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Valuable Insights on the Super-Infection Model of Immune System T (IT) Cells for crHIV-1 Gene Therapy

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Abstract. Development of crHIV-1 vectors has been tested in vitro, but the requirements for a crHIV-1 vector to proliferate and persist in vivo have not been fully explored. The aim of this study is to construct an expanded mathematical model to better simulate the mechanism. The expanded gene therapy model representing a super-infection from crHIV-1 on I_t and corresponding equations will be investigated using Matlab. The HIV-1 set point has been significantly lowered down to 10^2 grade and Matlab plots have been reproduced with almost the same trends. Results from super-infection Model showed significantly improved HIV-1 set point reduction compared to basic one. Thus, crHIV-1 super-infection, which is likely to occur, improves therapy.

Keyword: CrHIV-1 vector, viral inhibition, super-infection, Matlab simulation, steady state and stability analysis, setting point

1. Introduction

Although countless efforts have been taken to deal with HIV-1 infection for decades, there is no efficient treatment for AIDS [1]. Currently, the most widely used treatment is highly active antiretroviral therapy (HAART) by HIV-1 drugs. The HAART has been proven to effectively reduce the amount of active virus, in some cases, lower to undetectable level by current blood testing techniques [2]. However, HAART is highly toxic, expensive and appears to lose efficiency due to some long-lived and latently infected cells [3], [4]. Nowadays, a gene therapy is studied as an alternative method to cope with HIV. The main principle of the gene therapy is to subdue the gene expression of HIV by introducing conditionally replicating HIV-1 vector (crHIV-1) gene therapy vectors to interfere with wild-type HIV-1 replication [5], [6]. Although the crHIV-1 vectors has been developed in vitro, the requirements for a crHIV-1 vector to persist and proliferate in vivo remain partially unclear [7]. Luckily, a widely accepted and experimentally verified differential equation model of HIV-1 infection mechanism has been studied in vivo dynamics [8]. However, the original model has failed to address the super-infection of crHIV-1.

In this study, the super-infection of crHIV-1 will be introduced to the original model for more accurate simulation result. The effects of crHIV-1 gene therapy on the HIV-1 infected cells will thus be examined to find the optimum set point, and extensive numerical simulations will be performed to explore and determine the sensitivity of the set point to given parameters. Further investigation for describing the crHIV-1 viruses' steady state and stabilities analysis will also be derived.

2. Materials & Methods

The original model is a set of six equations, namely three equations describing densities of uninfected CD_{4+} T cells (T), productively infected T cells (I) and free circulating virus (V), and three equations describing the population of crHIV-1 therapy virus (V_t), therapeutically infected cells (I_t), and cells were

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dually infected with both HIV-1 and crHIV-1 therapy virus (I_d). Furthermore, two experimentally engineered parameters were introduced: the extracellular measure efficiency of crHIC-1 mediated viral inhibition (D) and intracellular concentration of crHIV-1 mRNA relative to HIV-1 mRNA (P) to increase its validity, shown in Fig. 1.

