CD4^+$ T-cells is not significant (dashed-dotted line); only infection through polarized secretion of viral envelopes (path 1), as well as through intracellular viral stock transfer (path 2) are taken into account. Mitotic transmission is assumed to be restricted to the productively infected and latent $CD4^+$ T-cell populations (dashed line). Path 3 indicates clonal expansion of the memory CD4 pool, whereas path 4 denotes reversion to memory phenotype after single mitosis of the effector $CD4^+$ T-cell.

Modeling $CD8^+$ T-cell dynamics

The potent viral suppression typically observed after the peak of HIV-1 viremia is classically modeled in terms of host immune response,[40,138,152] as well as a direct consequence of the initial abrupt decline on $CD4^+$ T-cell count.[149,153] Down-regulation of the initial burst of viremia during primary HIV-1 infection is thought to be mediated predominantly by HIV-1–specific cytotoxic T lymphocytes,[20] since there is a well-established temporal correlation between the development of anti-HIV-1 CTL response and the decline in the initial viremia. As in Price et al.,[20] we assume that if CTLs are important in limiting viral replication, then this effect should be evident during this period of intense CTL activity on a large viral population. Clearly, the development of a lasting CTL response depends on host and viral parameters, as well as on initial

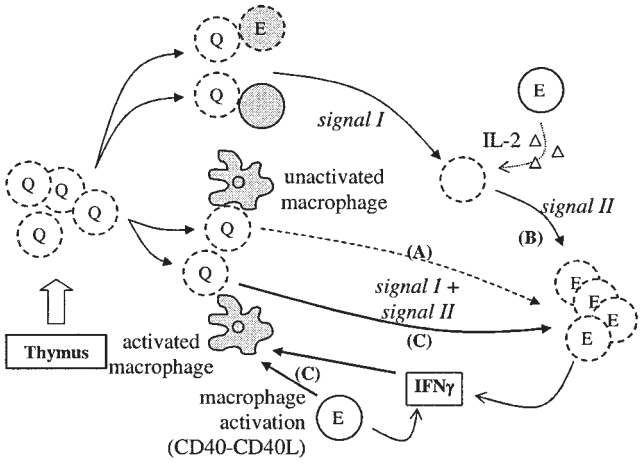


Figure 8. Activation, proliferation and differentiation model for CD8$^+$ T-cells.

E = effector T-cell; **Q** = quiescent/naive T-cell; CD8$^+$ T-cells represented with dashed circles; CD4$^+$ T-cells represented by continuous circles; unfilled symbols denote uninfected cell; filled symbols denote HIV-1 infected cell.

conditions. More specifically, the dynamics of HIV-1 and CTL depend on the balance between the rate of viral replication and the quality of the CTL response, mainly characterized by the CTL activation rate and the CTL death rate that are reflected in the viral load attained during primary infection.[4]

Typically, immune response is stronger when antigen levels are high since it takes time to develop.[51] In fact, results show that CTL activity is not substantial until after the peak viremia is reached due to the delayed dynamics related to the activation and proliferation processes. Presented results also support, but do not prove, that the end of the initial viremia is due to the synergy between target-cell limitation,[51,149] and the development of a strong CTL response.[63] However, while CTLs may shorten the lives of productively infected cells, these could be poor CTL targets since the HIV-1 cytopathic effects naturally shorten their lives.

After maturation in the thymus, naive CD8$^+$ T-cells, or *pre-CTLs,* emerge into circulation and require two signals for activation and differentiation into functional CTLs[84] (Figure 8). The first one depends on cell-cell contact and corresponds to recognition, by the pre-CTL, of viral peptide-MHC I complexes expressed on a nucleated infected cell. Signal 2 is provided by a professional APC through costimulation without (Figure 8; path A) or with uninfected CD4$^+$ T-cell help (system CD40-CD40L), and/or the latter in association with CTLs themselves (IFN-γ secretion) (Figure 8; path C), in which more efficient activation is obtained. Alternatively, it is assumed that signal 1 can be obtained from infected nucleated cells (other than macrophages) with expression of peptide-MHC I complexes. In this case, signal 2 consists of IL-2 secretion from uninfected helper CD4$^+$ T-cells,[84,154] whose activation can be stimulated by uninfected/infected APCs, if it is assumed that the ACMK mechanism is not totally effective at each cell-cell collision (Figure 8; path B). The ingestion of infected cells by professional APCs may be neglected for cell balance purposes, since these cells usually undergo apoptosis and therefore are already subtracted from infected cell populations.

Accumulated evidence indicates that there is a strong association between CTL activity and CD4$^+$ T-cell help (Figure 8; paths B and C).[70,101,154] Hence, the proposed model is flexible; it is neither exclusively dependent on stimuli from APCs, nor on CD4$^+$ T-cell help, as opposed to other approaches.[4,40,155] The proposed model represents combined physiological conditions *in vivo.* On the other hand, the memory CD8$^+$ T-cell pool is not considered here. This assumption is based on the following facts: (a) memory and effector CD8$^+$ T-cells are functionally similar[69] because both execute cytolytic functions; (b) in contrast to the memory CD4$^+$ T-cell pool, there is accumulated evidence that memory CD8$^+$ T-cells are not susceptible to infection,[156] and (c) CD8$^+$ T-cell provirus latent reservoirs originated from the reversion of effector CD8$^+$ T-cells are expected to be negligible in comparison with the CD4$^+$ T-cell pool, given the relatively less susceptibility of the effector CD8$^+$ T-cell pool to HIV-1 infection than that observed for CD4$^+$ T-cells.[156]

Major elimination pathways for CD8$^+$ T-cells are represented by apoptotic and infection mechanisms. Similar apoptotic routes proposed for CD4$^+$ T-cells have been recently reported for CD8$^+$ T-cells.[143] While excessive antigenic stimulation may result in the AICD mechanism,[143,157] the contraction of survival stimuli to insufficient levels may result in programmed (passive) cell death.[158] In addition, analogous mechanisms to ACMK for CD4$^+$ T-cells may also operate on uninfected CD8$^+$ T-cells.[143] In fact, although neither the Fas-FasL system nor crosslinking process have been observed for CD8$^+$ T-cells,[159] apoptotic routes that require cell-cell contact (macrophage/CD8$^+$ T-cell involving gp120/CXCR4 interactions[143]) or even pro proapoptotic soluble factors produced by macrophages were identified and demonstrated to act on CD8$^+$ (and CD4$^+$) T-cells,[159] thus, implying that ACMK mechanisms may still be independent of cell-cell contact.

The decrease in CD8$^+$ T-cell population due to CTL infection routes and up-regulation of CD4 after CD8$^+$ T-cell activation is documented.[65,118] According to Flamand et al.,[65] cell cultures stimulated with phytohemagglutinin (PHA) or *staphylococcal enterotoxin* (SEB) present similar behavior; double-positive cells are expressively detected around the 3rd day of culture, a peak of CD4 expression is verified next to day 6, after which there is a significant reduction and tendency to stabilization (Figure 9). This dynamic CD4 up-regulation may be approximated by Eq. 5, in which $t = 0$ corresponds to the initial time of cell stimulation. Since interindividual variability is expected, the proposed dynamics for the CD4 up-regulation on CTLs is clearly nonunique.

$$\tilde{\chi}_{CD4CD8} = \frac{\alpha_1}{\beta_1 + \delta e^{-(t^{\phi}-t_1)\Delta T_1}} - \frac{\alpha_2}{\beta_2 + \delta e^{-(t^{\phi}-t_2)\Delta T_2}} \quad t > 0 \quad (5)$$

Double positive cells would become susceptible to free HIV-1 infection,[65,143,156] and to cytopathic effects, as membrane lysis. Although CD4 up-regulation on CTLs has been expected to provide a larger CTL susceptibility to apoptotic processes commonly observed for CD4$^+$ T-cells, the occurrence of these mechanisms has not been properly evaluated and has not been observed *in vitro.*[159] Cell-to-cell transmission may be not considered, since it is assumed that after an immunologic interaction, the CTL remains intact.[85] In addition, it is assumed that infected CTLs are immunologically nonfunctional. Ultimately,

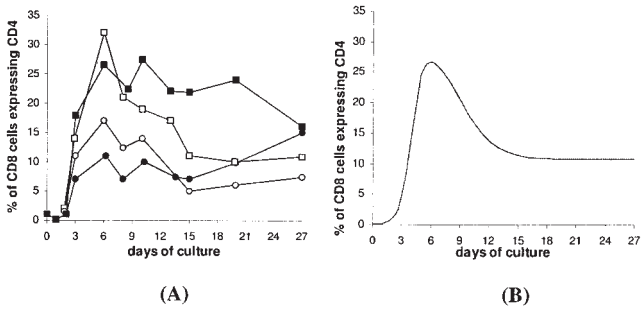


Figure 9. Dynamic model for CD4 up-regulation in CD8$^+$ T-cells under stimulation.

(A) Data from Flamand et al.[65] (B) Eq. 4 for $\alpha_1 = \alpha_2 = 8$, $\beta_1 = 0.26$, $\beta_2 = 0.40$, $\delta = 2$, $\theta = 1.4$, $\phi = 1$, $t_1 = 4$, $t_2 = 6$, $\Delta T_1 = 1.4$ and $\Delta T_2 = 1.8$.

infected CTLs are assumed to be susceptible to cytotoxic activity from uninfected (viable) CTLs[156] as observed in Figure 10.

Immune response model

The innate immune control model is presented in Figure 11. Peripheral blood monocytes are recruited to the LT compartment where innate immune reactions (stimulation by microbial products) provide the required stimuli for cellular maturation into macrophages, which are able to execute their immunologic functions as phagocytosis and APC. After antigen phagocytosis by an uninfected macrophage (thereafter, referred as APC), it activates and secretes IL-12 that promotes enhanced cytolytic activity of CTLs. In addition, IL-12 acts on antigen stimulated CD4$^+$ T-lymphocytes promoting their differentiation into helper 1 phenotype, whose typical function is to produce IFN-γ, a potent macrophage activator that is also secreted by

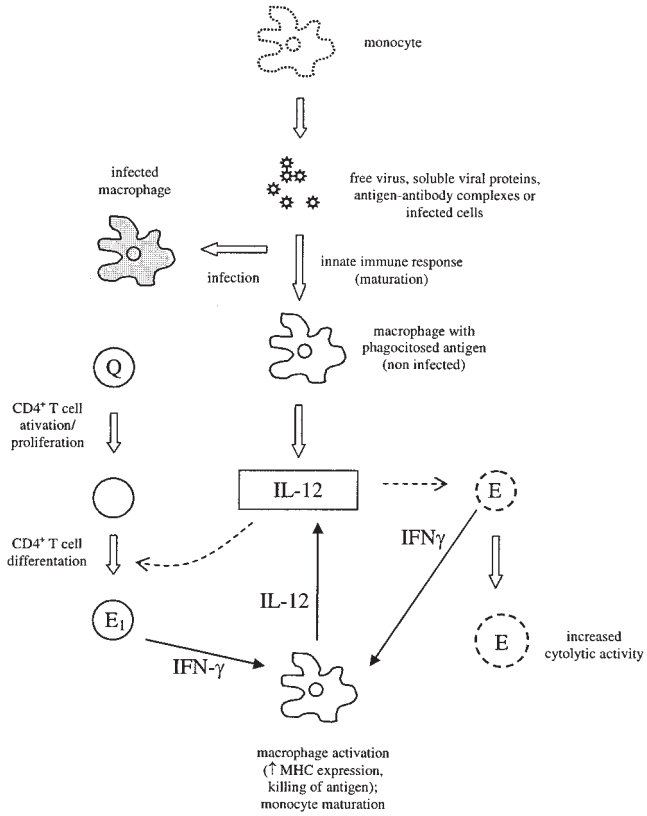


Figure 11. The innate immune response model.

