

## IMMUNOTHERAPY OF HIV-1 INFECTION

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### ABSTRACT

A number of lines of evidence suggest that immunotherapy with the cytokine interleukin-2 (IL-2) may boost the immune response to fight HIV infection.  $CD4^+$  T cells, the cells which orchestrate the immune response, are also the cells that become infected by the HIV virus. These cells use cytokines as signaling mechanisms for immune-response stimulation, growth and differentiation. Since  $CD4^+$  T cells are hampered due to HIV infection, normal signaling, and the resulting cascade, may not occur. Introduction of IL-2 into the system can restore or enhance these effects. We illustrate, through mathematical modeling, the effects of IL-2 treatment on an HIV-infected patient. With good comparison to existing clinical data, we can better understand what mechanisms of immune-viral dynamics are necessary to produce the typical disease dynamics.

*Keywords:* HIV,  $CD4^+$  T cells, turnover rates, production rates, interleukin, immunotherapy, mathematical model, ordinary differential equations (ODEs), lymph system.

### 1. Introduction

At present, the capacity to eliminate virus completely from an HIV-infected patient has not been demonstrated. HIV infection induces a condition of immunosuppression, among other disease traits. Most chemotherapies are aimed at killing or halting the pathogen, but a treatment which can boost the immune system can serve to help the body fight infection on its own. Efforts to boost the immune response while concomitantly reducing viral load (i.e. with antiviral drugs) are now being pursued. This brings new hope to the treatment of HIV infection, and it is this type of treatment which we explore.

Cytokines are protein hormones which mediate both natural and specific immunity. Cytokines are produced mainly by activated cells (lymphocytes) during cellular-mediated immunity. Interleukin-2 (IL-2) is the main cytokine responsible

for lymphocyte activation, growth and differentiation. It is produced by  $CD4^+$  T cells, and in lesser quantities by  $CD8^+$  T cells (cytotoxic T cells, or CTLs). This is important for AIDS studies since it is the  $CD4^+$  T cells that become infected with HIV. IL-2 acts on the same cells that produce it. Therefore it is referred to as an *autocrine* growth factor; although it can also act on nearby T lymphocytes (hence a *paracrine* growth factor).

Clinical trials have shown that there are immune-stimulation effects from treatment with interleukins [1,2]; although, as yet this immunotherapy has not received federal approval. IL-2 has been shown to enhance CTL activity, at different disease stages. Also, there is a restoration of defective natural killer (NK) cell activity as well as enhancement of polyclonal expansion of  $CD4^+$  and  $CD8^+$  T cells. Clinical trials show that there is a high correlation between low IL-2 concentrations and the decrease in  $CD4^+$  T cell numbers and with disease progression [3]. There is evidence for reduced IL-2 to undetectable levels in the lymph nodes at all stages of disease.

Since IL-2 has been shown to, at least partially, restore some of the immune functions known to be impaired by HIV infection, we explore the use of this cytokine through modeling.

Our objective is to develop a mathematical model of the dynamics of disease progression and IL-2 treatment of the HIV-infected immune system. Our model is based upon the key markers of HIV progression -  $CD4^+$  T cell levels and viral levels in the plasma, for which there exists extensive data. The typical dynamics of the disease progression, in an untreated individual, for these populations is shown in Fig. 1 [4,5].

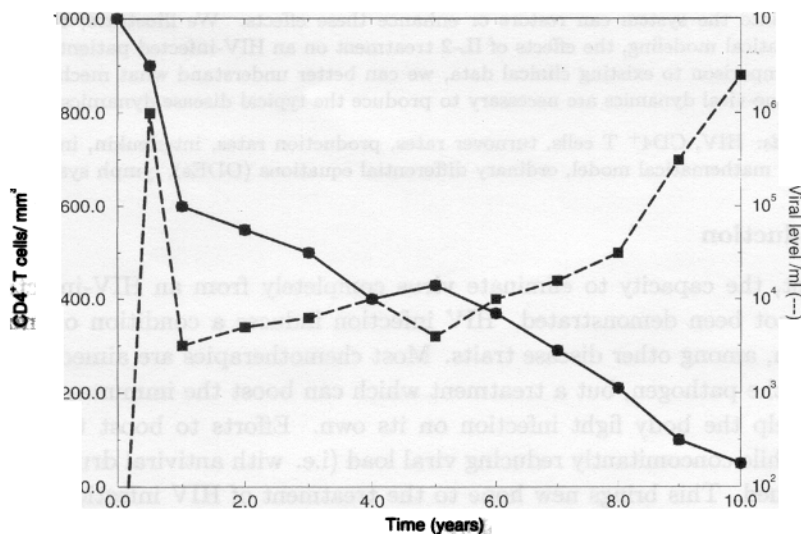


Fig. 1. Typical course of an HIV-infected individual is shown over 10 years. This data from [Pennisi and Cohen, 1996]. The typical markers of disease progression are the  $CD4^+$  T cell and viral counts.

