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HIV evolution and progression of the infection to AIDS

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HIGHLIGHTS

- ▶ A possible mechanism which enables HIV to break from immune control.
- ▶ Model describing evolving HIV and specific CTL response and helper T cells.
- ▶ Two thresholds: the immune activation threshold, the immunodeficiency threshold.
- ▶ Interval between thresholds corresponds to the asymptomatic stage of HIV infection.

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ABSTRACT

In this paper, we propose and discuss a possible mechanism, which, via continuous mutations and evolution, eventually enables HIV to break from immune control. In order to investigate this mechanism, we employ a simple mathematical model, which describes the relationship between evolving HIV and the specific CTL response and explicitly takes into consideration the role of CD4⁺T cells (helper T cells) in the activation of the CTL response. Based on the assumption that HIV evolves towards higher replication rates, we quantitatively analyze the dynamical properties of this model. The model exhibits the existence of two thresholds, defined as the immune activation threshold and the immunodeficiency threshold, which are critical for the activation and persistence of the specific cell-mediated immune response: the specific CTL response can be established and is able to effectively control an infection when the virus replication rate is between these two thresholds. If the replication rate is below the immune activation threshold, then the specific immune response cannot be reliably established due to the shortage of antigen-presenting cells. Besides, the specific immune response cannot be established when the virus replication rate is above the immunodeficiency threshold due to low levels of CD4⁺T cells. The latter case implies the collapse of the immune system and beginning of AIDS. The interval between these two thresholds roughly corresponds to the asymptomatic stage of HIV infection. The model shows that the duration of the asymptomatic stage and progression of the disease are very sensitive to variations in the model parameters. In particular, the rate of production of the naive lymphocytes appears to be crucial.

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1. Introduction

1.1. Background

The dynamics of human immunodeficiency virus (HIV) within a host, compared with other viral infections, demonstrates a few rather unusual features. Typically, HIV infection progresses through four distinctive stages (Levin et al., 2001; Pantaleo et al., 1993; Pantaleo and Fauci, 1995): after an instance of infection, there is a short (3–4 weeks on average) latent period (“establishing”). The latent period is followed by an acute influenza-like stage, which

lasts for 3–4 weeks on an average and is characterized by a rapid explosion of viral load, which may reach millions virus particles per milliliter of blood (Piatak et al., 1993). The acute period ends with an activation of the adaptive immune response that quickly suppresses the viral load to a very low level. However, in contrast to many other viral infections, in the case of HIV the immune response fails to completely clear the virus from the host body (this is viewed as the first unusual feature of HIV infection). Although the activated adaptive immune response causes a massive drop of virus load, HIV is still able to maintain its presence at very low (in some cases clinically undetectable) levels, and the disease enters the third stage when the infection exposes no symptoms. During the asymptomatic stage the viral load gradually grows (this growth is another unusual feature exhibited by HIV). If a case is left untreated, the asymptomatic stage lasts from a few weeks to 20 years or longer, and in

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some cases (“elite controllers” or “elite suppressors”), it is life-long. The asymptomatic stage ends when the immune system suddenly collapses, the virus load explodes and the disease enters its final stage, namely the acquired immunodeficiency syndrome (AIDS). Death of the patient then follows from opportunistic infections or cancer.

This scenario involves a number of questions, such as:

- (1) Why the immune system fails to completely clear virus, and what makes the survival of HIV under pressure of the immune response possible (even if it survives at a very low level)? For some viral infections a shift from an acute stage to a chronic stage is also possible, but in the case of HIV it is the only possible development (at least, there is no reliable reports of total recoveries).
- (2) Why during the asymptomatic stage, under a constant pressure of the immune response, the virus load slowly progressively grows?
- (3) What is causing the diversity of the disease progression? The most important and probably the most difficult question is, however.
- (4) What is the reason for a sudden collapse of the immune system and the development of AIDS?

Several factors, such as viral reproductive abilities, immune proliferative abilities, or an accelerated destruction of CD4⁺T cells caused by HIV, were suggested to explain the diverse disease progression (Alimonti et al., 2003; Altes et al., 2002; de Boer and Boerlijst, 1994; Galvani, 2005; Holmes et al., 1992; Ho et al., 1995; Mohri et al., 2001; Ribeiro et al., 2002; Tunetsugu-Yokota, 2005). However, the majority of mechanisms, which were proposed since the early 1990s in order to explain how HIV avoids the immune response and eventually overwhelms the immune system, are based on hypotheses, which involve the ability of HIV to evolve (Bittner et al., 1997; Iwasa et al., 2004, 2005; Nelson and Perelson, 1992; Nowak and Bangham, 1996; Nowak, 2006; Nowak et al., 1995). All viruses are able to evolve, and HIV is the fastest known evolving entity (Gamberg and Grant, 2000; Holmes et al., 1992; Moskophidis et al., 1995; Walker and Korber, 2001) producing many new variants within a single host every day (Rambaut et al., 2004). This extremely fast evolution and the astonishing viral diversity is a result of a combination of several factors specific to HIV (Rambaut et al., 2004), in particular (i) exceptionally fast reproduction cycle, with generation of about 10^{10} – 10^{12} new virions per patient per day (Perelson et al., 1996; Perelson and Ribeiro, 2008); (ii) very high mutation rate of about 3×10^{-5} mutations per nucleotide base per cycle of replication, and (iii) one of the highest known recombination rate, with about three recombinations per genome per replication cycle (Jost et al., 2002; Jung et al., 2002; Koelsch et al., 2003; Perelson and Ribeiro, 2008; Preston et al., 1988; Rambaut et al., 2004; Robertson et al., 1995; Zhuang et al., 2002). This exceptionally fast evolution rate leads to emergence of drug-resistant strains (Cohen and Fauci, 1998; Levin et al., 2001; Rambaut et al., 2004) and makes the development of an effective vaccine nearly impossible (Bittner et al., 1997; Iwasa et al., 2004, 2005; Nelson and Perelson, 1992; Nowak, 2006; Nowak and Bangham, 1996; Nowak et al., 1995). It also enables the virus to escape immune control (Allen et al., 2000; Barouch et al., 2002; Levin et al., 2001; Markham et al., 1998; Rambaut et al., 2004; Shankarappa et al., 1999; Wolinsky et al., 1996). The gradual growth of the viral load during the asymptomatic stage is also a consequence of evolution: organisms evolve towards higher fitness (Dempsey and Korobeinikov, submitted for publication; Gorban, 2007), which in this case is the reproductive ability of virus, and the viral load grows with the growth of the reproductive ability.