E = effector T-cell (overall pool); **E**$_1$ = effector T-cell (helper 1 only); **Q** = quiescent/naive T-cell; CD8$^+$ T-cells represented with dashed circles; CD4$^+$ T-cells represented by continuous circles; white symbols denote uninfected cells; shaded symbols denote HIV-1 infected cells.

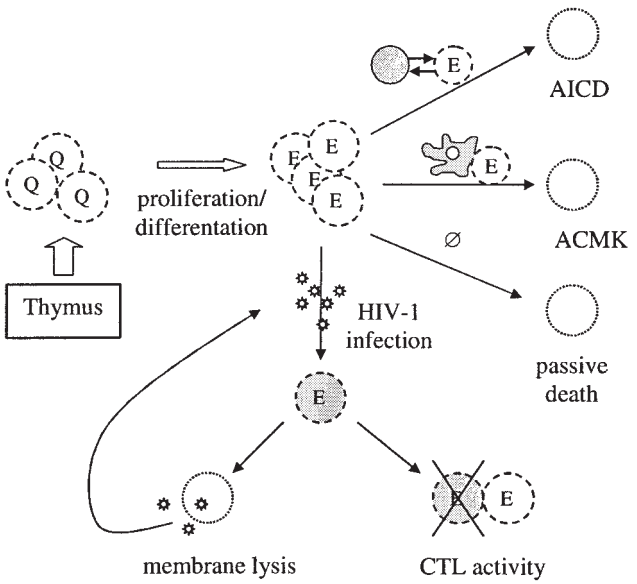


Figure 10. CD8$^+$ T-cell elimination model.

E = effector T-cell; **Q** = quiescent/naive T-cell; CD8$^+$ T-cells represented with dashed circles; CD4$^+$ T-cells represented by continuous circles; unfilled symbols denote uninfected cell; filled symbols denote HIV-1 infected cell.

activated CTLs in response to the IL-12 stimulation from macrophages. IFN-γ and CD40-CD40L interactions with (uninfected) helper CD4$^+$ T-cells induce conversion of monocytes into activated macrophages. In a simple model, effector CD8$^+$ T-cells may be considered as only having the CTL phenotype, whereas CD4$^+$ T-cells are exclusively helper.

The specific immune response model is based on cytolytic and noncytolytic mechanisms.[63–65,70,72,73,84] The cytolytic mechanism depends on the cell-cell contact between a cell that expresses peptide-MHC I complexes[160,161] on its surface (the target cell) and a viable (that is, uninfected) CTL. Moreover, this mechanism does not affect uninfected neighboring cells, does not cause injury to the CTL itself, is favored by IL-12 secretion from APCs, and induces target cell apoptosis.[84] Once in the differentiated state, it may be admitted that the CD8$^+$ T lymphocyte is effector, phenotypically a CTL, and is able to execute lysis of any infected cell that expresses the MHC-I-peptide complex on its surface.[84] Since the expression of the referred complex implies cell infection[162] by assuming that all nucleated cells possess HLA class I,[84] then all nucleated infected cells are subject to CTL activity. This process depends on the contact between the CTL and the infected cell,[63] and results in induced apoptosis of the target cell (before the peak of viral production by the infected target cell[64,72]). Furthermore, it does not affect neighboring cells that do not express

Table 1. HIV-1 Immune Control Model

Immune Control	Activity	Cell-Cell Contact	Mechanism	Target Cells	Stage	Affected Strain(s)
I	cytolytic	yes	apoptosis induction	$CD4^+$ T-cells, CTLs and monocytes/macrophages	Post-infection	all
II	non-cytolytic	no	secretion of CCR5 ligands	$CD4^+$ T-cells and monocytes/macrophages	Pre-infection	R5
III	non-cytolytic	no	unknown ("USF")	$CD4^+$ T-cells and monocytes/macrophages	Post-infection	all

the referred complex, does not cause injury to the CTL itself and is favored by IL-2 secretion from APCs.[84]

However, CTL responses are often lessened by viral escape mechanisms, such as mutation of CTL epitopes[160,163] and down-regulation of cell-surface major histocompatibility complex class I molecules by HIV-1.[20,152]

Recent studies have shown that the cytolytic mechanism, although probably predominant,[63] is not the only one that is responsible for HIV-1 immunologic containment.[63–65,70–73,156] Indeed, at least two other $CD8^+$ T-cell mediated antiviral activities have been described.[63,73,162] These mechanisms that have been neglected in mathematical models of HIV-1 pathogenesis and immunology, do not require cell-cell contact[63] and inhibit viral replication via soluble factors without death of the infected cell (Table 1). Although the possibility that these noncytolytic mechanisms might prevail in certain circumstances cannot be excluded,[70,73] experimental data suggest that their contribution is of the order of 1/1000 with respect to the cytotoxic activity.[63] Despite the inclusion of undifferentiated $CD8^+$ T-cells as potentially responsible by this antiviral noncytolytic activity,[73] a simple model may only regard CTLs.[72,73] Also, it may be assumed that noncytolytic activity is non HLA-restricted,[73] and triggered when peptide-MHC I complexes are recognized by CTLs.[63,154] Since the broad spectrum of secreted soluble factors are ligands to the CC chemokine family, as MIP-1α, MIP-1β and RANTES, the importance of their consideration for primary infection may be singular because of typical predominance of R5 strains during this period. This selective pressure mechanism may contribute to a continuous selection of X4 strains of HIV-1 throughout disease progression,[19] providing theoretical support for the typical evolution R5 $\rightarrow$ X4 from HIV-1 primary infection to AIDS (Section entitled "Coreceptor specificity"). Tomaras et al.[71] have shown that another efficient suppressing mechanism exists, is CTL mediated, does not depend on SDF-1 secretion, occurs after cellular infection, blocks viral intracellular cycle life at its final stage, and does not depend on the viral envelope such that it acts on both R5 and X4 (lymphocyte-tropic) HIV-1. A still unknown soluble factor (USF) has been suggested as responsible for this additional noncytolytic mechanism.[63] The cellular susceptibility to these aforementioned mechanisms is well documented on $CD4^+$ T-cells,[63,64,71,162] as well as on long-lived cell populations.[162] As pointed out by Chun et al.,[73] all of these CTL mediated suppressing mechanisms (Table 1) may have important implications for developing a therapeutic strategy.

State-of-the-Art in HIV/HIV-1 Modeling

To elucidate the complex dynamics associated with the immune system's delicate and intricate response to HIV-1 disease, mathematical models have proven to be an important tool. Extensive research on mathematical modeling of the immune system, and its interaction with HIV-1 can be traced back to over a decade ago.[1–3,29–33,35–52,164,165] Many of these works, especially those developed until 1995, have tended to explain the kinetics of $CD4^+$ T-cell depletion or viral dynamics, from which the paradigm of HIV-1 pathogenesis has conclusively changed from one of prolonged viral quiescence to one of astonishing viral replication.[33,34]

Particularly motivated by previous results on viral dynamics, and due to an increasing availability of treatment alternatives, this scenario has changed. Antiretroviral therapy has become the core of the discussion and optimal control of the drug chemotherapy is currently an active and challenging area of research.[1,45,48] On the other hand, while numerous models have been proposed to capture many of the features of progression to AIDS that follows the initial viral spike,[39,40,47] much less work specifically directed to the primary HIV-1 infection has been reported in the literature.[42,51] The lack of models is further aggravated if the objective is to address the initiation of antiretroviral therapy during this stage.[5] Despite the important progress related to mathematical modeling of the postacute HIV-1 infection, the vast majority of these developments, which in principle could serve as a basis for derivation of acute phase models, suffer from important limitations due to excessive model simplifications that cause them to be inadequate as fundamental biomedical process models for HIV-1 drug chemotherapy planning. While simplified models are computationally preferable to perform numerical simulation[51] and optimization,[2] and mathematically attractive to estimate viral dynamics,[33,34] or to perform stability analysis,[31] further modeling is required by the practical need of real-world applications, as the optimal management of the drug chemotherapy.

Mathematical models of HIV-1 pathogenesis and immunology may have an ordinary differential equation (ODE) system structure, which corresponds to the vast majority of the simulation, as well as simple optimization models, or a differential-algebraic equation (DAE) system structure, typically required in more sophisticated optimal control models intended to real-world applications, since the drug chemotherapy constraints must be properly posed as to obtain a mixed-integer nonlinear program (MINLP). In addition, models are predominantly deterministic rather than stochastic. Deterministic models differentiate from stochastic ones in the sense that their solutions are reproducible and independent upon random variations. On the other hand, stochastic modeling is useful not only for studying the trend and mean behavior of the process, but also for assessing the validity and usefulness of results from deterministic models.[44] In addition, it may be especially attractive in modeling the early stages of HIV-1 infection since at this time the numbers of infected T-cells and free HIV are usually very small. With respect to the modeling scope, strategic models, which constitute the vast majority of past and present devel-

opments, are intended to provide broad insights into the possible behaviors of the system based on simple assumptions, whereas tactical models are more complex representations that can be used for targeted predictions.[29]

Modeling techniques in AIDS pathogenesis

Interactions among immune system cells, as well as between these cells and free virions in the extra-cellular medium have capital importance on infection dynamics. The simplest and most reliable modeling strategy employed for the modeling of the aforementioned interactions is based on the mass action principle. Here, the rate of encounter between two identities (cells, viruses or cytokine molecules) is defined by the product of their densities multiplied by a (constant) proportionality parameter.[42,43,47]

According to this model, the virus-cell infection rate is a function of the probability of a physical encounter between a viable virion and a target cell. In particular, we have the bilinear term kVT,[45] where k denotes a constant parameter that may be posed as function of the coreceptor expression and regulation on the susceptible cell,[134] whereas V and T represent instantaneous concentrations of free HIV-1 and target cells, respectively. Since in general V and T are state variables and dynamic optimization is basically performed by converting the infinitesimal problem into an NLP formulation, some immediate implications of this formulation are as follows: (a) the model is nonlinear and nonconvex and (b) global optimal solutions are not guaranteed through conventional algorithms,[88,90] unless global optimization methods, deterministic or stochastic,[166] are applied.

In principle, the rate of encounter between two entities should saturate for high density values of at least one of them,[47,114] as in stages of HIV-1 infection characterized by high levels of viral load (that is, primary infection or clinical AIDS). In this sense, logistic functions, as density-dependent functions,[45,47] are often incorporated to the modeling not only to represent these interactions, but also to provide phenomenological support to biological mechanisms as thymic generation or cellular proliferation. For example, due to the cytopathic activity of HIV-1, there is an incremented cellular production by the thymus in order to maintain thymopoiesis. Therefore, a simple logistic generation term that represents the referred event could be

$$\lambda = \lambda_0\left(1 - \frac{T}{T^{max}}\right) \tag{6}$$

where λ is the effective thymic generation rate, whereas λ_0 denotes its upper bound, T is the overall T-cell count in the host at a given time after HIV-1 infection and T^{max} represents the normal level of the T-cell pool in healthy individuals.

Lastly, since the death probabilities of viruses or cells are unknown, this event is admitted to occur according to a (constant) rate d. In other words, it is assumed that the death probability of the entity at time t is given by an exponential distribution with half-life $1/d$.