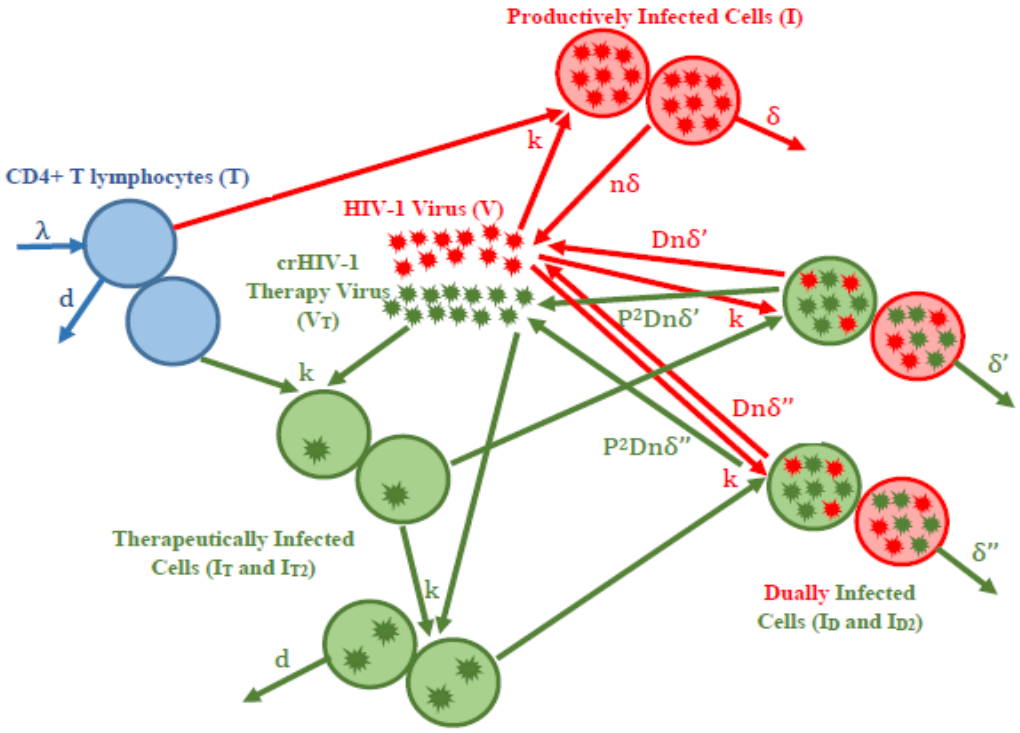

Fig. 1: Schematic Drawing for Super-infection Model of HIV-1 in Vivo Dynamics. The figure shows the overview of the biological process of gene therapy by different arrows and symbols respectively. Blue cells represent normally functioning T cells. Green cells represent therapeutically infected T cells by crHIV-1 virus. Red cells represent productively infected cells by HIV-1 virus. Cells mixed with both crHIV-1 and HIV-1 virus are dually infected cells labelled as green/red.

Both virus, crHIV-1 gene therapy virus (V_T) and HIV-1 virus (V) infects T cells (T) at the same rate. HIV-1 virus (V) converts T cells (T) into productively infected cells (I) which produce HIV virus (V), while crHIV-1 gene therapy virus (V_T) converts T cells (T) into harmless therapeutically infected cells (I_T) cells. Moreover, HIV-1 (V) may infect and convert I_T cells into dually infected cells (I_D) which produce both HIV-1 (V) and crHIV-1 (V_T). In this model, crHIV-1 virus inhibits HIV-1 virus production and reduces the production of productively infected cells (I) by competing for packaging HIV-1 mRNA transcripts [5]. Because crHIV-1 would not be produced without HIV-1 infection, we also assumed that I_T die at the same rate as T cells [6] besides the assumption mentioned in Table 1. The relative value of certain parameters could also be found in Table 2.

Table 1: Equations for super-infection Model of HIV-1 in vivo dynamics.

$\dot{T} = \lambda - dT - kVT - kV_TT$	$\dot{I} = kVT - \delta I$
$\dot{I_T} = kV_TT - dI_T - kVI_T - kV_TI_T$	$\dot{I_{T2}} = kV_TI_T - dI_{T2} - kVI_{T2}$
$\dot{I_D} = kVI_T - \delta' I_D$	$\dot{I_{D2}} = kVI_{T2} - \delta'' I_{D2}$
$\dot{V} = n\delta I + Dn\delta' I_D + D_2 n\delta'' I_{D2} - cV$	
$\dot{V_T} = P^2 Dn\delta' I_D + (2P)^2 D_2 n\delta'' I_{D2} - cV_T$	
$P \to 2P \;\; D \to D_2 \; D \le D_2 \le 2D \;\; \delta'' = D_2\delta$	

For the expanded model, HIV-1 nef (nef protein expression is initiated shorted after HIV infection, which internalize the CD [4], [5] receptor from the cell surface) is down-regulates CD_4 thus inhibiting HIV-1 super-infection. However, crHIV-1 can infect a cell multiple times since it does not encode nef. To better mimic the real physiological situation, we introduced two new equations to the expansion model. For simplicity, we have limited our consideration to the case of crHIV-1 dual infection (i.e. super infection of 2 crHIV-1) where super-infection produces a different species of dually infected cell, a cell that has altered P and D values [8] (illustrated in Fig. 1).