It is not clear, however, how virus diversity and evolution are linked to the abrupt collapse of the immune system and the development of AIDS. A number of hypotheses were suggested to explain this phenomenon. None of these, however, is entirely satisfactory.

An early hypothesis that linked virus evolution and the development of AIDS was proposed by Nowak and collaborators (Nowak et al., 1991), who suggested that the viral explosion at the end of the asymptomatic stage may be explained by an increase of the HIV variants' diversity. This conclusion is based on an assumption that virus evolution increases the strain diversity, and that the need to respond to each mutant strain eventually leads to exhaustion of the immune system. It was conjectured that there is a diversity threshold, which is reached at a later stage of the asymptomatic period; the immune system collapses and the virus load explodes when the viral diversity exceeds this threshold. This theory satisfactorily explains the variability of the asymptomatic phase and the development of AIDS. However, as pointed in Nowak (2006) and Rambaut et al. (2004), the model with a high level of viral strains does not foresee a faster progression for the patients with a higher antigenic diversity compared with that for the patients with a lower diversity. This implies that the viral diversity is unlikely to be the only reason for the collapse of immune system and development of AIDS. More recent theoretical and clinical studies show that virus evolution increases the diversity to a considerably lower extent than it was expected (Dempsey and Korobeinikov, submitted for publication; Gorban, 2007), and that the relationship between genetic diversity and disease status is more complicated (Markham et al., 1998; McMichael and Phillips, 1997; Shankarappa et al., 1999; Wolinsky et al., 1996). Now the link between the diversity and AIDS is unclear.

A hypothesis, which also links the diversity and the development of AIDS, was suggested by Nelson and Perelson (1992), they considered a possibility that the immune response preferably arises against the fast-replicating strains of HIV, and hence the slow-replicating strains can escape immune response more efficiently. This hypothesis, however, is unable to explain the bursts of virus load and the sudden collapse of the immune system in the later period of the disease development.

Galvani (2005) proposed an alternative mechanism, suggesting that the immune system collapses as a result of the functional deterioration of CD8⁺ and B cells, caused by accumulations of deleterious mutations during HIV infection. It is not clear, however, why such a deterioration is not observed on a comparable scale in course of other viral infections.

Krakauer and Nowak (1999) suggested a possible mechanism for CD4⁺T cells depletion. They suggested that, in conditions when cytopathogenicity of HIV is not very high and levels of infected cells are relatively low, the cytolytic effect of the virus is unlikely to be the cause of the dramatic reduction of the CD4⁺T cells level, which is usually observed by the end of the asymptomatic stage. Krakauer and Nowak hypothesized, accordingly, that a CTL-induced pathology (a “bystander effect”) can be responsible for the bulk of the killing. A reduction of CTL response can be beneficial under these conditions. This interesting hypothesis did not receive the attention it deserves and was not further developed mostly because its proving or disproving is rather a challenging task for the modern experimental science.

Wodarz et al. (1998) distinguished between two general classes of viruses, namely (i) viruses infecting the cells that are not involved in immune response and (ii) viruses infecting helper cells, and pointed that a high rate of replication and a low level of cytopathogenicity can promote exhaustion of CTLs. The most important target of HIV is CD4⁺T cells, which are helper cells for immune response, and thus HIV belongs to the second category. There is sufficient

evidence that the activation of immune response depends on levels of $CD4^+$ T cells (Altes et al., 2002; Norris and Rosenberg, 2002). Individuals, who maintain higher levels of cytotoxic T-lymphocytes (CTL), also exhibit high levels of $CD4^+$ T cells and comparatively low viral loads (Janewa et al., 2004). Although HIV does not target CTL directly, it impairs immune response indirectly by decreasing the number of $CD4^+$ T cells. Wodarz (2003, 2007), Wodarz and Nowak (1999) and Wodarz et al. (2002, 1998) proposed a number of mathematical models to demonstrate that an evolutionary increase of viral infectivity can induce the CTL exhaustion and can ultimately lead to the immunodeficiency. Developing this hypotheses, Iwami et al. (2009a, 2009b) considered immune impairment effects over HIV infection and suggested a model where the immune impairment rate is explicitly represented by a parameter. Assuming that the immune impairment rate gradually increases through viral evolution, and hence CTL proliferation decreases, Iwami et al. (2009a, 2009b) were able to predict the development of AIDS. A deficiency of this model is that the immune impairment rate, as it was defined in the model, is difficult to validate and estimate by experiments.

The above-mentioned and other studies utilized the concept of multi-strain modeling and are usually focused on evolution of the basic reproduction number R_0 and the diversity of viral population (Iwasa et al., 2004, 2005; Nowak and May, 2000; Nowak et al., 1995; Wodarz et al., 1998). However, HIV's primary targets are immune cells, and hence the role of these cells and the ability of the immune system to respond adequately, rather than the proliferation of the virus, should be investigated. In this paper we concentrate on the ability of the immune system to respond efficiently, and in particular on the role of $CD4^+$ T cells in the activation of the specific adaptive immune response, and suggest a mechanism for the collapse of the immune system. In order to demonstrate how continuous viral evolution leads to an ultimate collapse of the immune system, we employ a simple mathematical model of HIV infection, which explicitly incorporates the role of $CD4^+$ T cells (T helper cells) and describes the interactions between $CD4^+$ T cells, HIV and CTL response. We follow the basic assumption that virus evolves towards higher replication rates. Analysis of the model shows that there are two thresholds in the disease progression from an initial infection to AIDS, such that the effective cell-mediated immune response can only be established when HIV reproduction rate is between these two thresholds. When continuous viral evolution results in appearance of mutant strains with replication rates beyond the second threshold, the immune system collapses, and the immune response cannot be established due to the shortage of $CD4^+$ T cells.

1.2. The role of $CD4^+$ T cells in immune response

HIV infects $CD4^+$ T cells, macrophages and dendritic cells, which express CD4 molecules on their surface. $CD4^+$ T cells are a subtype of the helper T lymphocytes (also known as T helper cells and Th cells), and are major targets of HIV. As a result of HIV infection, levels of $CD4^+$ T cells decrease through (i) direct viral killing of infected cells, (ii) increased rates of apoptosis in infected cells, and (iii) killing of infected $CD4^+$ T cells by cytotoxic T lymphocytes (CTL). It is commonly believed that the depletion of $CD4^+$ T cells is the principal reason for the collapse of immune system; when the levels of $CD4^+$ T cells drops from around of 1000 cells per mm^3 (the normal level for a healthy individual) to about 200 cells per mm^3 , the cell-mediated immunity is lost, and the body becomes progressively more vulnerable to opportunistic infections. The T helper cells have no cytotoxic or phagocytic activity towards pathogens, and they do not kill infected cells or pathogens (some infected cells can be killed by $CD4^+$ T cells via the Fas/Fas ligand pathway; however, this, is a mechanism of self-

regulation of the immune system, rather than targeting pathogens). Instead, T helper cells are involved in activating and directing other immune cells: they are essential in activating and proliferation of CTL and B cells, in determining B cell antibody class switching, and in maximizing bactericidal activity of phagocytes. It is noteworthy that activation of helper T cells requires a much weaker activation stimulus than activation of cytotoxic T cells. The role of $CD4^+$ T cells in regulating and amplifying the immune response is vital, and a decline in their number results in deficits in humoral and cell-mediated immunity, opening an opportunity for opportunistic infections.