General HIV/HIV-1 dynamic modeling

The deterministic mathematical modeling of the HIV dynamics focuses on two major cases. These are: (1) perturbation of the clinical pseudo-steady, and (2) representation of the overall progression of HIV disease from initial infection with HIV to full-blown AIDS, because models are more applicable to represent the changes in mean cell numbers when population sizes are large.[44,47]

In this sense, while primary developments on the interaction of HIV and a simplified immune system have been reported since the late 1980s,[164,165,167] distinct activation states for $CD4^+$ T-cell clones were only mathematically formalized in more recent work by McLean and collaborators.[29,30,32] In McLean and Kirkwood,[29] infection and destruction of activated helper T-cells by HIV, and the growth of a free HIV population are considered in order to investigate the circumstances under which HIV can destabilize persistent infections by a general replication antigen and destroy immune memory, as well as to illustrate the impact of antigenic stimulation of infected T helper cell clones upon HIV replication rates. This investigation is extended[30] to study synergistic effects that would arise if HIV replication were enhanced by the activation of helper T-cells specific to other pathogens. This work suggests that there is a correlation between higher levels of activated helper T-cells (but not just all T-cells[168]), and disease progression. Moreover, it is assumed that there is a threshold number of activated helper T-cells above which the HIV infected immune system is unable to control pre-established pathogens; this threshold defines the boundary between a suppressed but still functioning immune system and the vicious circle of CD4 depletion that characterizes the final stages of AIDS. In a very comprehensive development[31] a set of models for the interaction of HIV with $CD4^+$ T-cells regarding three major T-cell phenotypes, namely naïve, activated and memory under distinct infection states as well as several biological issues, as time-dependent virus production or infection of precursor cells is proposed and analyzed. Although the loss of immune memory through infection of $CD4^+$ T-cells has also been modeled by McLean and coworkers,[29,30,32] the novelty of the model from Essunger and Perelson[31] relies on the explicit treatment of a latent stage in HIV-1 infection. In addition, these authors examine a new scenario in which resting $CD4^+$ T-cells also are considered susceptible to HIV-1 infection through the introduction of the *transiently infected cell clone*. Consistent with clinical observation,[33] a general feature of these models is that the decline in the total T-cell population is correlated with the rise in free HIV-1 and infected cells. The reasoning is based on a model with five-state variables (naïve, activated and memory $CD4^+$ T-cells, interleukin-2 concentration and a general antigen) that incorporates new data and theory about memory cells. McLean[32] discusses long-term immunological memory in terms of the biological significance of a pathway, whereby memory cells can lose their phenotypic and functional differences to return to the resting state. By using a potent protease inhibitor in order to disturb the balance between virus production and clearance, conclusive kinetic data on HIV-1 pathogenesis is first derived on the basis of nonlinear fitting of simple ODE systems by Ho et al.[33] and Wei et al.,[34] the most cited scientific medical research published in 1995.[47] These authors estimate as $2 \cdot 10^9$ the number of $CD4^+$ cells produced and destroyed each day and as 2 ± 0.9 days the half-life of virus-producing cells. Moreover, the half-life of HIV-1 is estimated as 6 h or less.[35] From these results, it was clear that with the rapid turnover of HIV-1, generation of viral diversity,

and the increased opportunities for viral escape from therapeutic agents are unavoidable sequelae.[33] As a consequence, it was suggested that the best treatment strategy could be one that initiates as early in the course of infection as possible[33,150,151] investigate HIV-1 infection kinetics in tissue cultures. On the basis of previous models for the course of HIV infection and disease progression, different patterns of $CD4^+$ T-cell decline after primary infection are examined[39] by considering different values of biologically interpretable parameters. Perelson et al.[110] extend Ho et al.[33] and Wei et al.[34] show that with combined therapy the initial drop in plasma viral load is followed by a slower second-phase decay of plasma viremia, whose major contributor is the loss of long-lived infected cells ($t_{1/2}$ of 1–4 weeks) whereas the activation of latently infected lymphocytes ($t_{1/2}$ of 0.5–2 weeks) is minor. Root-Bernstein and Merrill[37] discuss the need for cofactors (that is, any non-HIV substance which would result in an increase in the activation of immune response, and through it, increased activation of the $CD4^+$ T-lymphocytes) in the AIDS pathogenesis. Mittler et al.[41] extend Perelson et al.[35] to account for the influence of delayed viral production (due to intracellular and pharmacological delays) on viral dynamics in HIV-1 infected individuals in order to produce more accurate estimates about the half-life of free virus based on a experimental methodology similar to that of Ho et al.[33] and Wei et al.[34] Mathematical models of the asymptomatic phase of HIV-1 infection are proposed[40] assuming that HIV-1 infection is limited either by the availability of target cells, according to predator-prey models, or by a specific anti-HIV-1 cellular immune response, similarly to developments related to the acute phase of the infection. Perelson and Nelson[47] summarize and extend some of their high-profile work[43] and other contributions,[34] in which mathematical models for HIV dynamics, viral generation time and drug chemotherapy are discussed and mathematically analyzed. Based on previous models,[29,31,32,40] Wodarz et al.[138] conclude that macrophage infection may be essential for the successful establishment of HIV-1 in the acute phase of infection by acting as a viral reservoir. Regarding only three model states (healthy and infected $CD4^+$ T-cells and free virus), Culshaw and Ruan[49] discuss the existence and stability of the infected steady-state. Then the authors introduce a discrete time delay into the model to describe the time between infection of a $CD4^+$ T-cell and the emission of viral particles on a cellular level.

Deterministic models may be supplemented with noise terms, rendering them a stochastic differential equation (SDE) structure.[5] Because the HIV-1 epidemic is basically a stochastic process, the random nature of the process may affect the natural course of HIV-1 infection. This is especially important during the early stages of the infection, since at this time the numbers of circulating infected T-cells, as well as free HIV-1 are usually very small. It seems that the development of primary infection presents two regimes: a very early stochastic process, and a subsequent (quasi) deterministic one.[151] When HIV-1 particles enter the human body, there is a positive probability that the virus either grows by infecting susceptible $CD4^+$ T-cells, or is cleared from the human body before further infection takes place.[52] However, little work has been directed to the stochastic modeling of HIV dynamics. Merrill[181] calculated the probability of viral extinction using a branching process. The stochastic approach of Tan and Wu[44] mimics more closely the randomness of the HIV-1 infection process by allowing for a positive probability of viral extinction early in the infection process. Despite the limited number of published works, this research area should receive, as in the case of early HIV-1 population dynamics,[52,151] as well as for chemotherapy evaluation studies, substantial attention in the next years, due to the following reasons[44]:

- one may study the probabilistic properties and mean behavior of the process, as well as the validity and usefulness of results from deterministic models;
- one may readily assess the effects of the stochastic nature of the process and the random variation in many of the risk variables on the future course of the epidemic;
- stochastic models are needed to develop state-space models (Kalman-filter models as suggested by Tan and Xiang[48]) for the HIV-1 pathogenesis, which combine information from both the stochastic system model and statistical information, based on observed system data.

Early HIV-1 dynamics modeling

Unlike general HIV-1 modeling that addresses long-term disease progression, less work on early events in HIV-1 infection has been reported. Phillips[149] developed the first model specifically directed to HIV-1 primary infection, which was derived from the simplest versions of the HIV models from McLean and collaborators. The dynamic model is defined by four equations that represent the number of activated, uninfected $CD4^+$ T-lymphocytes, latently infected cells and free HIV. Although the patterns generated by the model approximate those actually observed in patients, the rapid decline in virus concentration after its high peak a few weeks after initial infection was not a result of the introduction of any term that modeled immune response; instead, the decrease was simply a result of population dynamics that suggests that the appearance of the HIV-specific immune response is a consequence of the high level of virus, rather than the cause of the decline from this high level. On the other hand, while the magnitude of the viral load depletion during the late stages of the acute phase appears to vary substantially among patients, the role of the immune control is nowadays questioned by many authors, for which the host immune response seems to be a crucial factor in controlling viral replication during HIV-1 primary infection.[27,42,63–65,72,73,101,104,156,158,178,179] Nelson[42] develops a more sophisticated model that suggests that the viral load suppression is a consequence of the reactivation of immunologic memory cells. The poorer this is, the higher the steady-state viral level.[42] However this model, as well as the ones by Phillips[149] and Stilianakis et al.,[38] has not explicitly considered the CTL cytolytic action or even the presence of other immune cells. Monteiro et al.[179] utilize the oversimplified two-state variable model (virus and virus-specific immune cells) for primary HIV infection from Nowak[182] to describe the arise of a escape mutant in a typical progression. Differently, by proposing a model with three states (uninfected and productively infected $CD4^+$ T helper lymphocytes and free virus), Stafford et al.[51] incorporate, by manipulating the infected $CD4^+$ T-cell elimination rate term, the aforementioned cytolytic effect according to the immunologic control principle.[105] Other mechanisms, as antibody-dependent cell-mediated cytotoxity (ADCC) or natural killer cell activity, have also been proposed as coadjuvant factors that control initial expansion of HIV-1

infection.[183] In summary, whether the fall in viral load results from structured immune response (CTL antiviral activity) associated or not to the rarefaction of target cells or from other factors, it remains unsatisfactorily explained.[5] Results based on both mechanisms suggest, but do not conclusively prove, that there are contributions from the two mechanisms; the limitation of target cells that respond for a major part of the observed fall in viral load after its peak, and the cytotoxic activity driving the stabilization of the pathogen population at a given plateau concentration level.[40,51]

Another important factor that may contribute to the inability of $CD8^+$ T-cells to control HIV-1 primary infection is the failure in efficient trafficking into the LT, where overwhelming HIV-1 replication occurs. A substantial reduction of the lymphoid tissue pre-CTL population that has been recently recruited from the peripheral blood may be related to the selective exhaustion of initially expanded HIV-1-specific CTL clonotypes. Hence, it is likely that exhaustion of virus-specific CTL clones has a major impact on the viral containment since their overall precursor frequency that affects the quantitative potential of the immune response is compromised in magnitude (and competence, if T-cell repertoire is also modeled) during the early stages of HIV-1 infection. Indeed, the inability of $CD8^+$ T-cells to express the homing receptors (particularly CCR7) required for binding with HEVs due to chronic stimulation with HIV-1, and other antigens may be detrimental to the host in HIV-1 infection by converting LT into relatively immunologically privileged sites.[103] Major changes in cytokine production and in the activation level of $CD4^+$ T-cells may well be occurring and mediating significant changes in $CD4^+$ and $CD8^+$ T-cell counts. Stafford et al.[51] consider that the decreasing levels of naive $CD4^+$ T-cells found in the periphery subsequently to HIV-1 infection reflects their activation, HIV-1 induced cell death and mainly sequestration in the lymph nodes. Conversely to the asymptomatic phase of HIV-1 infection, when the overall $CD4^+$ memory pool may become exhausted mainly because of chronic antigen persistence and inflammation, the $CD4^+$ memory T-cell count fluctuation in lymphoid tissues during primary HIV-1 infection may result from reactivation and migration to the periphery,[103] rather than elimination due to HIV-1 cytopathic effects on this long lived cell pool. In this sense, several theories have been proposed in order to explain the behavior of $CD4^+$ T-cell and viral counts during HIV-1 primary infection. Since productively infected cells correspond to a minor fraction of the overall cellular population, the classic viewpoint that the extent of $CD4^+$ T-cell depletion is due to HIV-1 cytopathic effects may be improbable. Indeed, a more consistent explanation for changes in $CD4^+$ T-cell numbers during HIV-1 primary infection relies on experiments carried on with SIV that have shown that they are caused by perturbations in the trafficking of these lymphocytes between the peripheral blood and the lymphoid tissue.[42]

Stochastic modeling concerned with early events has also been proposed.[5,52,151] In Tuckwell and LeCorfec,[151] a multidimensional diffusion process model that includes activated uninfected $CD4^+$ T-cells, latently and actively infected $CD4^+$ T-cells and free virions in plasma is proposed and stochastic effects are assumed to arise in the process of infection of $CD4^+$ T-cells and transitions may occur from uninfected to latently or actively infected cells by random mechanisms. The fundamental problem of this approach is that instead of examining the random nature of the variable, the approach simply adds Gaussian random noise to the state variables and wrongly assumes their independence. As a consequence, state variables have positive probabilities to take negative numbers and their variances are not necessarily inflated.[52] In Wick and Self,[5] a branching-process model of early events in HIV or SIV infection is proposed with the objective of studying the influence that the time of appearance of virus-specific antibodies or cytotoxic cells, or of administration of anti-retroviral chemotherapy, has on the probability of progression to a chronic infection. In Kamina et al.,[52] certain features and consequences of the Tuckwell and LeCorfec's model[151] are assessed in the context of the stochastic approach from.[44]