In this representation the  $CD4^+$  T-cell count declines approximately linearly from  $1000/\text{mm}^3$  to  $0/\text{mm}^3$  over 10 years. At the same time there is a gradual increase in the viral count during the asymptomatic stage of the disease and then a rapid increase of several orders of magnitude during the last stage of AIDS. Once we have a model that mimics clinical outcomes for infection with HIV, we can then explore the role of IL-2 immunotherapy.

These questions concerning immunotherapy can be formulated with differential equations models. The solutions of these differential equations can then be simulated to check for consistency with known and hypothesized dynamic information. HIV infection offers the opportunity of developing such models because of the unique character of its disease progression and the extensive data available for designing and comparing models. In this paper we will formulate a model of HIV progression (Sec. 2), use it to explore treatment with immune-system enhancing drugs such as interleukin-2 (Sec. 3), and, in Sec. 4, we include a discussion of the implications of our model for immunotherapy as well as for the long-term dynamics of HIV disease progression.

## 2. A Model of HIV Progression

In our model of HIV progression we consider the uninfected  $CD4^+$  T cell population,  $(T(t)/\text{mm}^3)$ , and the free virus population  $(V(t)/\text{ml})$  interacting in the plasma. In earlier work, [6–9], we included the class of infected  $CD4^+$  T cells, but our objective here is to demonstrate that the dynamics of HIV progression in the plasma can be based upon simple assumptions about the interactions of uninfected  $CD4^+$  T cells and free virus. Since there is extensive data for these two populations during the progression, the model simulations can be compared to data. The equations of the model are as follows:

$$\frac{dT(t)}{dt} = s_1 - \frac{s_2 V(t)}{b_1 + V(t)} - \mu T(t) - kV(t)T(t), \quad (1)$$

$$\frac{dV(t)}{dt} = \frac{gV(t)}{b_2 + V(t)} - cV(t)T(t). \quad (2)$$

In (1) the term  $s_1 - \frac{s_2 V(t)}{b_1 + V(t)}$  represents the source/proliferation of uninfected  $CD4^+$  T cells which includes both an external (not plasma) contribution of cells from sources such as the thymus and lymph nodes, and an internal (plasma) contribution from  $CD4^+$  T cell differentiation. This T-cell source deteriorates during the progression with limiting value  $s_1 - s_2$ . In (1) there is a natural loss  $-\mu T(t)$  of uninfected  $CD4^+$  T cells that is not influenced by the presence of the virus (this could include natural death or migration out of the plasma into the lymph). There is also a loss  $-kV(t)T(t)$  from the uninfected class of  $CD4^+$  T cells that become infected by virus; this is assumed proportional to the product of uninfected  $CD4^+$  T cells and virus (i.e. mass action).

In (2) there is a source of virus  $\frac{gV(t)}{b_2 + V(t)}$  that accounts for viral contributions to the plasma from both external compartments such as the lymph system as well

as virus produced by infected cells in the plasma. It is known that most of the virus resides in the lymph system which becomes saturated in the asymptomatic phase of the disease [10–12]. We thus choose this viral-source term in saturated form with limiting value  $g$ . In (2) there is also a loss rate of virus  $-cV(t)T(t)$ , that incorporates removal of virus due to all the components of the immune response, as well as viral death. We thus assume that the viral clearance rate depends on the  $CD4^+$  T cell level, which although is not directly responsible for clearance of free virus (apart from the infection process itself), represents the general capacity of the immune system to eliminate virus in the plasma. This idea has been recently confirmed in [26]. We further assume that this capacity diminishes during disease progression, since it is proportional to the  $CD4^+$  T-cell level. This follows since the number of  $CD4^+$  T cells has been shown to be the best predictor of the capacity of the immune system [c.f. 13].

Table 1. Variables and parameters.