The Th immune response can be differentiated according to which type of the response is activated: Type 1 Th responses are critical in controlling intracellular infections via cytotoxic T lymphocyte (CTL)-mediated mechanisms (cell-mediated responses), whereas Type 2 helper responses is characterized by the activation of B-cells, which produce neutralizing (killing) antibodies (humoral immunity) (Maloy et al., 2000). The factors that determine which type of response will be activated are not fully understood (Maloy et al., 2000); however, in general, Th1 responses are more effective against intracellular pathogens (viruses, including HIV, and bacteria that are inside host cells), while Th2 responses are more effective against extracellular bacteria, parasites and toxins. For this reason, in this paper we concentrate on the cell-mediated response, disregarding humoral immunity.

Cytotoxic T cells (also known as CTL, $CD8^+$ T cells, or killer T cells) are a major element of the cellular immune response. CTL is a subgroup of T lymphocytes, which are able to kill cells infected with viruses or other pathogens with a release of cytotoxins (perforin, granulysin and granzyme), which induce apoptosis (programmed cell death). Activation of CTL into effector cells depends on interactions between molecules expressed on the surface of the T cell and molecules on the surface of an antigen-presenting (infected) cell and often requires T helper cells stimulation. CTL are capable to inflict extensive damage to body tissue during an infection, and to limit the extents of this damage, CTL activation is tightly controlled and is considerably more difficult, compared with $CD4^+$ T cells activation; it generally requires a very strong antigen activation signal, or additional activation signals provided by helper T cells. Once activated, the CTL start to proliferate, rapidly increasing the number of CTL specific for the target antigen. When an infection is cleared out, the majority of the activated CTL (as well as $CD4^+$ T cells) will die, with a few remaining as memory cells. Schematic interaction between virus, target cells and CTL is shown in Fig. 1.

B cells (a subtype of lymphocytes) play a crucial role in the humoral immune response. Activated B cells produce antibodies (large Y-shaped proteins, which serve to identify and neutralize foreign objects), each of which recognizes and binds to a unique specific antigen, making them easier targets for phagocytes, and

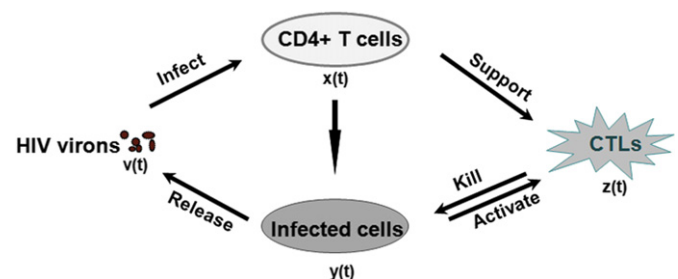


Fig. 1. Schematic delineation of interactions between $CD4^+$ T cells, HIV and CTL: HIV infects healthy $CD4^+$ T cells; infected $CD4^+$ T cells produce free virus particles; CTL are activated by contacts with infected cells and are capable of killing of infected cells; CTL need support of healthy activated $CD4^+$ T cells for activation and proliferation (see Norris and Rosenberg, 2002; Wodarz and Jansen, 2001).

triggers the complement cascade. Once a B cell encounters its specific antigen (in its native form as no antigen presenting is needed for B cell activation) and receives additional signals from a helper T cell of Th2 type, it proliferates and matures into a short living effector cell (the life span is 2–3 days), known as a plasma cell, which secretes antibodies. About 10% of plasma cells will survive to become long-lived antigen specific memory B cells; these cells can be called upon to respond quickly if the same pathogen re-infects the host.

2. Model

Typical mathematical models of a viral infection (including these for a HIV infection) describe interaction of four (sometimes three) variable quantities, namely the concentrations of healthy (and susceptible) target cells, infected cells, free virus particles and immune response agents (CTL, and sometimes also B cells and antibodies) (Nowak and Bangham, 1996; Nowak et al., 1995; Nowak and May, 2000). It is usually assumed that free virus particles infect healthy cells (at the rate, which depends on both concentrations), that the infected cells produce the free virus particles, and that the immune response agents kills the infected cells (sometimes also the free virus particles). The proliferation of immune response agents is usually assumed proportional to their current concentration, the concentration of infected cells, or a product (or a more complicated nonlinear function) of both (see de Baer and Perelson, 1998; Wodarz et al., 2001 for comparison and discussion of models). An apparent deficiency of such a model is that it disregards the role of $CD4^+$ T cells in immune response. This neglecting is acceptable for the majority of viral infections, where the $CD4^+$ T cell level remains approximately constant throughout the course of infection. For HIV infection, however, $CD4^+$ T cells are the target cells, and a decrease of their levels affects the efficacy of immune system.

To overcome this deficiency, in this paper we assume that the activation and proliferation of CTL depend on all three above-mentioned concentrations, namely on the current concentration of CTL, the concentration of infected cells (antigen presenting cells) and the concentration of healthy $CD4^+$ T cells. Accordingly, we describe the dynamics of HIV infection by the following system of ordinary differential equations:

$$\begin{aligned}\dot{x}(t) &= \lambda - \alpha xv - dx, \\ \dot{y}(t) &= \alpha xv - ay - pyz, \\ \dot{v}(t) &= ky - uv, \\ \dot{z}(t) &= cxyz - bz.\end{aligned}\quad (1)$$

Here $x(t)$, $y(t)$, $v(t)$ and $z(t)$ are the concentrations of healthy $CD4^+$ T cells, infected $CD4^+$ T cells, free virus particles and antigen-specific CTL, respectively. We assume that the healthy $CD4^+$ T cells are generated at a rate λ and die at a rate dx (and hence their average life span is $1/d$); free virus particles infect the healthy cell at a rate αxv ; the infected cells die at a rate ay and are killed by CTL at a rate pyz . Free virus particles are produced by infected cells at a rate ky and are removed at a rate uv . The specific CTL proliferate at a rate $cxyz$ (which is proportional to the levels of healthy $CD4^+$ T helper cells, antigen-presenting cells and CTL) and decay at a rate bz .