HIV-1 Drug Chemotherapy Modeling

A number of mathematical models have been developed that incorporate the therapy effects on HIV-infected individuals since Agur[184] and Perelson[165] ([1–3,10,14,30,32–34,36,38,46,48,50,185–187]) Cojocaru and Agur[185] have extended the approach by Agur[184] by considering AZT chemotherapy implications, and the optimal tradeoff between drug toxicity on uninfected immune cells and chemotherapy efficiency through cell cycle drug protocols. In Perelson,[165] stability analysis of the immune system state is performed under a scenario of AZT chemotherapy through a model based on the number of free HIV produced per infected $CD4^+$ T-lymphocyte. Descriptive models for the competitive interaction of AZT-sensitive and AZT-resistant strains of HIV-1 may also be found in McLean and Nowak.[30] Kirschner and Perelson[10] propose a model for the immune system response to HIV regarding AZT chemotherapy in which, by dealing with estimates of an efficacious therapy strategy towards the increase/retention of the $CD4^+$ T-cell pool, the authors have demonstrated the effectiveness of early treatment (i.e., before AIDS). In Kirschner and Webb,[1] AZT based chemotherapy models are considered in order to investigate the following issues: (a) frequent vs. infrequent dosing periods, (b) early vs. late treatment, and (c) high vs. low doses. According to their results, the periodicity with which a given drug dose is daily administered is irrelevant, that is, whether one receives a single 500 mg dose once a day or 100 mg doses five times a day, the overall result is the same. Second, in contrast to Ho et al.,[33] simulated results indicate that chemotherapy treatment should not begin before the final decline of T-cells (not until the $CD4^+$ T-cell count falls below approximately 300 cells/mm^3). Finally, Kirschner and Webb[1] compared dosage and frequency and did not identify clear benefits when a large dose of chemotherapy is administered. However, the Kirschner and Webb's model has not addressed the question of drug resistance. Also evaluating AZT based chemotherapy benefits, the hybrid ODE model of Hraba and Dolezal[28] includes immature and mature $CD4^+$ T-cells, as well as HIV-nonspecific $CD8^+$ T-cells, mature HIV-specific $CD8^+$ T-cells and HIV products regarding several feedback mechanisms that regulate the production and maturation of T lymphocytes. The homeostatic mechanism activation through anti-CD8 antibody administration is also considered regarding the possibility that the typical high levels of $CD8^+$ T-cells in HIV infected patients could block the effectiveness of this mechanism. The model proposed by Hraba and Dolezal[28] considers immature and mature $CD4^+$ (Q and E) and $CD8^+$ T-lymphocytes ($\check{C}$ and C), the amount of

HIV products (V), and the number of cytotoxic T cells (C_{HIV}), and is described as follows

$$\frac{dQ}{dt} = \frac{\lambda + f[(Q_0 - Q) + (C_0 - C)]}{f_g} - a_Q Q - k_Q V C_{HIV} Q$$

$$\frac{dE}{dt} = a_Q Q - d_E E - k_E V C_{HIV} E$$

$$\frac{d\check{C}}{dt} = \frac{2}{3}\frac{\lambda + f[(Q_0 - Q) + (C_0 - C)]}{f_g} - a_{\check{C}}\check{C}$$

$$\frac{dC}{dt} = a_{\check{C}}\check{C} - (d_C - a_{CD8})C$$

$$\frac{dV}{dt} = V[\theta - \xi - \gamma C_{HIV}]$$

$$\frac{dC_{HIV}}{dt} = \Lambda V[\varepsilon\lambda_C + a_C C_{HIV}] \cdot \left(\frac{E}{E_0}\right)^{\nu} - (d_c - a_{CD8})C \quad \text{(M5)}$$

with

$$f_g = \begin{cases} 1 & \text{if } \ln(V/V_0) < L \\ h\ln(V/V_0) & \text{if } \ln(V/V_0) \geq L \end{cases}$$

and

$$Q_0 = Q(0),\ E_0 = E(0),\ \check{C}(0) = \left(\frac{2}{3}\right)Q(0),$$

$$C_0 = \left(\frac{2}{3}\right)E(0),\ V_0 = V(0) \quad \text{and} \quad C_{HIV_0} = C_{HIV}(0)$$

where λ is the influx of Q cells, that is, the rate of differentiation of Q cells from the stem cells. The value a_Q is the rate of maturation of Q cells into E cells, and d_E is the rate of natural death of E cells; the quantities $a_{\check{C}}$ and d_C are defined in a similar way. Furthermore, f is the amplifying coefficient of the linear feedback effect of E and/or C cell decrease on the influx of Q and $\check{C}$ cells at time t. The quantity $k_Q V C_{HIV} Q$ is the rate of elimination of Q cells due to the amount of HIV products V, and the number of cytotoxic T cells C_{HIV} at time t. Analogously, $k_E V C_{HIV} E$ is the rate of elimination of E cells. The value V_0 is the function of the infectious dose of HIV, θ characterizes the growth rate of HIV, and γ is the rate of inactivation of HIV products mediated by cytotoxic C cells. Analogously to λ, λ_C is the influx of C_{HIV} precursors, ε their maturation rate, α the proliferation rate of C_{HIV} cells under the antigenic stimulation by HIV products and helper T cell influence, and d_c their natural death rate. The coefficient ν on the (E/E_0) term is introduced to characterize the intensity of this helper effect. E_0 is the count of peripheral blood helper T cells in a healthy person. The value h characterizes HIV-constraining intensity on the Q and $\check{C}$ cell influx. Value L defines the level, where such constraining (limiting) effect of fg starts. Finally, the effects of therapeutic interventions are described by the following parameters: ξ—HIV elimination rate by AZT or passive immunization; Λ—immune response-enhancing factor, and a_{CD8}—elimination rates of C and C_{HIV} cells by anti-CD8 antibodies.

Nowak et al.[36] have developed a mathematical framework for studying the decline of the wild type strain of HIV-1, and the concomitant rise of mutant viruses during treatment in free plasma viral population, productively infected cells, long-lived infected cells and cells that carry defective provirus. This model, in conjunction with experimental data, has also provided new estimates of the *in vivo* kinetics of HIV-1 turnover: latently infected cells have a half-life of about 10 to 20 days, and most of the infected PBMC harboring replication defective provirus have a half-life of about 80 days. In Wein et al.,[3] an optimal control problem based on an ODE model that tracks the dynamics of uninfected and infected $CD4^+$ T-cells and free plasma virus, and allows the virus to mutate into various strains, is proposed and solved through approximation techniques that employ perturbation methods in conjunction with principles from dynamic programming.[53] However, no attempt has been made to generate a model of realistic size. Also focusing on optimal control, Kirschner et al.[2] employ an existing model[165] to deal specially with the dynamics of early initiation of HIV drug chemotherapy. With respect to an optimal treatment strategy, their results present agreement with those from Ho et al.[33] indicating that earliest, nevertheless moderate, drug chemotherapy may be of interest when the objective of treatment is solely based on an increase or retention of the $CD4^+$ T-cell count. Differently from Wein et al.,[3] the work of Kirschner et al.[2] applies optimal control techniques to determine analytical solutions and then numerical methods to simulate different outcomes. Regarding specific modeling related to the development of mutations in the RT gene, Stilianakis et al.[38] demonstrate that the rebound of the HIV-1 load during zidovudine (AZT) chemotherapy is due to the outgrowth of wild-type virus and the first drug-resistant mutant (AAA $\rightarrow$ AGA at codon 70), whereas that in the case of lamivudine (3TC) it can only be due to the drug resistant mutants.

Also adapted from a simple model of HIV-1 dynamics,[35] Kepler and Perelson[113] have addressed the question of the HIV-1 sanctuaries by considering a two-compartment model (peripheral blood and central nervous system), differing only in size and drug concentration, with exchange of virus, but not of target cells between them, to show that drug concentration heterogeneity may induce the evolution of HIV-1 drug resistance. Wein et al.[45] analyze the transient and steady-state behavior of generalizations of the model proposed in Perelson et al.[110] in order to predict whether these drug regimens can eradicate HIV-1 or maintain viral loads at low levels regarding under constant immune response. Also, based on the models of Perelson and coworkers,[35,110] Ding and Wu[46] derive approximation formulas for the relationships between biphasic viral decay rates and treatment effects in order to provide a justification for using both viral decay rates in evaluating the antiretroviral treatment efficacy. In Tan and Xiang,[48] the model of Perelson et al.[35] is extended to a stochastic formulation to develop a state-space model for the HIV infection under drug chemotherapy. Moreover, the authors propose procedures for estimating and predicting the numbers of infectious free HIV and non-infectious free HIV, as well as the numbers of differ-

ent T-cell clones through the extended Kalman filtering method. Wodarz and Nowak[4] have designed an explicit immune response model, which considers CTL precursors, as well as CTL effector cells, in order to analyze how specific antiviral treatment regimes lead to the establishment of effective immune responses and long-term control of HIV. However, the potential evolution of CTL escape mutants, which may imply in loss of virus control, has not been addressed. Changes in HIV-1 dynamics regarding a discrete delay in the initiation of virus production are considered in Nelson et al.,[50] that address dynamics occurring only after drug treatment based on less than perfect protease inhibitors.

Certainly, the main deficiency of available models intended to the optimal control of HIV-1 infection relates to the absence of a realistic mathematical treatment of the HIV-1 chemotherapy. This is caused by two major factors. The first concerns the proposition of a suitable mathematical treatment of the HIV-1 mutation. Several factors are known to contribute to the generation of new viral variants throughout the course of HIV-1 disease and to affect the speed at which these variants evolve. One is the expressive error-prone nature of the viral reverse transcriptase, which lacks proofreading functions that result in nucleotide substitutions, deletions and insertions. A rate of $3.4 \cdot 10^{-5}$ misincorporation per replication cycle in vivo has been suggested.[38,77]

A second factor is the astonishing viral production (about 10^{10} virions/day), and the elevated number of replication cycles that sustain the HIV-1 infection *in vivo* (about 300 per year).[77] The third and most important factor is the rapid selection of fittest viruses that are able to survive in an adverse environment of host immune responses and/or HAART. Recombination is a fourth factor that significantly contributes for the HIV-1 genomic variability.[77]

Typically, HIV-1 drug resistance results from multiple mutations in the *pol* region. However, there are substantial difficulties facing the interpretation of genotypic information, especially in the case of multiple mutations, since the large number of individual mutations known to affect HIV-1 drug resistance interact in complex ways; some mutations cause resistance on their own, some in combination; some cause resistance to a number of different drugs; some cause resistance to one drug and reverse it to another.[8] Therefore, translating genotypic information into a reliable prediction of the drugs that the virus would be able to resist and those to which it would be sensitive is an exceptionally challenging task. Moreover, there is no systematic approach to quantitatively predict the resulting phenotypic resistance originated by multiple substitutions. This is normally performed through subjective judgment, use of rule-based algorithms or data bank for direct comparison, as *Virtual*Phenotype™.[170]

Similarly to Shafer,[8] one can assume that the phenotypic resistance associated to the ordered pair (*mutation, drug*) does not depend on the set of mutations in which it is inserted, that is, the individual phenotypic resistance provided by mutation A when associated to a set of mutations α is the same as that when A is associated to a distinct set of mutations β. In addition, it may be assumed that the overall phenotypic resistance to drug *d* originated by multiple mutations corresponds to the summation of individual contributions of each mutation. Here, the individual contribution of a given mutation should refer to experimental values determined on a mutant directly generated by single substitution in the wild-type sequence (HXB2).