<u>Dependent Variables</u>	<u>Initial Values</u>
$T$ = Uninfected $CD4^+$ T cell population	$1000/\text{mm}^3$
$V$ = HIV population	$10^3/\text{ml}$
<u>Parameters and Constants</u>	<u>Values</u>
$s_1$ = source/production of $CD4^+$ T cells	$2.0 \text{ mm}^3 \text{d}^{-1}$
$s_2$ = source/production of $CD4^+$ T cells	$1.5 \text{ mm}^3 \text{d}^{-1}$
$\mu$ = death rate of uninfected $CD4^+$ T cell population	$0.002 \text{d}^{-1}$
$k$ = rate $CD4^+$ T cells becomes infected by free virus $V$	$2.5 \times 10^{-4} \text{ mm}^3 \text{d}^{-1}$
$g$ = input rate of external viral source	$30 \text{ d}^{-1} \text{ mm}^3$
$c$ = loss rate of virus	$0.007 \text{ mm}^3 \text{d}^{-1}$
$b_1$ = half saturation constant	$14.0 \text{ mm}^3$
$b_2$ = half saturation constant	$1.0 \text{ mm}^3$
$r(t)$ = Interleukin treatment function	$c_1 t e^{(-c_2 t)} \text{d}^{-1}$
$c_1$ = treatment parameter	given in figure legends
$c_2$ = treatment parameter	given in figure legends

In Table 1 we provide a list of parameters for (1) and (2). Previously, we have modeled the HIV-immune system dynamics using a third ordinary differential equation (ODE) monitoring the change in the HIV-infected class of T cells. A discussion of the choice for parameter values for the three-ODE model is given in those papers [6–9]. Here, we choose rate constants in a similar way, adjusting for the fact that we have two equations rather than three. The model assumptions incorporate key aspects of the HIV-immune dynamics, and we are able to therefore accurately simulate disease progression. In Fig. 2 we provide a simulation of the solution to (1) and (2) with initial values  $T(0) = 1000/\text{mm}^3$  and  $V(0) = 3 \times 10^3/\text{ml}$ .

We remark that we do not model the initial viremia that occurs in the early weeks of infection, but begin the progression at the “set-point” established after

the initial viremia [14]. The correlation of this viral set-point to the duration of the progression indicates that the dynamics of the post-set-point HIV progression can be modeled by autonomous differential equations; that is, equations without explicit time dependence in the terms or parameters.

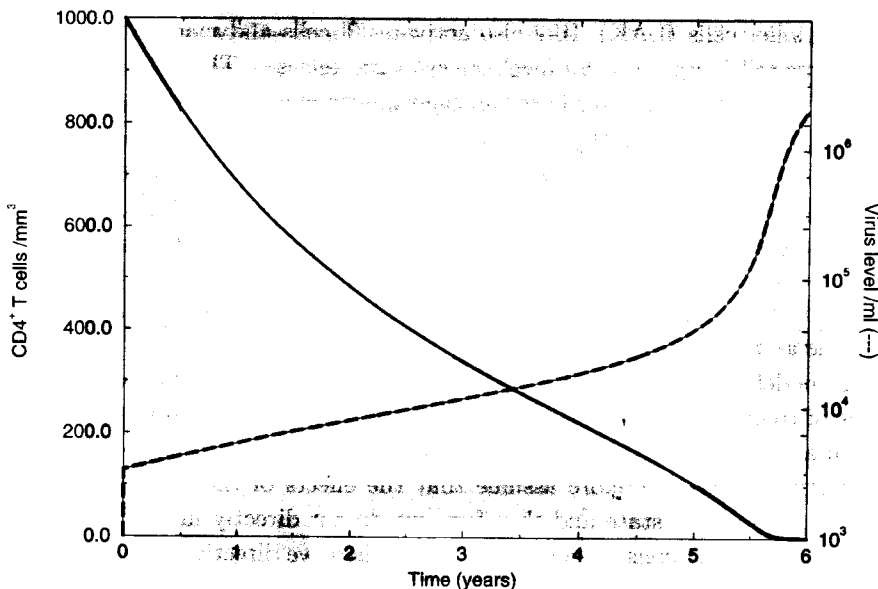


Fig. 2. Disease progression. The numerical simulation of the model (1)-(2) with parameter values from Table 1.