Compared with other virus dynamics and immune response models (Funk et al., 2005; Iwami et al., 2009a, 2009b; Iwasa et al., 2004, 2005; Li and Shu, 2011; Nowak and Bangham, 1996; Nowak and May, 2000; Wodarz, 2003), this model explicitly takes account of the function of $CD4^+$ T helper cells (so-called “target cell dependence in immune activation” Regoes et al., 1998), postulating that

the activation and proliferation of the antigen-specific CTL response are proportional to the numbers of both healthy and infected $CD4^+$ T helper cells. (In the form $cxyz$, the factor $y(t)$ accounts for antigen presenting; that is we assume that the level of antigen-presenting cells is proportional to that of infected cells.) In order to simplify further analysis, we use the form $cxyz$, rather than $c(x+y)yz$, disregarding the contribution of infected $CD4^+$ T cells in CTL activation/proliferation on the basis that for HIV the levels of infected $CD4^+$ T cells are considerably lower than these of uninfected cells. Below we will show that this assumption is justified.

This system can be further simplified, if we take into consideration that an average life span of virus particles is usually significantly shorter than that of infected cells. It can be assumed, therefore, that, compared with a “slow” variation of the infected cells level, the virus load $v(t)$ relatively quickly reaches a quasi-equilibrium level. The equality $\dot{v}(t) = 0$ holds in the quasi-equilibrium state, and hence $v(t) = ky(t)/u$. This assumption is referred to as “separation of time scales” and is in common use in the virus dynamics (see e.g. Iwasa et al., 2005; Wodarz et al., 2002; Regoes et al., 1998; Vargas-De-León and Korobeinikov, 2011; Wodarz and Thomsen, 2005). We have to stress that this assumption does not imply that the virus concentration $v(t)$ remains constant; on the contrary, it is assumed to be proportional to the varying concentration of infected cells $y(t)$. The system (1) can now be reformulated as a system of three differential equations

$$\begin{aligned}\dot{x}(t) &= \lambda - \beta xy - dx, \\ \dot{y}(t) &= \beta xy - ay - pyz, \\ \dot{z}(t) &= cxyz - bz,\end{aligned}\quad (2)$$

where new per capita infection rate $\beta = \alpha k/u$ describes both the probability of an infecting contact and the reproduction of virus.

2.1. Equilibrium states and qualitative behavior of the model

The dynamics of system (2) is determined by two numbers, namely

$$R_0 = \frac{\lambda\beta}{ad}, \quad Q_0 = \frac{c\lambda}{b\beta} \frac{R_0 - 1}{R_0}.$$

Here, R_0 is the basic reproduction number of infected cells (that is, R_0 is an average number of infected cells produced by a single infected cell introduced into entirely healthy environment), and Q_0 is the basic reproduction number of immune response (that is, an average number of CTL produced by a single CTL introduced into a system where healthy and infected cells are at their equilibrium levels). The number Q_0 is defined at equilibrium levels of healthy and infected target cells; both these levels depend on R_0 , and hence Q_0 depends on R_0 as well. This implies that the concept of basic reproduction number of immune response is meaningless, when a pathogen is incapable to establish and persist within a host. The incapability of a pathogen to persist means that $R_0 < 1$ for this particular pathogen, and Q_0 takes a negative value in this case.

It is noteworthy that the dynamics of 4-dimensional system (1) is entirely determined by these two numbers as well, and that for both systems these numbers coincide (for system (1), parameter β should be substituted by its definition $\beta = \alpha k/u$).

Depending on values of R_0 and Q_0 , system (2) (as well as system (1)) has following equilibrium states:

1. The model always has an infection-free equilibrium state $E_0 = (x_0, y_0, z_0)$, where $x_0 = \lambda/d$ and $y_0 = z_0 = 0$. This equilibrium state is the unique equilibrium state of the model when $R_0 \leq 1$.

2. When $R_0 > 1$, the model also has an *immune-absence equilibrium state* $E_1 = (x_1, y_1, 0)$, where

$$x_1 = \frac{a}{\beta} = \frac{x_0}{R_0}, \quad y_1 = \frac{d}{\beta}(R_0 - 1), \quad z_1 = 0. \quad (3)$$

3. When $Q_0 > 1$ (and hence $R_0 > 1$), then, in addition to the infection-free and immune-absence equilibrium states, the model has a positive (interior) *immune-presence equilibrium state* $E^* = (x^*, y^*, z^*)$, where

$$\begin{aligned} x^* &= \frac{c\lambda - b\beta}{cd} = \frac{b\beta}{cd} \left(Q_0 - 1 + \frac{Q_0}{R_0 - 1} \right), \\ y^* &= \frac{bd}{c\lambda - b\beta} = \frac{d}{\beta} \left(Q_0 - 1 + \frac{Q_0}{R_0 - 1} \right)^{-1} = \frac{b}{cx^*}, \\ z^* &= \frac{b\beta^2}{cdp}(Q_0 - 1). \end{aligned} \quad (4)$$

For $0 < R_0 \leq 1$, the infection-free equilibrium state E_0 is the unique equilibrium state of the system. For this range of R_0 , the equilibrium state is asymptotically stable (it can be conjectured that it is the global attractor of the system: phase trajectories with non-negative initial conditions eventually converge to this equilibrium state). The concentration of healthy $CD4^+$ T cells reaches its maximum possible level $x_0 = \lambda/d$ in this equilibrium state. When $R_0 > 1$ and $Q_0 \leq 1$, the model has two equilibrium states: in addition to the infection-free equilibrium state E_0 , an immune-absence equilibrium E_1 appears. For these parameters values, the infection-free equilibrium loses its stability and turns into a saddle point, whereas the immune-absence equilibrium E_1 is asymptotically stable. The immune-absence equilibrium state corresponds to a situation where both healthy and infected $CD4^+$ T cells are present, while the antigen-specific CTL response is not activated. A failure to activate the specific cell-mediated immune response may correspond either to an initial phase of HIV infection, when immune response is not yet activated because of low levels of the antigen-presenting cells, or to the later period of infection, when there is a shortage of $CD4^+$ T cells. Later we consider both these possibilities in detail.