Table 2: Explanation of parameters and values used in the crHIV-1 super-infection gene therapy model

Parameter[a]	Biological interpretation	Value (units)[b]	Reference(s)[c]
λ	Birth rate constant of uninfected $CD4^+$ T cells (T)	31 (cells/[μl × day])	45
d	Death rate of uninfected $CD4^+$ T cells (T)	0.02 (1/day)	36
k	Infection rate of activated $CD4^+$ T cells per virion	1.875×10^{-4} (μl/virions)	no data
δ	Death rate of HIV-1-infected cells (I)	0.7 (1/day)	21,51
δ'	Death rate of dually infected cells (I_D)	δ' = D×δ (1/day)	††
n	Burst size (no. of virions released from HIV-1-infected cell) (I)	200 (†)	17
c	Clearance rate of HIV-1 (V) and crHIV-1 (V_T) virions	30 (1/day)	21,47
P	Fold increase in crHIV-1 mRNA concentration compared to HIV-1 mRNA in cytoplasm of dually infected cells (I_D)	1–100 (†)	††
D	Antiviral effect: fractional therapeutic downregulation that crHIV-1 achieves on HIV-1 virion production from I_D cells	0.0–1.0 (†)	††
T_0	Initial concn of uninfected $CD4^+$ T cells in plasma	800 (cells/μl)	40
V_0	Initial HIV-1 set point (before crHIV-1 administration)	10^5 (virions/ml)	40

[a] Numerous other parameter values were also tested and were not found to produce qualitatively different results. The ranges tested for each parameter are as follows: λ 20 to 50; k, 10^{-3} to 10^{-5}; δ, 0.4 to 0.9; n, 100 to 1,000; T_0, 500 to 1,200; and V_0, 10^4 to 10^7. Furthermore, analytical studies that did not include the viraleclipse phase used c = 3 instead of c = 30. Examples can be found in the SI (http://genomics.lbl.gov/suppinfo/JVI/index.html).

[b] †, Dimensionless parameter.

[c] ††, crHIV-1 can be genetically engineered to change the value of this parameter (see the text), but in vivo measurements have not been carried out.

3. Result

Steady state solutions for equations 1 to 8 were determined to relate the HIV-1 set point to the experimentally controllable parameter P and D. A new, reduced viral set point was described by the expanded crHIV-1 gene therapy model solution. The introduction of crHIV-1 to an HIV-1 infected individual would eventually lead the system to either a new steady state (i.e. crHIV-1 therapy virus propagates), or the original HIV-1 infection set point (i.e. crHIV-1 dies out). The new HIV-1 set point achieved after crHIV-1 therapy was investigated to predict the effect of crHIV-1 gene therapy on HIV-1 virus infected individuals. Incorporating the normal value for CD_{4+} T-cell and HIV-1 concentration in blood plasma into the Expansion Model, we calculated a steady state of HIV-1 in vivo dynamic. The new established steady-state value of V (the HIV-1 set point) as a function of parameters P and D, compared to the previous model were presented. Both figures were presenting steady-state values for $1 < P < 100$ and $0 < D < 1$ for each of them being divided into 100 units.

4. Discussion

In this study the effect of crHIV-1 gene therapy on the HIV-1 in vitro setting point was examined by constructing and analysing a novel expanded mathematical model based upon the basic model of HIV-1 in vivo dynamics, adding I_{D2} and I_{T2} to the system, which represents a dual super-infection from crHIV-1 on I_T. The goal of this therapy is not to fully inhibit HIV-1 but to prevent disease progression by reducing the HIV-1 set point low enough for the patient to be asymptomatic [9]. Algebraic analysis showed that increasing the intracellular mRNA concentration of crHIV-1 relative to that of HIV-1 is the most important consideration for establishing a persistent crHIV-1 infection and reducing the HIV-1 set point. By applying proper parameter values [10], the HIV-1 set point has been significantly lowered down to 10^2 grade and Matlab plots have been reproduced with almost the same trends.