For the disease-free state  $\bar{T} = \frac{s_1}{\mu}$  and  $V = 0$  in (1) - (2), an analysis of the Jacobian yields the eigenvalues  $\{-\mu, \frac{g}{b_2} - c\bar{T}\}$ . It then follows that the disease free state is stable when  $g < cb_2\bar{T}$ . If this inequality is reversed, then the virus population will always grow from even very small levels until the inevitable CD4<sup>+</sup> T-cell collapse. This inequality implies that in order to eradicate the disease or even to halt disease progression treatment must either sufficiently suppress all viral production ( $g$ ), boost the CD4<sup>+</sup> T-cell count ( $\bar{T}$ ) or the immune response ( $c$ ), or a combination of these. For the parameter values in Table 1 the system (1)-(2) has no steady-state with  $V > 0$ , and the system has the following asymptotic behavior as  $t$  increases:  $V(t)$  increases without bound,  $T(t)$  decreases to zero,  $V'(t)$  increases to  $g - \frac{c(s_1 - s_2)}{k}$ ,  $T'(t)$  converges to zero, and  $V(t)T(t)$  converges to  $\frac{s_1 - s_2}{k}$  since the system can never stabilize to a positive equilibrium in the presence of virus.

### 3. A Model of Immunotherapy

Cytokines are protein hormones which mediate both natural and specific immunity. In natural immunity, they are most often produced by macrophages/monocytes in

response to antigen-stimulated T cells as part of specific immunity. Most cytokines in specific immunity are produced by activated lymphocytes. Interleukin-2 (IL-2) is the main cytokine responsible for lymphocyte activation, growth and differentiation. It is produced by CD4<sup>+</sup> T cells, and in lesser quantities by CD8<sup>+</sup> T cells.

IL-2 is known under high concentrations to stimulate the growth of natural killer cells (NK) and their cytolytic functions; hence, producing the lymphokine-activated killer cells (LAK). IL-2 also activates T cells and macrophages together with an overall increase in all levels of cytokine release. The rationale for using cytokines in treatment is thus based on their ability of enhancing these components of cellular-mediated immunity.

There is evidence that IL-2 also induces HIV production. Results indicate that the negative effects are most pronounced when HIV levels are high, and CD4<sup>+</sup> T cell counts are below 200/mm<sup>3</sup>. In asymptomatic patients, however, IL-2 does not result in a sustained increase in HIV replication. There are also other harsh side effects which can result in cessation of treatment. These include, capillary leakage syndrome as well as other toxic side effects.

Our model of immunotherapy is based upon patient data from two recent clinical studies of treatment with interleukin-2 given in [1,2]. In both studies, patients had been on a variety of antiviral treatments for at least one month prior to beginning this treatment. We therefore assume that the effects of the antiviral treatments are in a quasi-steady state and therefore, we do not directly model that aspect of treatment. We have considered other models where we directly model the effects of antiviral treatment on the course of the disease progression [c.f. 7-9].

In [1] 31 HIV infected patients were treated with 6 cycles of IL-2 with each cycle consisting of 5 days of intravenous infusions separated at two month intervals. Patients had CD4<sup>+</sup> T cell counts with a mean of 427/mm<sup>3</sup> (range 188 to 753) at the start of treatment. The mean viral count at the start of treatment for these patients was approximately  $2 \times 10^4$ /ml (with range  $5 \times 10^3 - 9.5 \times 10^4$ /ml). The patients experienced a mean CD4<sup>+</sup> T-cell count increase up to 916/mm<sup>3</sup> at month 13. No significant change was seen in the mean of the viral counts during the 12 month period of the study. The mean dose level per cycle decreased from 76 million IU for cycle 1 to 39 million IU for cycle 6.

The corresponding model of bi-monthly infusions of IL-2 for this study is as follow:

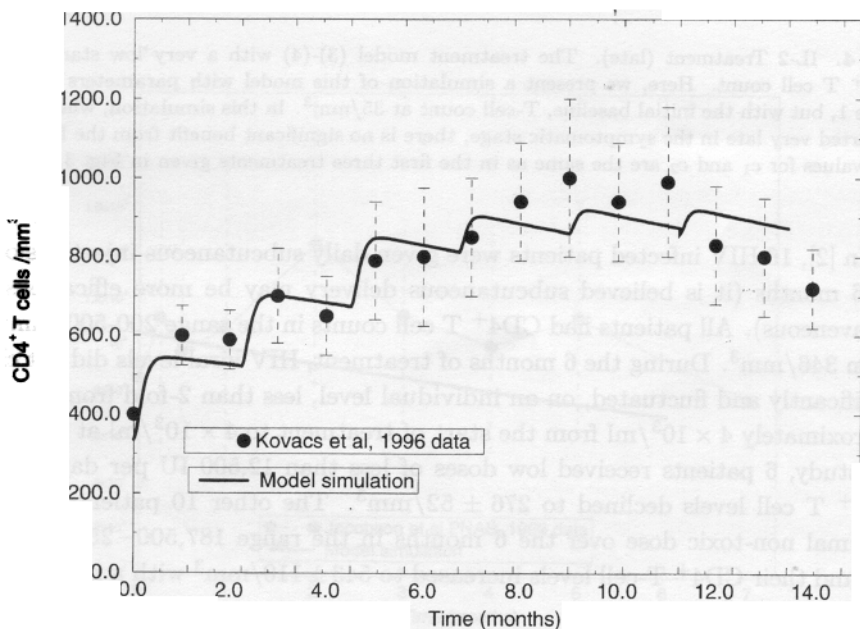
$$\frac{dT(t)}{dt} = s_1 - \frac{s_2 V(t)}{b_1 + V(t)} - \mu T(t) - kV(t)T(t) + r(t)T(t), \quad (3)$$

$$\frac{dV(t)}{dt} = \frac{gV(t)}{b_2 + V(t)} - cV(t)T(t), \quad (4)$$

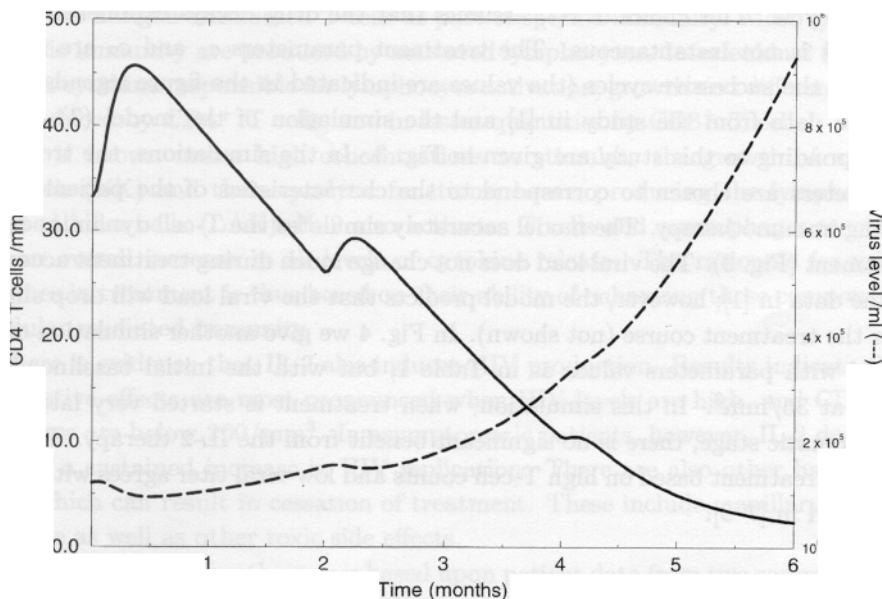
where in (3) it is assumed that the enhancement of the immune system through IL-2 results in an increase in the CD4<sup>+</sup> T cells proportional to the population of these cells at the rate  $r(t) = c_1 t e^{(-c_2 t)}$ /day, where  $t$  is reset to zero at the start

of each cycle. This choice of  $r(t)$  assumes that the drug decays exponentially, but its effect is not instantaneous. The treatment parameters  $c_1$  and  $c_2$  are reduced through the successive cycles (the values are indicated in the figure legends).

The data from the study in [1] and the simulation of the model (3) and (4) corresponding to this study are given in Fig. 3. In the simulations, the treatment parameters are chosen to correspond to the characteristics of the patients in [1] during immunotherapy. The model accurately simulates the T-cell dynamics during treatment (Fig. 3). The viral load does not change much during treatment according to the data in [1]; however, the model predicts that the viral load will drop slightly over the treatment course (not shown). In Fig. 4 we give another simulation of this model with parameters values as in Table 1, but with the initial baseline, T-cell count at  $35/\text{mm}^3$ . In this simulation, when treatment is started very late in the symptomatic stage, there is no significant benefit from the IL-2 therapy. This idea of early treatment based on high T-cell counts and low viral titer agrees with results presented in [7–9].