Distinct nucleotide changes in the RT gene occur with different probabilities.[38] Point mutations G→A (G = guanine, A = adenine) have the greatest probability of occurrence,[8,38,76,77,83,171] corresponding to about 50% of all pair base substitutions.[38,83] Stilianakis et al.[38] determined that the mutation frequence of G→A is $\mu_1 = 10^{-5}$ per nucleotide. C→T (C = citosine, T = timine),[83] A→G[38,171] and T→C transitions correspond to the next relevant frequencies of substitution in the *pol* gene[83] that are approximately 10–50% of the G→A rate. Extrapolating Jong et al.[171] and Stilianakis et al.[38] to other mutations not originally considered in these references, one can categorize mutation probabilities into three ranges: $\mu_1 > \mu_2 > \mu_3$, imposing $P(G\rightarrow A) = \mu_1$, $P(A\rightarrow G) = P(C\rightarrow T) = P(T\rightarrow C) = \mu_2$ and $P(C\rightarrow A) = P(A\rightarrow C) = P(T\rightarrow A) = P(A\rightarrow T) = P(C\rightarrow G) = P(G\rightarrow C) = P(G\rightarrow T) = P(T\rightarrow G) = \mu_3$, since transversions (pirimidine (C or T) → purine (A or G) and vice-versa) emerge as events 2 to 20 times less probable than transitions (pirimidine → pirimidine; purine → purine).[83,171] Stilianakis et al.[38] assume $\mu_2 = \mu_1/2$ and $\mu_3 = \mu_1/10$, whereas in others[171] such parameters are estimated as $\mu_2 = \mu_1/2$ and $\mu_3 = \mu_1/4$. Under experimental support[83] G→A substitution has been shown significantly more frequent than other transitions. Hence, one can impose $\mu_2 = \mu_1/4$ and $\mu_3 = \mu_1/15$. In addition, one can assume that (a) the mutation frequency in a given codon that codifies the RT enzyme is defined on the basis of the frequencies of nucleotide substitutions, (b) the mutation frequency in a given codon does not depend on the occurrence of mutation in other position(s), and (c) these results do not depend on the HIV-1 subtype B isolate. Although the *pro* gene (responsible for the codification of protease) is located between the *gag* and *pol* genes in all retroviral genomes, the variable orientation of the reading structures in this region does imply that the *pro* gene is expressed with relevant difference in each group of the *Retroviridae* family.[76] In the case of HIV-1, the *pro* gene is located in the *pol* structure and neither *pro* nor *pol* should be strictly considered distinct genes.[76] Therefore, one can extend the previous assumptions to mutations that confer resistance to PIs (Protease Inhibitors).

A number of sequence data are available for the polymerase (RT and protease) genes of different HIV-1 subtypes and several mutations in the *pol* gene that confer drug resistance in the wild-type subtype B background have been mapped by the Stanford University and the Los Alamos National Laboratory Databases. Since over one hundred nucleotide substitutions that confer increased resistance to the conventional HIV-1 drug chemotherapy have been documented, a realistic multivariant mathematical model that considers all potential combinations of these mutations (Eq. 7), should result in a problem that is computationally intractable.[172]

$$\text{Number of HIV-1 variants} = \sum_{p=1}^{n} \frac{n!}{p!(n-p)!} \qquad (7)$$

where n is the number of mutations.

In order to reduce problem dimensionality, the concept of *pseudovariant* is introduced. In this model, it is assumed that the viral population is homogeneous with respect to the *pol* gene at each instant of the time horizon after HIV-1 infection. In other words, the pseudovariant *pol* gene sequence is as-

sumed to reflect the arithmetic mean of the *pol* gene sequence of each virion within the (truly heterogeneous) viral population. Therefore, this yield

$$\sum_{c \in S_{c,s}} p_c^s(t) = 1 \quad \forall\, s \subset \{S^{RT} \cup S^{PR}\},\ \forall\, t \tag{8}$$

For example, position 101 is characterized by the K101E mutation; that is, codon 101 may be a lysine (K), the wild-type nucleotide sequence, or a glutamic acid (E), that is the mutated nucleotide sequence that confers increased resistance to EFV. In terms of pseudovariant modeling, this results in the following constraint

$$p_{K,t}^{101} + p_{E,t}^{101} = 1,\ \forall\, t \tag{9}$$

The second factor for the absence of a realistic mathematical treatment of the HIV-1 chemotherapy concerns drug pharmacology. Despite the expressive number of models intended to examine the benefits of drug chemotherapy on the HIV-1 infection, there are few detailed reports on mathematical modeling directed to practical purposes of optimal control of HIV-1 infection. Of interest to the optimal control of HIV-1 pathogenesis are: (a) the drug antiviral action, which may be quantified by the drug concentration necessary to suppress 50% of the viral replication *in vitro* (IC_{50}); (b) the cellular and viral variants over which the drug action is more specific; (c) the synergistic/competitive/prohibitive relationships among drugs; (d) the rate of drug penetration in the central nervous system; (e) the magnitude of drug side-effects; (f) the drug resistance of non wild-type strains, and (g) the drug cost, if overall treatment cost is to be minimized.

A major strategy in the fight against AIDS may consist in the prevention of the emergence of the more pathogenic CXCR4-using strains of HIV-1.[121,133] In this sense, a model that focuses on the relationship between the antiviral action of the drug and the viral phenotype preferentially affected has been proposed.[15] According to this model, AZT is preferably active on activated cells that express CCR5, which are more susceptible to NSI HIV-1 infection, whereas ddI acts preferably on quiescent cells characterized by important CXCR4 expression that are substantial targets of SI HIV-1 strains.

HIV-1 drugs may present synergistic, competitive or prohibitive relationships. Prohibitive associations among drugs according to the Brazilian Health Ministry[7] are summarized in Table 2. It is important to note that, for optimization purposes, these decisions can be modeled through integer (binary) variables constraints and expressed in mixed-integer programming (MIP) form.

Synergy (Competition) results from the fact that some drugs may increase (decrease) the absorption rate or elimination rate of other drugs. Indeed, since the metabolism represents an important elimination route for HIV-1 drugs, the competition among drugs for association to the catalytic region of the enzyme responsible for this process is probably the main cause for changes in the dynamics of elimination of these drugs[60] and essential for defining a feasible chemotherapy strategy. These relationships have important implications on the optimality of the corresponding scheduling problem (as defined in the Introduction Section) since pharmacokinetic data is very useful in the optimization of the dosage and its frequency.[173] For example, when drug A coadministered with drug B provides drug B bioavailability raising (that is, the fraction of a drug dose that reaches the systemic circulation), the dosage of the drug A may be reduced in order to minimize not only the therapy cost, but the potential side-effects associated to it.

HIV-1 drug chemotherapy may produce important side effects. Clearly, if the intensity of the side-effects is neglected during drug chemotherapy planning, one may conclude that the optimal regime (for HIV-1 eradication) is such that it implies essentially the death of the patient because of the side-effect associated to the chemotherapy rather than of the HIV-1 disease. In this scenario, the solution would consist of saturating the patient with anti-HIV-1 drugs. The relationships between anti-HIV-1 drugs and their side effects are relatively well documented[174] and bounds on the chemotherapy side-effect intensity are necessary. In this sense, the following is considered[174]:

- The observed frequency of the side effect within a population and
- The relative magnitude of the side-effect.

Clearly subjective, the side effect should be qualified by its magnitude. For example, "fatigue" should be less undesirable than "nausea" or "diarrhea." Table 3 summarizes observed frequencies[174] and suggests the relative magnitude of some typical side-effects associated to HIV-1 drug chemotherapy.

A simple approach is to assume that (1) data from Table 3 correspond to the standard dosage of each antiretroviral, and (2) such frequencies are affected by the interactions among different antiretrovirals administered simultaneously. Note that the presented information is subjective and other viewpoints concerning the undesirability of the cited side-effects can be also used. Based on the parameters of Table 3, a methodology for evaluating the overall drug chemotherapy side-effect at a given time is given by M6

$$S_e = \sum_{d=1}^{D} \bar{e}_d \frac{C_d^{PB}}{C_{ss0_d}^{PB}} \le S_e^{\max}$$

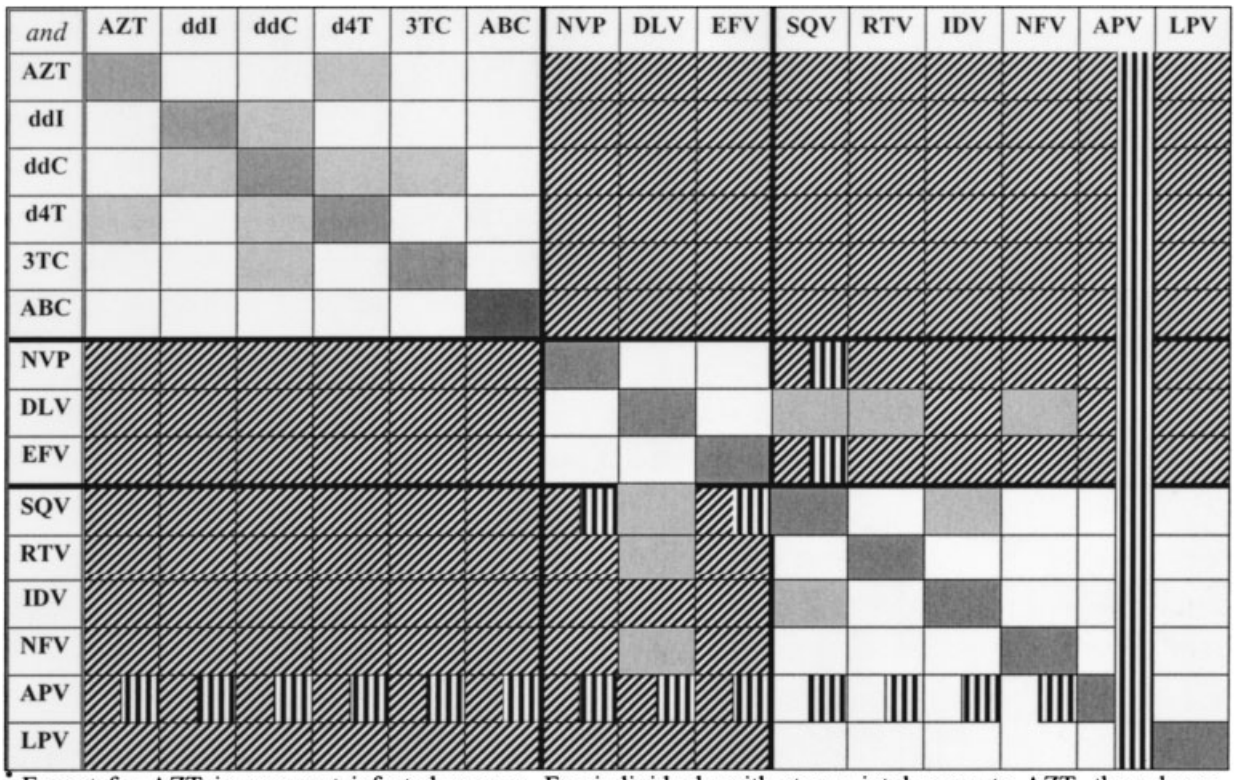
$$e_d = \sum_{j \in J_d} (h_{d,j}^{se} \cdot f_j^{se}) \quad d \in D$$

$$\bar{e}_d = \frac{e_d}{\max\limits_{d \in D}(e_d)} \quad d \in D \tag{M6}$$

In M6, d ($d = 1, 2, \ldots, D$) is the index that denotes HIV-1 drugs, J_d is the set of side-effects related to drug d, S_e is the magnitude of the side-effect of the HIV-1 drug chemotherapy at time t, e_d ($\bar{e}_d$) is the magnitude (normalized magnitude) of the side-effect caused by standard dosage of drug d, C_d^{PB} is the peripheral blood concentration of the drug d at time t, $C_{ss0_d}^{PB}$ is the mean peripheral blood concentration of the drug d at time t at standard dosage, S_e^{max} is the acceptable upper bound for the side-effect intensity, $h_{d,j}^{se}$ is the frequency of individuals that present side-effect j when subject to drug d at standard regime, and f_j^{se} is the relative magnitude of side-effect j ("undesirability"). This model is based on the additivity of the observed frequencies and magnitudes of side-effects, and considers that the magnitude of the side-effect is proportional to the amount of drug that is administered.