When $Q_0 > 1$ (and hence $R_0 > 1$ necessary holds), the system has three equilibria: in addition to the infection-free and immune-absence equilibrium states, an asymptotically stable immune-presence equilibrium state E^* , where all three components of the system are present, appears in the non-negative quadrant. The infection is chronic with a low level of viral load in this equilibrium state. It can be expected that in the immune-presence equilibrium state the infected cells' level is lower and the healthy cells level is higher than these in the immune-absence equilibrium state. Indeed, it can be seen that condition $Q_0 > 1$ is, in fact, equivalent to $x^* > x_1$. Furthermore, it is easily checked that $y^* < y_1$ holds for $Q_0 > 1$. The immune-presence equilibrium state is asymptotically stable when it exists and presumably it is the global attractor of the system. The infection-free equilibrium is unstable (in fact, it is unstable for all $R_0 > 1$), and the immune-absence equilibrium also loses its stability when Q_0 exceeds 1. The stability analysis of equilibria is given in Appendix.

Above we mentioned that we disregard the contribution of infected $CD4^+$ T cells in CTL proliferation on the basis that the levels of the infected cells are considerably lower than the levels of uninfected cells and hence $x + y \approx x$. Indeed, by (4), $y^* = b/cx^*$ holds in the immune presence equilibrium state. Here, by Table 2 and Iwami et al. (2009a), $b = 0.025$ and $c \sim 1$, whereas the concentration of helper T lymphocytes in a healthy individual is of

order 1000 cells per mm^3 of blood and about 200 cells per mm^3 by the end of the asymptomatic stage. Even if we take into consideration that this concentration is the sum of both, healthy and infected cells, that is $x^* + y^* = x^* + b/cx^* = 200$ holds, it still gives extremely small proportion of infected cells in the total helper T lymphocytes population. Furthermore, by (3), $y_1/x_1 = d(R_0 - 1)/a$ holds in the immune absence equilibrium state. For HIV, $d/a \sim 0.025$, and hence R_0 should be of the order 40 to make the levels of x_1 and y_1 of a comparable magnitude. This implies that $x + y \approx x$ holds, and that the use of the form $cx y z$, rather than $c(x + y)yz$, is justified throughout HIV progression to AIDS, up to the ultimate collapse of immune system.

3. Virus evolution and dynamics of the model

Compared with many viruses, HIV is known to be one of the fastest evolving entities. As we mentioned in the Introduction, a very high genetic variability of HIV is a result of combination of its fast replication cycle, a high mutation rate and recombinogenic properties of reverse transcriptase (Rambaut et al., 2004; Robertson et al., 1995; Perelson and Ribeiro, 2008). It may be expected that a mutant virus differs in any of the virological and immunological parameters. However, to be successful a mutant virus must outcompete the resident virus, and hence the basic assumption for the virus evolutionary dynamics is that during infection a virus continuously evolves towards higher replication rates (Asjo et al., 1986; Dempsey and Korobeinikov, submitted for publication; Gorban, 2007; Neher and Leitner, 2001; Tersmette et al., 1989; Wodarz et al., 1998). Clinical data (Rinaldo et al., 1995) and mathematical modeling (Dempsey and Korobeinikov, submitted for publication; Gorban, 2007) support this hypothesis. It was suggested that initially, immediately after the infective event, HIV has a relatively low affinity towards T cells and a higher affinity for macrophages. This implies that the concentration of infected $CD4^+$ T cells is also comparatively low, and consequently a killing rate of $CD4^+$ T cells by the immune system is relatively low as well. At this stage the low-rate loss of $CD4^+$ T cells can be compensated for via the production of new helper T cells from the thymus. However, as a result of evolution, at some stage the virus becomes lymphotropic (likely due to a change in the co-receptors it binds to during infection and probably as a result of evolution towards higher specialization) and begins to infect $CD4^+$ T cells far more efficiently. At this point, the portion of infected $CD4^+$ T cell grows and the total $CD4^+$ T cell level begins to decrease, and eventually the immune system is overwhelmed.

In system (2), a single parameter β describes properties of virus, and hence in this model framework the virus evolution is described by a gradual increasing of the per capita infection rate β . Compared with a duration of HIV infection, virus mutation (and evolution) can be considered as a continuous process. Compared with virus replication, mutation is slow process, and the overall dynamics of HIV infection with virus evolution can be regarded as a slow-fast system, where the virus evolution is slow while the virus dynamics is fast. For a slow-fast system, it is usually assumed that the "slow motion" is sufficiently slow and, apart from a few singular points of bifurcation, the "fast motion" has enough time to converge to a stable quasi-equilibrium state (when it exists). These quasi-equilibria states, combined for different values of the slow variable, form a stable "slow" manifold. If the fast motion converges to the slow manifold, then, as the slow variable varies, the whole system evolves remaining on (or near) this manifold. We, therefore, can limit our study to the equilibrium dynamics of the system.

As we mentioned above, the equilibria of system (2) (as well as system (1)) are entirely determined by the numbers R_0 and Q_0 .

Both numbers are functions of β

$$R_0 = \frac{\lambda}{ad}\beta, \quad Q_0 = \frac{c\lambda}{b} \left(\frac{1}{\beta} - \frac{ad}{\lambda} \frac{1}{\beta^2} \right) = \frac{c\lambda}{b} \left(1 - \frac{ad}{\lambda} \frac{1}{\beta} \right).$$

The basic reproduction number of virus R_0 grows with β linearly, whereas $Q_0(\beta)$ is a more complicated nonlinear function (see Fig. 2). It is easy to see that $\lim_{\beta \rightarrow +0} Q_0(\beta) = -\infty$ and $\lim_{\beta \rightarrow +\infty} Q_0(\beta) = +0$. Furthermore, there is $\beta_0 = ad/\lambda$ such that $Q_0(\beta_0) = 0$ (and hence $R_0(\beta_0) = 1$); $Q_0(\beta) < 0$ for all $\beta < \beta_0$ (that is, for all $R_0 < 1$), and $Q_0(\beta) > 0$ for all $\beta > \beta_0$ (that is, for all $R_0 > 1$). Function $Q_0(\beta)$ satisfies

$$\frac{dQ_0}{d\beta} = \frac{c\lambda}{b} \frac{1}{\beta^2} \left(2 \frac{ad}{\lambda} \frac{1}{\beta} - 1 \right),$$

and hence $Q_0(\beta)$ reaches its maximum value $\max(Q_0) = c\lambda^2/4abd$ at $\beta^* = 2ad/\lambda$. Depending on the value of $Q_0(\beta^*)$, there are two generic cases, namely: (i) $Q_0(\beta^*) \leq 1$ and (ii) $Q_0(\beta^*) > 1$.