**Fig. 3.** IL-2 treatment. The treatment model (3)-(4) is compared with data from [1]. The model parameters are chosen to correspond to the characteristics of the patients in [1] and the treatment parameters are chosen to simulate the changes in CD4<sup>+</sup> T cell counts during the 6-cycle, bi-monthly, infusion immunotherapy. The treatment function is:  $r(t) = c_1 t e^{-c_2 t}$ , where  $c_1$  and  $c_2$  vary over the course of the six treatments, beginning with a large dose and ending with a smaller dose, as follows: treat 1:  $c_1 = 0.08$ ,  $c_2 = 0.4$ ; treat 2:  $c_1 = 0.05$ ,  $c_2 = 0.4$ ; treat 3:  $c_1 = 0.04$ ,  $c_2 = 0.4$ ; treat 4:  $c_1 = 0.03$ ,  $c_2 = 0.5$ ; treat 5:  $c_1 = 0.02$ ,  $c_2 = 0.5$ ; treat 6:  $c_1 = 0.02$ ,  $c_2 = 0.5$ . Shown here are the T-cell data and corresponding simulation.

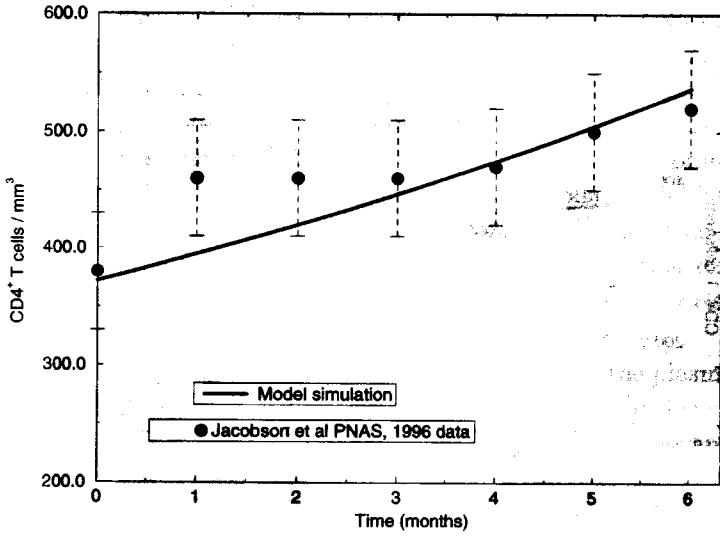


**Fig. 4.** IL-2 Treatment (late). The treatment model (3)-(4) with a very low starting level of CD4<sup>+</sup> T cell count. Here, we present a simulation of this model with parameters values as in Table 1, but with the initial baseline, T-cell count at 35/mm<sup>3</sup>. In this simulation, when treatment is started very late in the symptomatic stage, there is no significant benefit from the IL-2 therapy. The values for  $c_1$  and  $c_2$  are the same as in the first three treatments given in Fig. 3.

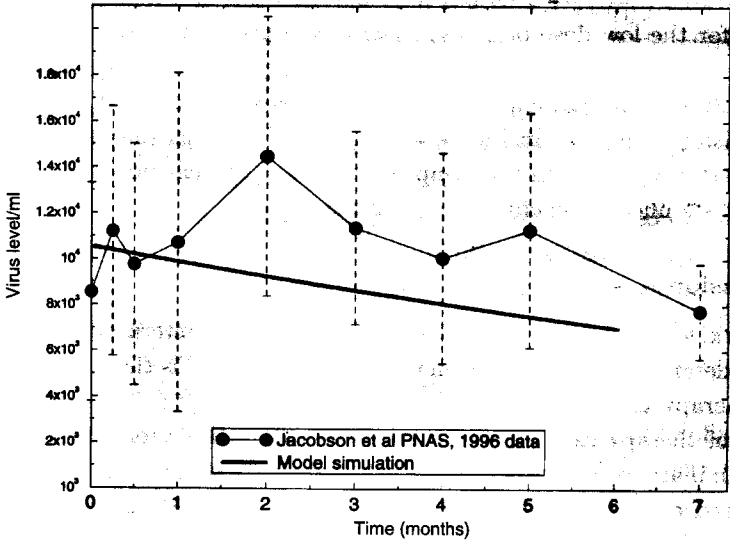
In [2], 16 HIV infected patients were given daily subcutaneous injections of IL-2 for 6 months (it is believed subcutaneous delivery may be more efficacious than intravenous). All patients had CD4<sup>+</sup> T cell counts in the range 200-500/mm<sup>3</sup> with mean 346/mm<sup>3</sup>. During the 6 months of treatment, HIV viral levels did not change significantly and fluctuated, on an individual level, less than 2-fold from a mean of approximately  $4 \times 10^3$ /ml from the start of treatment to  $4 \times 10^3$ /ml at the end. In the study, 6 patients received low doses of less than 12,500 IU per day and their CD4<sup>+</sup> T cell levels declined to  $276 \pm 52$ /mm<sup>3</sup>. The other 10 patients received a maximal non-toxic dose over the 6 months in the range 187,500–250,000 IU per day and their CD4<sup>+</sup> T-cell levels increased to  $543 \pm 110$ /mm<sup>3</sup> with a mean monthly gain of 27/mm<sup>3</sup> per month.