For the super-infection model, when $P<3.5$ (more HIV-1 than crHIV-1 was produced from I_D cells), crHIV-1 virus dies out (the red region in Fig. 2). As P is increased in value beyond 3.5 (i.e., a crHIV-1 hypothetically engineered to have threefold-higher cytoplasmic mRNA concentration), crHIV-1 virus reaches a steady state of coexistence with HIV-1, and the HIV-1 set point is reduced compared to the original pre-therapy set point. Furthermore, reduction in the HIV-1 set point is influenced by the parameter P when certain value of D is used. As P increases in value (increased crHIV-1 production from I_D cells), the HIV-1 set point is reduced. It can also be influenced by the parameter D (therapeutic reduction in HIV-1 titer produced). When $D>0.15$, as D decreases in value (reduced HIV-1 production from I_D cells), the HIV-1 set point is also reduced. However, if HIV-1 down-regulation is highly efficient ($D<0.15$), crHIV-1 propagation itself is constrained by the dearth of HIV-1 packaging proteins in the cell. In fact, as D is decreased to 0.15, viral production from I_D cells becomes so efficiently inhibited that these cells stop producing virus altogether and crHIV-1 infection and propagation within the individual die out. Importantly, I cells still produce HIV-1 at the same rate, allowing the HIV-1 set point to rebound to the pre-therapy level. We refer to this rebound effect as crHIV-1 “shooting itself in the molecular foot” [5]; crHIV-1 inhibits HIV-1 so efficiently that its own production is inhibited. Very importantly, if P and D are optimized, crHIV-1 gene therapy can reduce HIV-1 set point to levels comparable to HAART reduction. The optimal value of D is approximately 0.15-0.18 for the parameter values used here (the lowest region in Fig. 2). It should also be noted that, as mentioned above, the altered death rate algebraically cancels out of the steady-state solutions of all populations (except the I_D population). This occurs because viral production and cell death are reciprocally linked: increasing cell death increases the viral production term by exactly the same amount that infected cell lifetime is decreased [11].

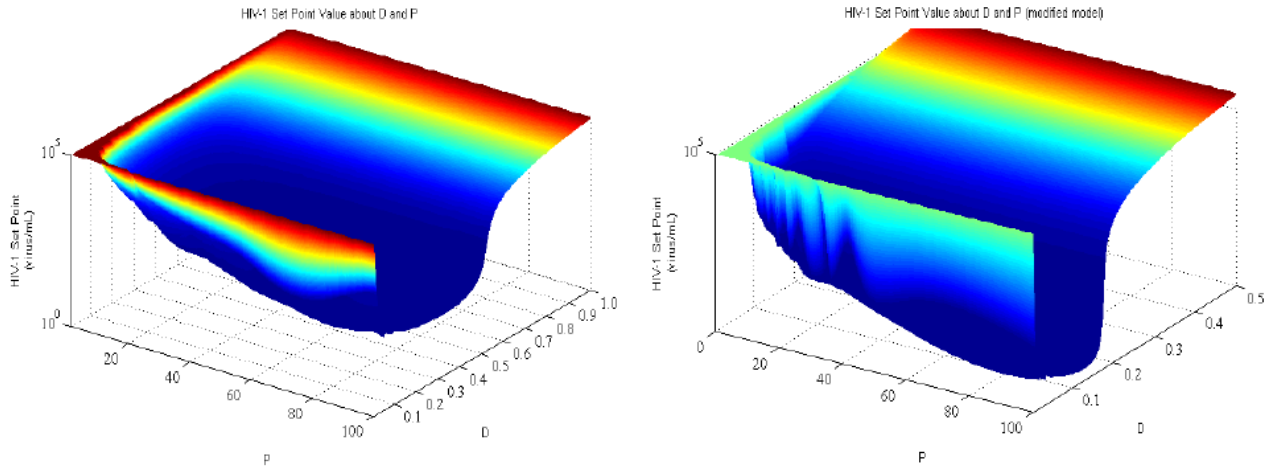

Fig. 2: HIV-1 Set Point with all D & P Values for Original Model and Super-Infection (Dual) Model. The U curve represents the conditionally replicating HIV-1 vector (crHIV-1) gene therapy vectors interfering with wild-type HIV-1 replication.

Representative simulations demonstrate how the new reduced HIV-set point was reached after the administration of the crHIV-1 compared to the basic model discussed in the previous study (shown in the Fig. 3). The basic trend for the new simulation result was the same compared to the previous one containing six

equations. However, due to the different P and D values, a relatively larger drop for the lowest set point could be observed in the revised model. Also, new established steady-state had a relatively lower value without rebound. The simulation results highlight the critical importance of maximizing the ratio of crHIV-1 to HIV-1 virus generated from dually infected cells [5]. This ratio can be improved by either increasing the quantity of cytoplasmic crHIV-1 mRNA or increasing the efficiency of their infection into viron particle [12]. The simulation model and its corresponding results could be further tested in vivo and as a result, the study and development of this therapy is relatively crucial for treatment to the HIV-1 infection.