The resulting model is formulated as a nonlinear hybrid

Table 2. Prohibitive Chemotherapy Schemes[7]*



* Except for AZT in pregnant infected women. For individuals with strong intolerance to AZT, the scheme d4T+3TC+ABC may be considered, except for patients with renal or hepatic insufficiency.

Legend:

NRTIs	Three or more NRTIs are prohibited, except AZT+3TC+ABC, which is also prohibited for patients with renal or hepatic insufficiency
NNRTIs	Two or more NNRTIs are prohibited
PIs	Three or more PIs are prohibited, except schemes where RTV is used as pharmacological adjuvant
	Prohibitive combination
	Monotherapies are prohibited
	Prohibited when not associated with AZT and 3TC
	Prohibited for double therapy schemes
	Not prohibited if combined with RTV

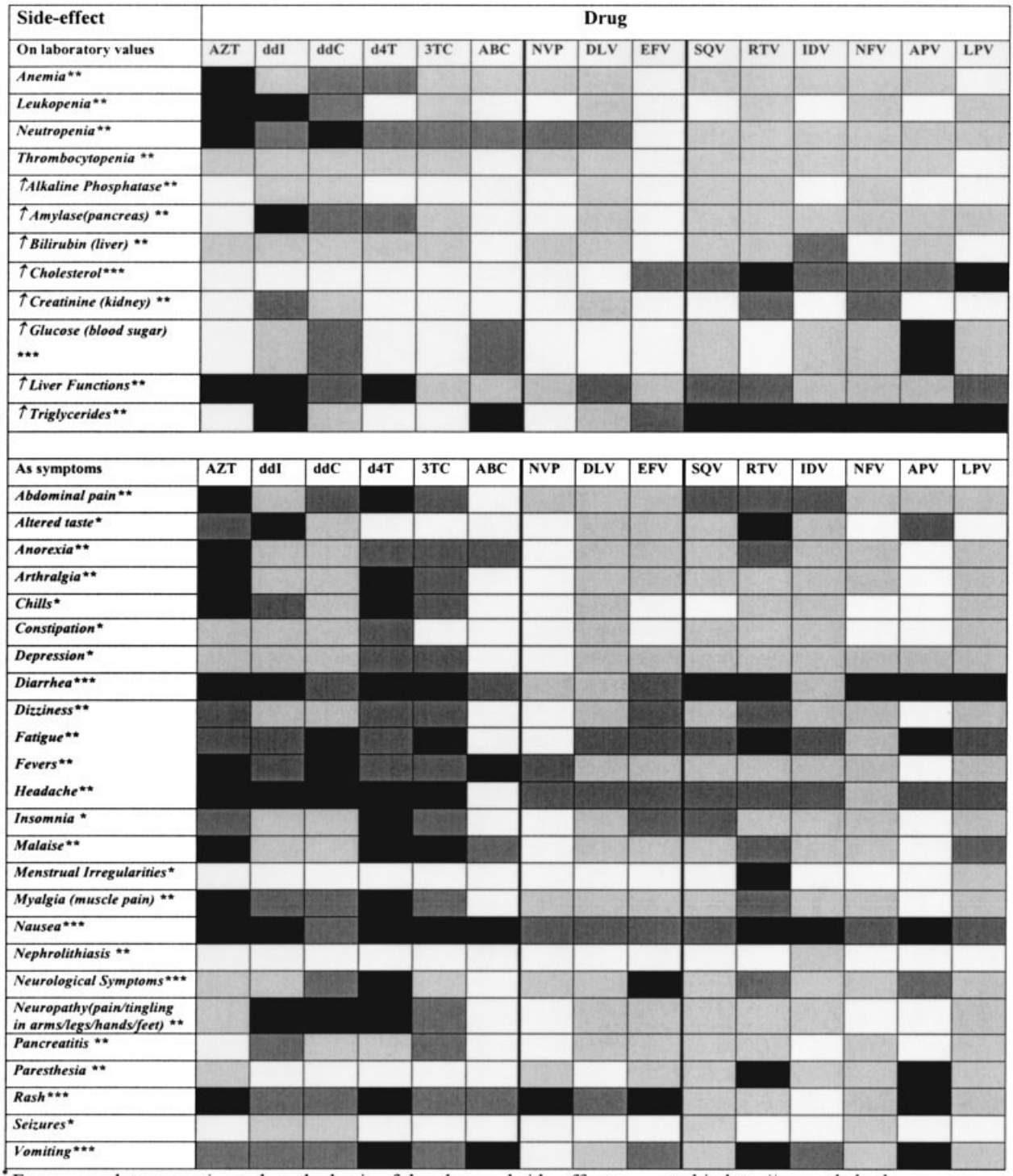
discrete-continuous DAE system, whose conversion into a finite dimensional formulation results in a nonlinear nonconvex MINLP. Because of its highly combinatorial feature, the model is NP-hard.[172,175] These models, in general, require careful initialization.[176] Moreover, global optima are not guaranteed through conventional algorithms. Since the problem has high dimension, as well as the combinatorics that result from the realistic number of available chemotherapy alternatives, the computational effort may be very expensive.

Phenomenologically, the most notable simplification is related to the HIV-1 phenotypic resistance. As discussed, it is considered that under a multiple mutation scenario, the overall phenotypic resistance of the pseudovariant is given by the summation of the individual contribution of each elementary (or *canonic*) mutation on the basis of a wild-type background. We denote this as canonic mutation additive approach (A.A.). According to this methodology, the phenotypical resistance due to M mutations is estimated by

$$r_f = r_{IC50_{wt}} + \sum_{m=1}^{M} r_m^i$$

where r_f = overall phenotypic resistance (relative to wild-type HIV-1 resistance), $r_{IC50_{wt}}$ = natural phenotypic resistance (that is, 1) and r_m^i = phenotypic resistance **increase** due to canonic mutation m. An alternative approach for estimating HIV-1 phenotypic resistance due to multiple mutations is the *mutation scoring* (MS) developed by Shafer.[8] According to this methodology, constant values are assigned to each mutation already documented and the overall phenotypic resistance of a given sequence with

Table 3. Frequencies and Estimated Relative Magnitude of Major Antiretroviral Chemotherapy Effects*



Frequency data are estimated on the basis of the observed side-effects reported in http://www.thebody.com.

Legend:

	Side-effect reported in more than 15% of individuals.
	Side-effect reported from 5% up to 15% of individuals.
	Side-effect reported in less than 5% of individuals.
	Side-effect not reported in individuals.
***	Side-effect considered "strongly undesirable"
**	Side-effect considered "undesirable"
*	Side-effect considerable "weakly undesirable"

Table 4. Mathematically Estimated and Experimental Data on Resistance Due to Multiple Mutations[8]*

Drug	Main Mutations	Evaluated Variant	Method	Experimental ($xIC_{50_{wt}}$)	Estimated A.A. ($xIC_{50_{wt}}$)	Estimated Mutation Scoring ($xIC_{50_{wt}}$)	Deviation A.P. (%)	Deviation M.S. (%)
AZT	L210W, T215Y	HXB2-210-215	Virco	10	7.4	47	−26%	+370%
	M41L, T215Y	HXB2-41-215	Virco	26	7.4	50	−71.5%	+92%
	M41L, D67N, K70R, T215Y	HXB2-41-67-70-215	Virco	24	17.4	90	−27.5%	+275%
	M41L, D67N, K70R, M184V, L210W, T215Y	HX-41-67-70-184	Virco	12	10.7	87	−10.8%	+625%
ddI	L74V, M184V	MV156wk24	Virco	4.2	3.2	70	−23.8%	+1567%
	K65R, L74V, M184V	HXB-65-74-184	R.P.	6.0	5.6	100	−6.7%	+1567%
	K65R, M184V	MV-101wk24	Virco	3	3.6	50	+20%	+1567%
d4T	M41L, T215Y	HXB2-41-215	Virco	2.6	<3.9	37	?	+1323%
	V75T, T215Y	HXB2-75-215	R.P.	3.3	4.6	75	+39.4%	+2173%
	M41L, T215Y, K219Q	DK-6384	Virologic	2.0	<5.6	49	?	+2350%
3TC	Q151M, M184V	WH-151-184	Virologic	>100	101	75	?	?
ABC	K65R, L74V	HXB-65-74	R.P.	3.6	1.1	45	−69.4%	+1150%
	Q151M, M184V	WH-151-184	Virologic	20.9	4.1	60	−80.4%	+187%
	K65R, M184V	MV-101wk24	Virco	4.4	2.4	40	−45.4%	+809%
	K65R, Y115F, M184V	MV-140wk16	Virco	9.5	3.5	75	−63.1%	+689%
NVP	K103N, Y181C	NL43-nnrt12	Virologic	>500	>149	120	?	?
	Y181C, M230L	NL43-M230L-181	Virologic	>780	138	120	?	?
	L100I, K103N	LB-100I-103N	Virco	110	>54.3	100	?	−9%
DLV	K103N, M230L	NL43-M230L-103	Virologic	>250	125	130	?	?
	Y181C, M230L	NL43-M230L-181	Virologic	>250	90	130	?	?
	K103N, Y181C	LB-103N-181C	Virco	270	100	120	−62.9%	−55.5%
EFV	K103N, V108I, P225H	LB103N-108I-22	Virco	625	36.8	100	−94.1%	−84%
	K103N, P225H	LB103N-225H	Virco	100	36.2	90	−63.8%	−10%
	K103N, V108I	LB-103N-108I	Virco	68	36.6	70	−46.2%	+2.9%
SQV	G48V, L90M	NL43-PRI-7	Virologic	12.9	12	85	−6.9%	+608%
	L10I, G48V, L90M	YG-NL1-SQV	Gong00	8.0	12	87	+50%	+986%
RTV	K20R, M36I, I54V, A71V, V82A, L90M	RTV-306FU	Poppe97	64	12.8	73	−80%	+14%
	L10I, M46I, I54V, A71V, V82A	SB-PtD-w79	Virologic	33.7	6.8	61	−79.8%	+81%
	L10I, K20R, M36I, M46I, I54V, A71V, V82A, L90M	SB-PtE-w16	Virologic	260.9	11.6	85	−95.6%	−67.4%
IDV	I54V, L63P, V82A	PtlW12	Condra96	4	4.1	47	+2.5%	+1075%
	L10I, L63P, A71V, G73S, I84V, L90M	DK-6694	Virologic	8.1	25.8	58	+218%	+616%
	L10I, V32I, M46L, L63P, A71V, V82A, L90M	RC-V020853	Virco	21	9.8	83	−53.3%	+295%
NFV	D30N, M46I, L63P, N88D	NL43-PRI-2	Virologic	24.7	12.2	102	−50.6%	+313%
	D30N, A71V, N88D	DK-623	Virco	31.4	12	77	−61.8%	+145%
	D30N, L63P, V77I, N88D, L90M	NP-12	Virologic	67	16.2	128	−24.2%	+91%
APV	M46I, I47V	HXB2-46-47	Partaledis	1	2	45	+100%	+4400%
	L10F, M46I, I47V, I50V	IIIB-10-46-47-5	Partaledis	200	4	97	−98%	−51.5%
	L10F, V32I, M46I, I47V	RC-V207648	Virco	16	5	67	−68.8%	+318%
	L10F, V32I, M46I, V82I	YG-RF3-APV	Gong-00	82	7	52	−91.5%	−36.6%
LPV	L10F, V32I, M46I, I47V, I84V	NL43-P14	Carillo-98	46	9.7	30	−78.9%	−34.8%

*Data on wild-type strain (HXB2) and Virco methodology was considered whenever available in order to minimize discrepancies due to employment of different viruses and experimental techniques.

multiple mutations is then estimated through the summation of the individual scores associated with each detected substitution. However, the quantification of the overall HIV-1 phenotypic resistance due to the combination of two or more mutations is non trivial, and there is no general function that is able to accurately predict the resulting resistance provided by a set of mutations. Indeed, as showed in Table 4, the phenotypic resistance predicted by the proposed methodology may, in some cases, differ significantly from experimental results.