In the first case, that is if $Q_0(\beta^*) \leq 1$, the immune presence equilibrium E^* does not exist for all $\beta > 0$, and the cell-mediated immune response cannot be established for any β (see Fig. 2a). The inequality $Q_0(\beta^*) \leq 1$ is equivalent to $4ab/c \geq \lambda^2/d$. Potentially, such a situation can arise if the level of antigen-presenting cells (and the viral load) is extremely low. Such a development is unlikely, but it may occur, for instance when processes other than cytotoxic T-cells (for instance, humoral immunity or apoptosis) destroy infected cells and limit the dispersion of virus such that cytotoxic T-cells are not activated. The second possibility is that the ratio c/b , which represents the quality of the cell-mediated immune response, is small (that is, $c/b \leq 4ad/\lambda^2$ holds); individuals in this conditions may be considered as primary immunodeficiency syndrome patients (Yin et al., 2001).

The case when $Q_0(\beta^*) > 1$ is of our immediate interests, as in this case the cell-mediated immune response exists for some β . Condition $Q_0(\beta^*) > 1$ implies that there are β_1 and β_2 , such that $Q_0(\beta_1) = Q_0(\beta_2) = 1$ hold, and the immune presence equilibrium E^* exists for $\beta_1 < \beta < \beta_2$ (see Fig. 2b). It is easy to verify that

$$\beta_1 = \frac{c\lambda - \sqrt{c^2\lambda^2 - 4abcd}}{2b}, \quad \beta_2 = \frac{c\lambda + \sqrt{c^2\lambda^2 - 4abcd}}{2b}. \quad (5)$$

We define β_1 and β_2 as the *immune activation threshold* and the *immunodeficiency threshold*, respectively. If immune response can be established (that is, if $Q_0(\beta^*) > 1$), then, depending on β , the dynamics of the system exhibits four distinctive types of behavior. When $\beta < \beta_0$, all solutions of system (2) converge to the healthy equilibrium state E_0 , and the number of infected CD4⁺T cells tends to zero. If CD4⁺T cells were the only target cells for the virus, then the infection with this β would eventually disappear. However, CD4⁺T cells are not the exclusive target of HIV. The CD4⁺T cells are an opportunistic target of HIV at this stage, and apparently the virus can survive and successfully proliferate using other cells, such as macrophages, B lymphocytes and dendritic cells. It is likely that a

primary infection occurs when per capita infection rate β towards CD4⁺T cells is in this interval.

At $\beta = \beta_0$, the first bifurcation occurs: as β exceeds β_0 , the asymptotically stable immune-absence equilibrium state E_1 appears in the positive octant, and solutions of system (2) converge to this equilibrium state. For $\beta_0 < \beta < \beta_1$, the specific CTL response is not activated. This interval of β might correspond to the acute phase of HIV infection, where the virus load is high.

At $\beta = \beta_1$, the second bifurcation occurs, and the asymptotically stable immune-presence equilibrium state E^* appears in the positive domain. When $\beta_1 < \beta < \beta_2$, solutions of system (2) converge to this equilibrium state. This implies that the effective specific CTL response is established and reduces virus load. The level of activated CTL in this equilibrium state is $z^* = (-b\beta^2 + c\lambda\beta - acd)/cdp$, which reaches the maximum value at $\beta_m = c\lambda/2b = (\beta_1 + \beta_2)/2$. (It is noteworthy that $\beta_m \neq \beta^* = 2ad/\lambda$.) As β increases beyond β_m , the level of CTL decreases, until eventually β exceeds β_2 .

The third bifurcation occurs at $\beta = \beta_2$; at this bifurcation point the positive immune-presence equilibrium state E^* disappears (it shifts into a negative octant of the 3-dimensional xyz-space). When $\beta > \beta_2$, solutions of system (2) converge again to the immune absence equilibrium state E_1 , which is now (globally) asymptotically stable. This implies that, due to low levels of CD4⁺T cells, the specific cell-mediated response cannot be established for newly appearing mutant strains. As β increases further, virus load, which is not any more controlled by the cell mediated immune response, eventually explodes, and the patient enters the AIDS phase. This is congruous with clinical observation that AIDS is characterized by a high virus load and CD4⁺T cells depletion (Janewa et al., 2004).

The progression of HIV infection through these stages is summarized in Table 1 and Fig. 3; in Fig. 3, solid lines show evolution of the equilibrium levels of healthy and infected CD4⁺T cells with respect to β , as the stable steady state of the model evolves as $E_0 \rightarrow E_1 \rightarrow E^* \rightarrow E_1$. (Note that the scales for healthy and infected CD4⁺T cells in Fig. 3 are different.)

The four distinctive types of the model behavior clearly correspond to actual stages of HIV infection. As we mentioned in the Introduction, HIV typically progresses through four distinct stages (Levin et al., 2001), namely establishing, when virus enters and colonizes a host (Stage 1); acute HIV syndrome, when virus

Table 1

Stable equilibrium states and equilibrium levels of variables for model (2) for varying β .

β	Steady state	CD4 ⁺ T cells level	Infected cells level	CTL response level
$(0, \beta_0]$	E_0	λ/d	0	0
$(\beta_0, \beta_1]$	E_1	a/β	$\lambda/a - d/\beta$	0
(β_1, β_2)	E^*	$\frac{c\lambda - b\beta}{cd}$	$\frac{bd}{c\lambda - b\beta}$	$\frac{\beta}{p} \left(\frac{\lambda}{d} - \frac{a}{\beta} - \frac{b\beta}{cd} \right)$
$[\beta_2, +\infty)$	E_1	a/β	$\lambda/a - d/\beta$	0

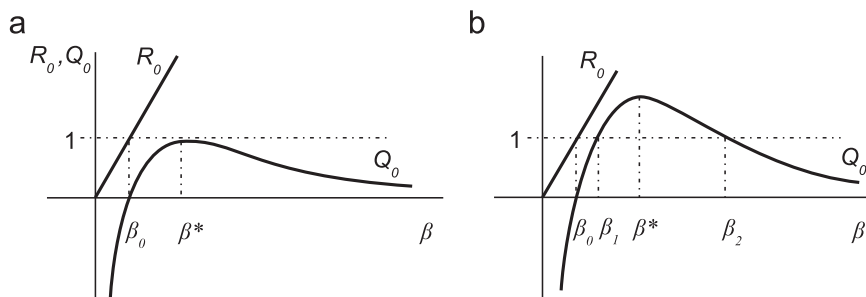


Fig. 2. Functions $R_0(\beta)$ and $Q_0(\beta)$ with respect to β ; here, $Q_0(\beta^*) < 1$ in (a), and $Q_0(\beta^*) > 1$ in (b).

proliferates to high densities (Stage 2); asymptomatic period when infection is chronic, circulating virus remains at low gradually increasing levels, and disease symptoms are absent (Stage 3); symptomatic AIDS period (Stage 4). Assuming that the per capita rate of infection β is continuously increasing throughout a period of infection, each of these HIV stages can be characterized by and associated with an interval of β , as is shown in Table 1 and in Fig. 3.