The data for this study and the corresponding model simulations are given in Fig. 5. The model of daily injections of IL-2 for the study in [2] is again given by (3) and (4), where the treatment function  $r(t)$  is taken to be constant (assuming the treatment can be approximated by a continuous process):  $r(t) = .003$  for the maximal, nontoxic dose (Figs. 5a,b) and  $r(t) = 0.0001$  for the low dose patients (Fig. 5c). The effects of treatment on the viral burden are presented in Fig. 5b. These are averaged-viral concentrations for the high-dose patients (viral data was



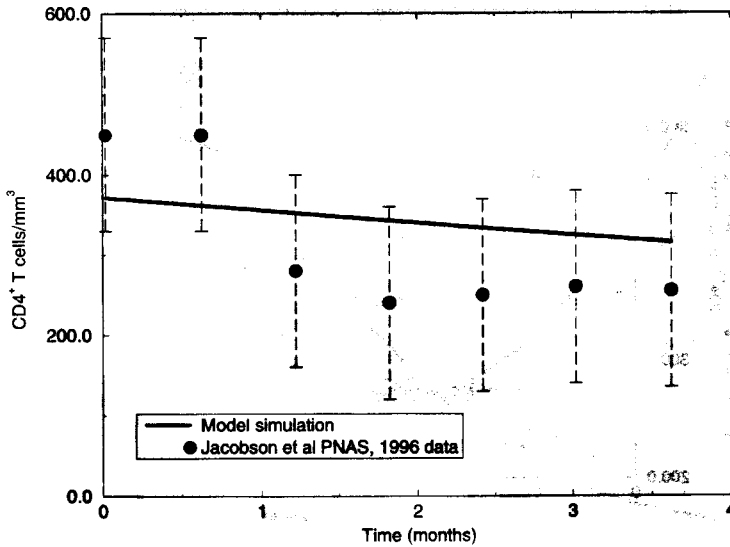


(a)



(b)

**Fig. 5.** IL-2 Treatment (continuous). The treatment model (3)-(4) is compared with data from [2]. There are two treatment scenarios here. The model of daily injections (continuous infusions) of IL-2 for the study in [2] is again given by the model (3)-(4), where the treatment functions are chosen constant:  $r(t) = 0.003$  for the maximal, nontoxic dose (Panel 5a,b) and  $r(t) = 0.0001$  for the low dose patients (Panel 5c). Data were presented in [2] only for the viral burden during IL-2 treatment for the high-dose case. We present it, together with the model simulation, in Panel 5b.



(c)

Fig. 5 (Cont'd)

not given for the low dose patients). The model parameters (except for the treatment function  $r(t)$ ) are chosen as in the bi-monthly infusion model above, since the characteristics of the patients in the two studies are similar. The late treatment scenario also fails here as in Figure 4 (not shown). In this simulation, when treatment is started very late in the symptomatic stage (T cells below  $100/\text{mm}^3$ ), there is again no significant benefit to the IL-2 therapy.

#### 4. Discussion

In this work, we present and study a simple model for the interaction of CD4<sup>+</sup> T cells and virus describing the progression to AIDS. This model is then used to explore an immunotherapy treatment strategy, namely using the cytokine IL-2. We find that this type of therapy can be successful in delaying AIDS progression. This agrees with preliminary results from clinical trials. We also find that immunotherapy administered during the early stages of disease progression is the most beneficial for raising CD4<sup>+</sup> T-cell counts.