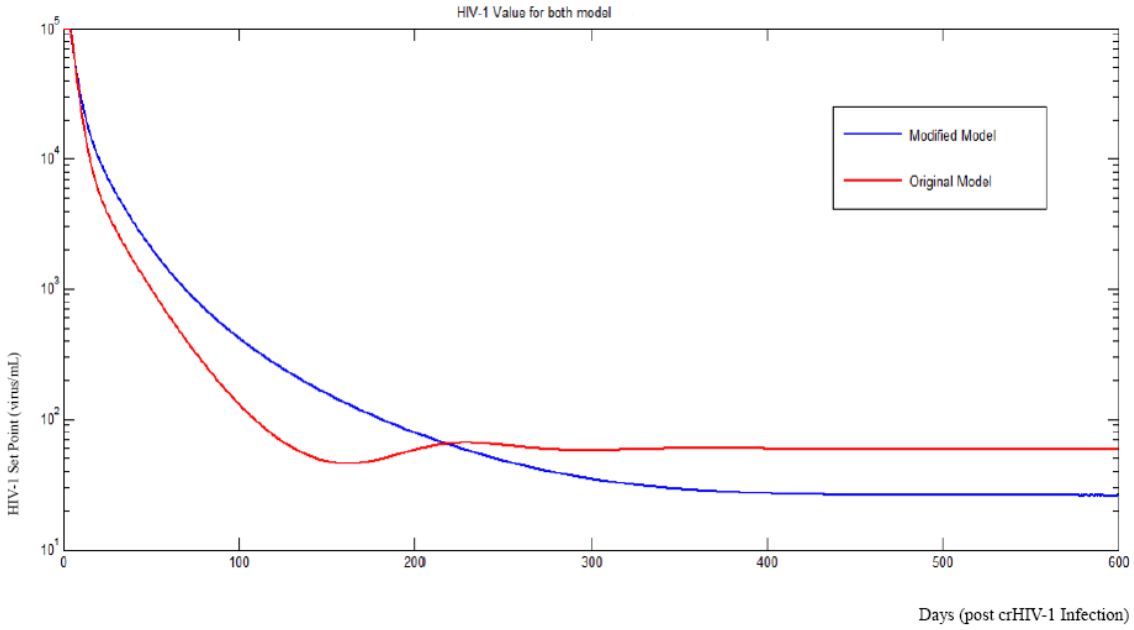

Fig. 3: HIV-1 Set Point Comparison between Original and Super-Infection Model (P=100 D=0.195). Certain rebound phenomenon observed on the original model can be partially eliminated by the modification on the super-infection model and even lower set point is being created simultaneously.

In order to simulate this model more efficiently, certain ideas were recommended to future group who wish to review this model. First, it might be more efficient to plot graphs by using a more powerful software, origin for example, rather than Matlab. Second, an ultra-powerful computer can be used to reduce the calculation time and to store much more data for analysis. Or it is better to lessen the data amount so as to shorten the simulation time. The specs of the computer being used in this study were i5-750 for CPU and ATI Radeon HD 4550 for graphic cards with 4 GB (2*2GB) of RAM, operated in 32-bit Window 7 system. Two of the PCs with listed specs were used in parallel for approximately 10 days to obtain the results while one of them ran out of the memory in the middle of the calculation (at 7th day) followed by reduced iteration time to ease the calculation. Furthermore, before simulation, it is suggested to confirm the proper initial values since a slight different in these values could result in a large variation in the final plots. Due to the certain limitation of the apparatus within the research centre where the authors conducted their project, the simulation results couldn't be further verified with in-vitro or in-vivo test. However, it is encouraged for the followed up researchers to dig deeper with triple infection model or even more verifying the same trend of the super-infection model and simulated data.

5. Future Perspective

There are different relative benefits and risks associated with crHIV-1 gene therapy. Conditionally replicating gene therapy vectors, once mobilized by wild-type HIV-1, have a distinct advantage over both autonomously replicating and non-replicating vectors from the standpoint of long-term therapeutic intervention for HIV-1. Self-replicating or competing HIV-1 therapy viruses have been theoretically examined but have the potential to outcompete HIV-1 and run the risk of establishing a new uncontrolled infection by the principle of competitive exclusion. However, the most significant problem of the crHIV-1 approach is the efficiency for its treatment. The development of this genetic therapy within the

HIV-1-infected population attracts significant concerns, but it may also be worthwhile to achieve the accompanying benefit: can we integrate and turn this gene therapy into pre-clinical vaccines among the next generation?

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