To verify the conclusion that the intervals of β in Table 1 and Fig. 3 directly correspond to the stages of HIV progression, we compare the relative lengths of these intervals. Table 2 gives estimated values of model parameters for three patients (these data are adopted from Iwami et al., 2009a) and the corresponding calculated values of the immune activation threshold β_1 and the immunodeficiency threshold β_2 . Fig. 3 relates the asymptomatic period with β evolving from the activation threshold β_1 to the immunodeficiency threshold β_2 , whereas the interval from zero to β_1 corresponds with the combined

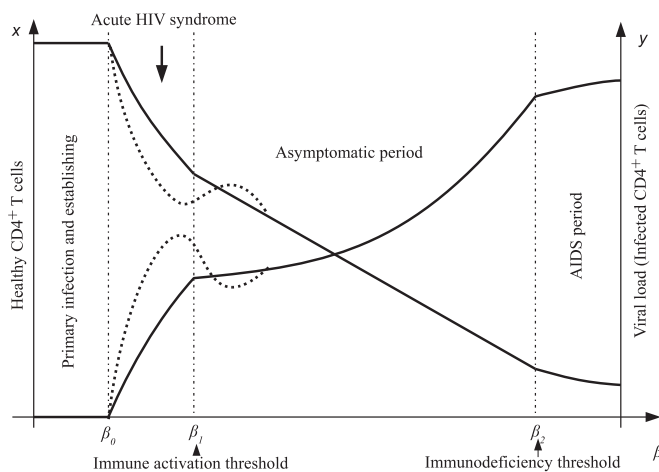


Fig. 3. Evolution of the levels of healthy and infected $CD4^+T$ cells with increasing β ; note that the scales for healthy and infected cells are different. The solid line is for the equilibrium levels, whereas the dotted line schematically shows the dynamic knock-outs from the equilibrium by supercritical bifurcations at β_0 and β_1 . (It is noteworthy, that CTL not only kill infected cells but also inhibit the virions' production. This implies that in the presence of CTL the parameter k is reduced, and hence the reproduction rate $\beta = \alpha k/u$ is low either. In small ambit of β_1 , the reduced by the presence of CTL β can become smaller than β_1 , which results in CTL vanishing again. Only when β exceeds β_1 a little bit, CTL can persist. This induces small oscillations and jumps near the immune activation threshold. Hence we use the dotted line to describe briefly the practical state of asymptomatic phase.)

Table 2
Estimations of the model parameters and the durations of asymptomatic stage for three patients.

Parameter	Description	#1	#2	#3
λ	Production rate of $CD4^+T$ cells (cells day^{-1})	0.13	0.2	0.065
d	Decay rate of healthy $CD4^+T$ cells (day^{-1})	0.013	0.02	0.0065
a	Death rate of infected cells (day^{-1})	0.4	0.8	0.43
p	Killing rate of infected cells by CTL (cells $^{-1}$ day^{-1})	0.016	0.016	0.016
c	Proliferation rate of CTL (cells $^{-2}$ day^{-1})	0.4	0.75	1.0
b	Decay rate of CTL (day^{-1})	0.05	0.05	0.025
β	The infection rate of HIV (cells $^{-1}$ day^{-1})	0.15	0.216	0.204
β_1	The immune activation threshold	0.042	0.082	0.044
β_2	The immunodeficiency threshold	1.0	2.918	2.556
$(\beta_2 - \beta_1)/\beta_1$	Relative duration of the asymptomatic stage	23.0	34.47	57.45
	Estimated duration of the asymptomatic stage (years)	3.1	4.64	7.73

The values of parameters λ, d, a, p, c, b , come from parts of the data in Iwami et al. (2009a), which are set to be estimated values based on 10 patients' virus concentration data in Stafford et al. (2000). β_1 and β_2 are directly calculated by (5). Note that we assume that the primary and acute stages (the progression to β_1) take 7 weeks on an average and the infectivity of virus is gradually increasing with an approximately constant rate throughout the period of infection. Hence, by the ratio of the lengths of intervals $[0, \beta_1]$ and $[\beta_1, \beta_2]$, we can estimate the length of asymptomatic period for three patients, respectively.

duration of the establishing and acute periods. Then a relative duration of the asymptomatic period to that of the combined duration of the establishing and acute periods is $(\beta_2 - \beta_1)/\beta_1$. For the data in Table 2, the asymptomatic period is 20–60 times longer than the combined duration of the establishing and acute periods.

The earliest clinical manifestation of HIV infection in most cases is an influenza- or mononucleosis-like illness, which usually occurs about 2–4 weeks after an infective event. A duration of this acute stage is 4 weeks on average. The acute viremia ends with the activation of the cell-mediated immune response and is followed by an asymptomatic stage, which typically lasts for several years. Assuming that the infectivity of virus is gradually increasing with an approximately constant rate throughout the period of infection, and comparing the lengths of intervals $[0, \beta_1]$ and $[\beta_1, \beta_2]$, we obtain an estimated length of asymptomatic period. Thus, if the progression to β_1 takes 7 weeks on average (this includes 3 weeks of the establishing plus 4 weeks of the acute stage), then the asymptomatic period lasts from 3 to 8 years (see Table 2). These estimations are in an agreement with clinical observations. This agreement can be even better, if we take into consideration that (i) evolution does not necessary have a constant speed and that (ii) for any infection some time is needed to rise a viral load to a level, where pathogen presenting can occur (for instance, for influenza it takes about three days). At this stage, however, it is difficult to provide more accurate figures, as more clinical data on the speed of evolution and on dynamics of virus at an early stage of infection are needed.

It is noteworthy that for this model the length of the asymptomatic period is determined mainly by the ratio of the parameters b and c . For three patients in Table 2 this implies that the patient 3, which has the largest ratio, has also a considerably longer asymptomatic period.