There have been many recent mathematical models of the HIV-infected immune system [10,15–20]. The most influential have been the quasi-steady state models that were used to determine the short-time effects of powerful antiviral drugs on the T-cell and viral populations. Many key hypotheses about immune-HIV dynamics have been proposed through these efforts. We attempt to address these key questions here by exploring existing ideas, and comparing the results with those of our model.

The first question we address is: why do high concentrations of virus continue to appear in the bloodstream at the end stage of disease, even though its primary target and source of production, the  $CD4^+$  T cells, become almost completely depleted? In our model (1)-(2), the reasons for the rapid growth of plasma virus during the end stage of disease are that since the supply of  $CD4^+$  T cells deteriorates over the long disease progression and thus the immune response collapses with the  $CD4^+$  T-cell collapse then the viral influx to the plasma compartment continues from the saturated, external, lymph source with no means of clearance. Other explanations for this dynamics have been proposed by the antigenic variation models of Nowak *et al.* [21,22] and the "sink" model of Ho *et al.* [14,15].

In the model of HIV progression given by Eqs. (1) and (2) the plasma  $CD4^+$  T cell population and plasma viral population cannot stabilize to positive values. The collapse of the  $CD4^+$  T cells to zero, together with the rapid appearance of virus at the end-stage of disease has been called a paradox, because the  $CD4^+$  T cells are the primary source of viral production. The interpretation of these phenomena provided by model (1)-(2) is that the virus detected in the plasma is produced primarily by external sources, such as those in the lymph nodes. This external contribution of viral production to the plasma does not increase rapidly at the end-stage of disease, but instead gradually approaches a saturated value. Also, the form of Eq. (2) implies that the external viral source  $\left(\frac{gV(t)}{(b_2+V(t))}\right)$  slowly increases and that the viral loss  $(-cT(t)V(t))$  slowly decreases (since it depends on  $T(t)$ ); hence the viral population can grow unbounded. The form of Eq. (1) then yields the eventual collapse of the  $CD4^+$  T cell population. Thus, the inability of the plasma  $CD4^+$  T cell population to stabilize to a positive value arises directly from the form of equations. Although the qualitative behavior of the solutions of the model (1)-(2) arises from the form of the equations that are based on assumptions of the known biological processes, the close fit of the model simulations to data argues the feasibility of this interpretation of HIV disease progression.

A second question we can address relates to the production rates of T cells: is there an above normal rate of  $CD4^+$  T cell turnover during the pre-symptomatic stage of the disease as suggested in [14-16,23,24]?

The "sink model" hypothesizes that the  $CD4^+$  T-cell population collapse occurs gradually through years of asymptomatic disease with high daily turnover rates of production and destruction of these cells. In our model, (1)-(2), this hypothesis is not required to explain the disease progression. In (1) we do not include a proliferation term, only a source term of new cells. Thus, the daily source of  $CD4^+$  T cells is not higher than the normal, non-infection value,  $s_1$ , but is instead lower than normal, due to the presence of infection, and decreasing to the value  $s_1 - s_2$ . This implies that the above normal turnover rate is not required to obtain the disease progression. In previous work by the authors [27] we included a proliferation term together with the source term; to obtain the observed disease progression, the proliferation rate had to be about twice that of normal. This proliferation term had

no significant effects on the disease progression simulations in this simplistic model, (1)-(2), hence we did not include it. In [25] it is claimed that telomere endings of  $CD4^+$  T cells in HIV infected patients show no shortening above normal. Telomere endings of chromosomes shorten each cell division and thus indicate no evidence for increased  $CD4^+$  T cell proliferation during HIV infection. Wolthers *et al.* [25] suggest instead that this depletion is due to the gradual decline of the primary source of these cells, rather than their high rate of destruction.

A third question we can ask is: do the daily loss rates of both viral and  $CD4^+$  T cells populations change during disease progression or do they remain constant throughout infection as suggested in [10]?

The linear, constant coefficient model in [10] assumes that the populations are in steady state, and such terms are useful for modeling short-term dynamics. The Eqs (1)-(2), however, model the long-term dynamics of HIV infection. During disease progression the  $CD4^+$  T cell and viral populations both change by three or more orders of magnitude in the plasma. The nonlinear, nonconstant coefficient model (1)-(2) gives accurate simulations of these extreme changes during HIV-disease progression. An essential element of this model is that the loss rate of plasma virus in (2) is dependent on the  $CD4^+$  T-cell count. This represents the capacity of the immune system to clear the virus, and diminishes as disease progresses.

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