Such discrepancies are specially verified in the case of multiple mutations that confer resistance to NNRTIs and PIs when, in general, negative deviations are observed. Moreover, there is no appreciable correlation between the number of canonic mutations involved, and the corresponding discrepancy between predicted and experimental phenotypic resistance data. An exhaustive medical validation of the proposed technology remains to be performed. A general purpose model capable of optimizing the short-term chemotherapy schedule for a given HIV-1 genotyping status on the basis of a DAE model that describes the HIV-1 pathogenesis is ultimately

desired; work towards this goal is under development.[134] Several features of the major developments in mathematical modeling of HIV-1 pathogenesis and immunology are summarized in Table 5 regarding (a) model structure and characterization, whether deterministic or stochastic; (b) infection stage addressed in the work; (c) human compartments under consideration (PB, LT and/or CNS); (d) inclusion of explicit (that is, CTL activity at least) or implicit (that is, through model parameter manipulations) immune response modeling; (e) inclusion of drug chemotherapy modeling at simulation or optimization (optimal control of the antiretroviral treatment) level; (f) inclusion of HIV-1 mutation modeling, and (g) cellular and viral populations under consideration.

Conclusions

The human immune system is complex and not fully understood, and no model, mathematical or otherwise, can capture every facet of its phenomenology.[32] Therefore, the optimal control of HIV-1 drug chemotherapy is an extraordinarily challenging task that encompasses several different disciplines that have as common goal the development of novel therapeutic strategies.

As described throughout this article, the understanding of the HIV-1 dynamics, the HIV-1 genomics, the HIV-1 drug pharmacokinetics and metabolism, as well as optimal control techniques play an important role for the *in vivo* drug chemotherapy effectiveness, since with the advent of potent treatment regimens, the eradication of HIV-1 seems feasible.[108,177] However, to achieve this objective, one of the most discouraging aspects is the persistence of HIV-1 latent reservoirs, as resting $CD4^+$ T-cells[142] that carry replication-competent HIV-1 from which mutation events lead to drug resistance.

The host immune response has been largely neglected in terms of mathematical modeling of HIV-1 pathogenesis, despite its foremost importance for the control of viral replication during the primary HIV-1 infection,[72,101,178] and extensively associated with the infection progression rate.[73,152] The few recent developments[4,40,51] are characterized by several deficiencies. Excessive simplification of the overall model,[179] of the immune response model,[39,40,138] or even the implicit consideration of the immune control through parameter manipulations without phenomenological support[151] are typical weaknesses. Ignoring immune response mechanisms as well as co-infection by secondary antigens in the context of primary HIV-1 modeling may be pointed out as another limitation of previous works.

Similarly, previous works that address the asymptomatic phase, the overall disease course or intended to examine the optimal drug chemotherapy planning suffer from an even larger set of shortcomings. The most critical model simplification concerns the inclusion of the $CD4^+$ T-cell pool as the only target of HIV-1 infection. However, other cells may become HIV-1-infected and exhibit different kinetic behavior. $CD8^+$ T-lymphocytes and, even more rarely B lymphocytes, as well as monocytes, macrophages and other components of the immune system are also typically neglected. Furthermore, these models have only dealt with infection of cells by free viruses, and the death of cells due to viral infection. Direct cell-to-cell viral transmission has been reported in cell culture and cited as a principal infection route,[66] as well as death of cells due to effects other than direct viral action. Phenomenological modeling of the evolution from R5 isolates to more cytopathic strains remains to be proposed. Lastly, the typical state variables of these models are almost exclusively intended to describe the HIV-1 infection in the peripheral blood, despite the fact that most of the infection seems to occur in the lymph nodes; moreover, the models ignore that initial $CD4^+$ T-cell levels in the peripheral blood are most likely affected by their exchange between the peripheral blood and the lymph nodes. Flamand et al.[65] suggest that CTL depletion does occur after HIV-1 infection and massive antigenic stimulation is undoubtedly responsible for specific CTL clone exhaustion, since it induces CD4 expression on CTL, rendering them susceptible to HIV-1 infection. On the other hand, while it has been shown that the frequency of HIV-1-specific CTL is considerably reduced with the initiation of HAART (possibly of the lack of available antigen in treated patients), it dramatically improves the effectiveness of autologous $CD8^+$ T-cells in suppressing viral replication in $CD4^+$ T-cells through cellular contact.[73] This suppressive mechanism may play an important role in the lymphoid tissue of infected patients, where both populations of cells share the same microenvironment.

On the other hand, these developments have provided relevant contributions for the understanding of the infection dynamics. The models discussed in this paper are relatively simple and further studies must be performed on the development of phenomenological immune response models for HIV-1 pathogenesis. This additional research must incorporate a more comprehensive and accurate modeling of the immune system, pharmacological issues related to each HIV-1 drug together with resistance effects and a realistic approach for integer decisions that considers prohibitive, synergistic and competitive interactions among available drugs. Furthermore, a far more complete understanding of the large-scale patterns of drug resistance evolution is crucial for the development of an effective strategy to reduce human mortality by HIV-1. More sophisticated mathematical models that account for spatial and temporal variations, in conjunction with improved experimental measurements of drug and viral levels in multiple compartments, will be of foremost importance in this ongoing effort.[113] A quantitative understanding of the role played by heterogeneity in drug levels and pathogen transport may be critical to control re-emerging infectious diseases.[46,113]

In this work, we examined the state-of-the-art in HIV-1 modeling, immunology and AIDS drug chemotherapy planning, as well as the foundations of the optimal control theory in order to provide an extended basis for the development of novel comprehensive phenomenological modeling that addresses some of the aforementioned deficiencies of the current models. These represent an important obstacle for the generation of an integrated tool for defining optimal real-world HIV-1 drug chemotherapy scheduling strategies.

In particular, the major contributions of this research are as follows: (a) since the real-world problem of the optimal control of HIV-1 pathogenesis is of multidisciplinary nature, several issues related to mathematics and immunology were presented, and a biology-oriented discussion was presented that focus on novel mechanisms recently identified that affect the course of HIV-1 infection; (b) development of a more comprehensive biological basis that addresses the complex and integrated human immunology for development of phenomenological mathematical models, which may serve to study the dynamics of other viral pathogenesis, as hepatitis-C. Here, we point out

Table 5. Summary of Mathematical Models of HIV-1 Pathogenesis and Immunology*

Model	Model Structure	Determin./ Stochastic	Model Compartments			Infection Phase Evaluated	Immune Response E/I	Chemotherapy S/O	Mutation Model	Explicit Model State Variables										
			PB	LT	CNS					m	$\bar{m}$	Q	M	E	$\bar{Q}$	$\bar{M}$	$\bar{E}$	$\bar{V}$	V	A	I
Anderson '89[164]	ode	D	x			I								x			x	x		x	
Reibnegger et al. '89[167]	ode	D	x			I								x				x		x	
McLean & Kirkwood '90[29]	ode	D	x			I							x	x				x		x	x
McLean & Nowak '92[30]	ode	D	x			I/II/III							x	x				x		x	x
Essunger & Perelson '94[31]	ode	D	x			I/II/III						x	x	x	x	x	x	x			
McLean '94[32]	ode	D	x			na						x	x	x						x	x
Ho et al. '95[33]	ode	D	x			II/III									x				x		
Wei et al. '95[169]	ode	D	x			II/III											x		x		
Hraba & Dolezal '96[28]	hode	D	x			I/II/III	E	S				x		x				x			
Kirschner & Webb '96[1]	ode	D	x			II/III	I	S						x			x				
Perelson et al. '96[36]	ode	D	x			II/III								x			x	x	x		
Phillips '96[149]	ode	D	x			I								x		x	x	x			
Spouge et al. '96[150]	ode	D		Tissue culture		I/II							x			x		x			
Kirschner et al. '97[2]	ode	D	x			II		O						x		x	x	x			
Nowak et al. '97[36]	ode	D	x			II/III		S	x		x			x		x	x	x			
Perelson et al. '97[110]	ode	D	x			II/III				x	x			x		x	x		x		
Root-Bernstein & Merrill '97[37]	ode	D	x			I						x		x			x				x
Stilianakis et al. '97a[38]	ode	D	x			II		S	x			x		x			x	x			
Stilianakis et al. '97b[39]	ode	D	x			I/II/III	I						x			x		x			
Wein et al. '97[3]	ode	D	x			II/III		O	x					x			x	x			
DeBoer & Perelson '98[40]	ode	D	x			II	E	S				x		x			x	x			
Kepler & Perelson '98[113]	ode	D	x		x	II		S	x					x			x	x			
Mittler et al. '98[41]	ide	S	x			II/III		S									x	x	x		
Murray et al. '98[42]	ode	D	x			I	I					x	x	x				x	x		
Tan & Wu '98[44]	sde	S	x			I/II/III								x		x	x	x			
Tuckwell & LeCorfec '98[151]	sde	S	x			I								x		x	x	x			
Wein et al. '98[45]	ode	D	x			II	I	S	x	x	x			x			x	x	x		
Ding & Wu '99[46]	lde	D	x			II		S		x	x			x			x	x	x		
Perelson & Nelson '99[47]	ode	D	x			II		S		x	x			x		x	x	x	x		
Tan & Xiang '99[48]	sde	S	x			II/III		S						x			x	x	x		
Wodarz and Nowak '99[4]	ode	D	x			I/II	E	S						x			x	x			
Wodarz et al. '99a[138]	ode	D	x			I/II/III	E			x	x	x		x			x				
Culshaw & Ruan '00[49]	ode	D	x			I								x			x	x			
Kamina et al. '01[52]	sde	S	x			I								x		x	x	x			
Monteiro et al. '00[179]	ode	D	x			I	E											x			
Nelson et al. '00[50]	ode	D	x			II/III		S						x			x	x	x		
Stafford et al. '00[51]	ode	D	x			I	I							x			x	x			
Wick & Self '00[5]	sde	S	x			I	I	S		x	x			x			x	x			

*ode, (non-linear) ordinary differential equation system; sde, stochastic differential equations; ide, integro-differential equation system (in Mittler et al.[41], the integral-differential equations are converted into an ode system); Ide, linear differential equation system (Ding and Wu[46] assumes that the target T cell pool is constant over the horizon (i.e., asymptomatic phase is under consideration). Therefore, a system of linear differential equations is generated); hode, hybrid ordinary differential equation system.

the development of biological models that incorporate, in an integrated way, *a multicompartment human body model,* which considers the peripheral blood, the lymphoid tissues and the central nervous system, a cellular apoptotic model that may contribute to T-cell depletion throughout the infection course, a comprehensive $CD4^+$ T-cell infection model that considers distinct cellular phenotypes and infection routes other than virus-cell, and an integrated model for the $CD8^+$ T-cell pool dynamics that regards its immunologic interactions with the monocyte-macrophage cell lineage. It is important to note that the contribution of the humoral immune response remains controversial, comparatively much less studied that the specific one and most probably of minimal impact[84]; (c) a unified comparison of the major mathematical developments in the area emphasizing main potentials and limitations that characterizes past work, and (d) the foundations for the development of a realistic HIV-1 drug chemotherapy model that must be incorporated into the human immunology model in order to compose an integrated tool that is able to define the optimal drug chemotherapy short-term schedule for AIDS infection as function of patient status at a given time.

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Notation

$\bar{E}_{CCR5}$ = vector of the absolute CCR5 expressions of each of RC clones[114]

E^L_{CCR5}, E^M_{CCR5} = absolute CCR5 expression (ABS/cell) on $CD4^+$ T-lymphocytes and monocyte-macrophage cell lineage, respectively

E^L_{CXCR4}, E^M_{CXCR4} = absolute CXCR4 expression (ABS/cell) on $CD4^+$ T-lymphocytes and monocyte-macrophage cell lineage, respectively

$E^L_{\%CCR5}$, $E^M_{\%CCR5}$ = percent of CCR5 positive $CD4^+$ T-lymphocytes and monocyte-macrophage cell lineage, respectively. Here, superscript $\sim$ denotes normalization in [0, 1]

$E^L_{\%CXCR4}$, $E^M_{\%CXCR4}$ = percent of CXCR4 positive $CD4^+$ T-lymphocytes and monocyte-macrophage cell lineage, respectively. Here, superscript $\sim$ denotes normalization in [0, 1]

$\tilde{I}^L_{R5}$, $\tilde{I}^M_{R5}$ = overall R5 HIV-1 normalized infectivity in [0, 1] on $CD4^+$ T-lymphocytes and monocyte-macrophage cell lineage, respectively

$\tilde{I}^L_{X4}$, $\tilde{I}^M_{X4}$ = overall X4 HIV-1 normalized infectivity in [0, 1] on $CD4^+$ T-lymphocytes and monocyte-macrophage cell lineage, respectively

$\tilde{I}^L_{R5X4}$, $\tilde{I}^M_{R5X4}$ = overall R5X4 HIV-1 normalized infectivity in [0, 1] on $CD4^+$ T-lymphocytes and monocyte-macrophage cell lineage, respectively

S_{CCR5} = R5 HIV-1 normalized infectivity in [0, 1] as exclusive function of relative CCR5 density on the target cell

S_{CXCR4} = X4 HIV-1 normalized infectivity in [0, 1] as exclusive function of relative CXCR4 density on the target cell

x_{CCR5} = percent of relative CCR5 density on the target cell

x_{CXCR4} = percent of relative CXCR4 density on the target cell ($=100 - x_{CCR5}$)

η_{R5} = R5 HIV-1 normalized infectivity in [0, 1] as exclusive function of absolute CD4 and CCR5 expressions on the target cell

χ^L_{CD4}, χ^M_{CD4} = absolute CD4 expression (ABS/cell) on $CD4^+$ T-lymphocytes and monocyte-macrophage cell lineage, respectively

$\tilde{\chi}_{CD4}$ = percent of CD4 positive cells within the monocyte-macrophage cell lineage population (note that this parameter is not considered for the $CD4^+$ T-lymphocyte pool, which is by definition CD4 positive)

$\tilde{\chi}_{CD4CD8}$ = percent of CD4 positive cells within the $CD8^+$ T-lymphocyte population

ϑ_{R5} = free fraction of 2D7 ABS (CCR5 coreceptors) on a cell

Abbreviations

ABS = antibody-binding sites
ACMK = autologous infected cell-mediated killing
AICD = activation-induced cell death
AIDS = acquired immunodeficiency syndrome
AM = alveolar macrophages
APC = antigen presenting cells
AZT = zidovudine
CNS = central nervous system compartment
CSF = cerebro-spinal fluid
CTL = cytotoxic T lymphocytes
DAE = differential-algebraic equations
DAOP = Differential-algebraic optimization problem
FDC = follicular dendritic cells
GRG = generalized reduced gradient algorithm
HAART = highly active antiretroviral therapy
HIV-1 = human immunodeficiency virus type 1
HLA = human leukocyte antigen
IL-2 = interleukin-2
IFN-γ = interferon γ
LT = lymphoid tissue compartment
MDM = monocyte-derived macrophages
MHC = major histocompatibility complex
MINLP = mixed-integer nonlinear programming
NLP = nonlinear programming
NN = neural network algorithm
NSI = nonsyncytium-inducing
ODE = ordinary differential equations
PB = peripheral blood compartment
PBL = peripheral blood lymphocytes
PBMC = peripheral blood mononuclear cells
PHA = phytohemagglutinin
SI = syncytium-inducing
SIV = simian immunodeficiency virus

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Appendix

Dynamics of cell susceptibility to HIV-1 infection

Joly and Pinto[134] proposed that the potential susceptibility degree of a target cell to HIV-1 infection after a collision between a viable viral envelope and the target cell, or equivalently, the HIV-1 infectivity as function of the superficial characterization of the cell in terms of CD4 marker, CCR5 and CXCR4 coreceptor expressions, is given according to Table A1 in the [0, 1] range.

Regarding the infection of $CD4^+$ T-cell by R5 isolates, the derivation of the overall infectivity model must consider: (a) the cell phenotype (naive/activated/memory), (b) the CCR5 positive fraction of the $CD4^+$ T-cell pool that has the specified phenotype, (c) the free fraction of CCR5 on the cell (noncytolytic immune response), (d) the cell susceptibility level as function of the interdependent relationship between absolute CD4 and free CCR5 expressions on the cell, and (e) the inhibition degree on R5 infectivity caused by CXCR4 coexpression on the CXCR4 positive fraction of the $CD4^+$ T-cell pool under consideration. Figure A1 presents a comprehensive scheme illustrating the R5 infectivity model derivation for the case, where the target cell is a $CD4^+$ T-lymphocyte. Clearly, the model must be composed by the summation of the terms identified by A, B and C, as simplified in (I) (function arguments not shown). The case for the X4 infectivity model derivation is analogous

$$\tilde{I}^L_{R5} = \tilde{E}^L_{\%CCR5} \cdot \eta_{R5} \cdot (S_{CCR5} \cdot \tilde{E}^L_{\%CXCR4} + (1 - \tilde{E}^L_{\%CXCR4})) \quad \text{(A1)}$$

The derivation of the overall infectivity model for the case of monocyte-macrophage cell lineage is similar, but involves two particularities. First, there are no discrete phenotypes to be considered, instead the model relies on a continuous approach composed by elementary time functions that describe dynamic efficiency profiles. Furthermore, the model contains an additional function describing the CD4 up-regulation during the monocyte maturation, since not all cells are CD4 positive. It is important to note that infectivity expressions for the monocyte-macrophage cell lineage are conservative and define an upper bound for $\tilde{I}$. Alternatively, lower bounds may be obtained by assuming random distribution of cell receptors. Mathematically, this is equivalent to replace the term $[\min(\bar{\chi}_{CD4}, \tilde{E}^M_{\%CR}(t)),\ CR = CCR5,\ CXCR4]$ that denotes restriction to infection by the limiting coreceptor, by $(\bar{\chi}_{CD4} \cdot \tilde{E}^M_{\%CCR5}(t))$.

Table A1. Proposed Infectivity Model for R5/R5X4/X4 HIV-1 Strains on $CD4^+$ T-Lymphocytes and Monocyte-Macrophage Cell Lineage

$CD4^+$ T-Lymphocytes
R5 strains: $\tilde{I}^L_{R5}(s) = \tilde{E}^L_{\%CCR5}(s) \cdot \eta_{R5}(\chi^L_{CD4}, \vartheta_{R5}E^L_{CCR5}(s)) \cdot (S_{CCR5}(\vartheta_{R5}E^L_{CCR5}(s), E^L_{CXCR4}(s)) \cdot \tilde{E}^L_{\%CXCR4}(s) + (1 - \tilde{E}^L_{\%CXCR4}(s)))$
X4 strains: $\tilde{I}^L_{X4}(s) = \tilde{E}^L_{\%CXCR4}(s) \cdot (S_{CXCR4}(\vartheta_{R5}E^L_{CCR5}(s), E^L_{CXCR4}(s), \chi^L_{CD4}) \cdot \tilde{E}^L_{\%CCR5}(s) + S_{CXCR4}(0, E^L_{CXCR4}(s), \chi_{CD4}) \cdot (1 - \tilde{E}^L_{\%CCR5}(s)))$
R5X4 strains: $\tilde{I}^L_{R5X4}(s) = \min(1, \tilde{I}^L_{R5}(s) + \tilde{I}^L_{X4}(s))$
Monocyte-Macrophage Cell Lineage
R5 strains: $\tilde{I}^M_{R5}(t) = \min(\tilde{\chi}_{CD4}, \tilde{E}^M_{\%CCR5}(t)) \cdot \eta_{R5}(\chi^M_{CD4}(t), \vartheta_{R5}E^M_{CCR5}(t)) \cdot (S_{CCR5}(\vartheta_{R5}E^M_{CCR5}(t), E^M_{CXCR4}(t)) \cdot \tilde{E}^M_{\%CXCR4}(t) + (1 - \tilde{E}^M_{\%CXCR4}(t)))$
X4 strains: $\tilde{I}^M_{X4}(t) = \min(\tilde{\chi}_{CD4}, \tilde{E}^M_{\%CXCR4}(t)) \cdot (S_{CXCR4}(\vartheta_{R5}E^M_{CCR5}(t), E^M_{CXCR4}(t), \chi^M_{CD4}(t)) \cdot \tilde{E}^M_{\%CCR5}(t) + S_{CXCR4}(0, E^M_{CXCR4}(t), \chi^M_{CD4}(t)) \cdot (1 - \tilde{E}^M_{\%CCR5}(t)))$
R5X4 strains: $\tilde{I}^M_{R5X4}(t) = \min(1, \tilde{I}^M_{R5}(t) + \tilde{I}^M_{X4}(t))$

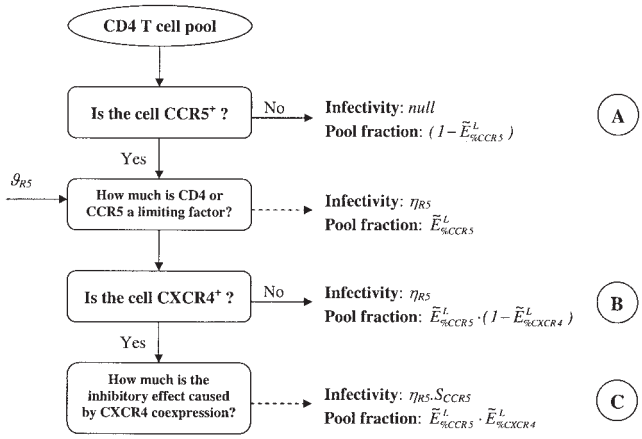

Figure A1. R5 infectivity model for $CD4^+$ T-lymphocytes.

Finally, it has been demonstrated that prototype dual-tropic HIV-1 isolates, such as strains 89.6 and DH12, can use both CCR5 and CXCR5 as fusion coreceptors.[180] For simplicity, we conservatively assume that dual-tropic envelopes are equally able to infect a target cell either via CCR5 or CXCR4, such that the R5X4 strain infectivity is defined by the algebraic summation (bounded to [0, 1]) of individual components for R5 and X4 strains on the same target cell.

The major conclusions are as follows: (a) the infectivity of R5 isolates as function of relative CXCR4 (or CCR5) density does not depend on the ratio between CD4 ***R***eceptor antibody binding sites (ABS), and (CCR5 + CXCR4) ***C***oreceptor ABS (***R/C*** ratio), and is characterized by distinct behaviors. Specifically, the cell may be considered refractory to R5 infection for relative CXCR4 densities larger than approximately 92%. On the other hand, R5 infectivity is linearly incremented from this level ($x_{CCR5} = 8\%$) and saturates when relative the CCR5 density reaches 78%, approximately, (b) in contrast, there is no appreciable correlation between X4 envelope infectivity and relative CCR5 density on cells characterized by similar ***R/C*** ratios, in particular when ***R/C*** < 1 that corresponds to the case of higher degree of competition among coreceptors, (c) on the other hand, there is an strong correlation between X4 infectivity and relative coreceptor density. Furthermore, results suggest that the ratio between the rate of X4 infectivity and the rate of the relative CXCR4 density on the cell is constant and independent from the cell absolute CD4 expression. In addition, such constant is not related to the ***R/C*** parameter, (d) for a given relative CXCR4 density, the X4 infectivity level is an exclusive function of the absolute CD4 expression on the cell, as indeed suggested by Lee et al.[115] and already demonstrated by Platt et al.[114] in the analogous case of the CCR5 coreceptor, and (e) according to previous studies,[63,65] our results indicate a key role for cytolysis in the antiviral activity of CTL.

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