Compared with the solid lines in Fig. 3, which show evolution of the equilibrium levels of healthy $CD4^+T$ cells and virus load (which assumed to be proportional to the infected cells level), typical diagrams of HIV progression show a higher level of virus load and a lower level of $CD4^+T$ cells in the acute stage, where these diagrams usually depict a visible hump of the virus load and a symmetric drop of the $CD4^+T$ cells level. A reason for this discrepancy is that the system (2) is not remaining in an equilibrium (which is shown by the solid line) throughout all range of β . As we mentioned above, a slow-fast system remains in a stable quasi-equilibrium (on or near a stable “slow” manifold), as the slow variable varies within a range, which does not include singular points of bifurcation. At a bifurcation point, however, the rate of transformation of the solutions is infinite, and hence a

small variation of the bifurcation parameter (usually it is the slow variable) causes a disproportionately large outbursts of the fast variables, which are knocked off the stable equilibrium manifold by the bifurcation. At a point of supercritical bifurcation, a new stable equilibrium emerges from an existing equilibrium state (which loses its stability) and moves away of that with an infinite velocity. The velocity of fast motion is finite, and consequently the fast variables are left behind by the faster moving equilibrium state. As a result, the system is knocked off the stable slow manifold when the bifurcation parameter crosses the point of supercritical bifurcation, and a short transition period follows the bifurcation before the system returns to the slow manifold again. This knock-out off the slow manifold and the following transition period, however short it is, are accountable for these considerably higher levels of virus load (and, respectively, lower levels of CD4⁺T cells) that are observed in the acute stage of HIV infection. At the subcritical bifurcation, where a stable equilibrium state merges with an unstable equilibrium and disappears, there is no such a knock-out, and a system reasonably smoothly shifts from one equilibrium to the other.

For system (2), the supercritical bifurcations occur at the points β_0 and β_1 , whereas β_2 is the point of a subcritical bifurcation. As β approaches β_0 from below, the virus load is very low but positive. (We assume that for $\beta < \beta_0$, the virus proliferates in other cells such as macrophages or dendritic cells.) As β exceeds β_0 , a newly appeared stable equilibrium jumps off the xz -plane with infinitely large speed, while the virus load remains very low. The system now must move into the new equilibrium state undergoing a transition process, where the peak values of the virus load considerably exceed its equilibrium level. The second supercritical bifurcation at $\beta = \beta_1$, when an outbreak of CTL occurs, follows shortly after the first one. The interval between β_0 and β_1 is very small, compared with both $(0, \beta_0)$ and (β_1, β_2) , and the cumulative effect of these two successive outbreaks, of the virus load and the CTL, is mostly responsible for the distinctive hump of the viral load and the drop of CD4⁺T cells levels in the acute HIV stage. Dotted lines in Fig. 3 schematically show the actual levels of CD4⁺T cells and virus load with transitions after supercritical bifurcations.

4. Contribution of infected CD4⁺T cells in CTL proliferation

The previous discussion is based on the assumption that the contribution of infected CD4⁺T cells in CTL proliferation is negligible compared with that of healthy CD4⁺T cells. This assumption itself is based on the assumption that the lifespan of infected cells is considerably shorter than this of healthy cells (that is, $a \gg d$ holds). However, if the cytopathogenicity of virus is low, then in the absence of cell mediated response the lifespans of healthy and infected cells can be comparable, and the assumption $x+y \approx x$ may be incorrect. This is particularly relevant for later stages of the infection when levels of uninfected cells are comparatively low.

We assume now that infected cells participate in CTL proliferation, and that an infection with a virus reduces the ability of CD4⁺T cells to perform their function by a factor $\sigma \leq 1$ (that is, $\sigma = 1$ if the infection does not affect the cells capabilities). Then, under the assumptions in Section 2, CTL proliferation is governed by

$$\dot{z} = c(x + \sigma y)yz - bz. \quad (6)$$

When $\sigma = 0$ (that is when infected cells completely lose their immune-regulating abilities), this model is equivalent to model (2).

Eq. (6) describes immune response, and hence changes in this equation do not affect the infection-free and the immune-absence equilibrium states. Furthermore, the basic reproduction number of infected cells \tilde{R}_0 for this model is the same as that for model (2),

$\tilde{R}_0 = R_0 = \lambda\beta/ad$. The basic reproduction number of immune response \tilde{Q}_0 for this model is, however, different

$$\tilde{Q}_0 = \frac{c}{b}(x_1 + \sigma y_1)y_1 = Q_0 \left(1 + \sigma \frac{y_1}{x_1}\right) = Q_0 \left(1 + \sigma \frac{d}{a}(R_0 - 1)\right).$$

That is, $\tilde{Q}_0 = Q_0$ when $R_0 = 1$, and $\tilde{Q}_0 > Q_0$ for all $R_0 > 1$. Moreover

$$\frac{d\tilde{Q}_0}{d\beta} = \frac{c}{b} \left(2 \frac{d(a-\sigma d)}{\beta^3} - \frac{\lambda}{a\beta^2}(a-2\sigma d)\right) = \frac{c}{ab\beta^3}(2ad(a-\sigma d) - \lambda(a-2\sigma d)\beta),$$

and hence there is

$$\tilde{\beta}^* = \frac{2ad(a-\sigma d)}{\lambda(a-2\sigma d)},$$

such that $d\tilde{Q}_0/d\beta = 0$ when $\beta = \tilde{\beta}^*$. Note that

$$\tilde{\beta}^* = \frac{2ad(a-\sigma d)}{\lambda(a-2\sigma d)} = \beta^* \frac{a-\sigma d}{a-2\sigma d} = \beta^* \left(1 + \frac{\sigma d}{a-2\sigma d}\right),$$

and hence $\tilde{\beta}^* > \beta^*$ for all $a > 2\sigma d$; in this case $d\tilde{Q}_0/d\beta > 0$ for $\beta < \tilde{\beta}^*$, and $d\tilde{Q}_0/d\beta < 0$ for $\beta > \tilde{\beta}^*$. When $a \rightarrow 2\sigma d$ as it decreases, $\tilde{\beta}^*$ tends to infinity (and hence \tilde{Q}_0 grows for all $\beta > 0$). Furthermore, when $\sigma d \leq a < 2\sigma d$, there is no positive $\tilde{\beta}^*$, and $d\tilde{Q}_0/d\beta > 0$ holds (ensuring that \tilde{Q}_0 grows) for all $\beta > 0$. For all $a < \sigma d$ (that is when the lifespan of infected cells are longer than that of uninfected cells), there is $\tilde{\beta}^* < \beta^*$, and $d\tilde{Q}_0/d\beta > 0$ for all $\beta > \tilde{\beta}^*$. That is, inequality $a > 2\sigma d$ is necessary for the existence of an immunodeficiency threshold $\tilde{\beta}_{2,*}$.

For $\beta = \tilde{\beta}^*$

$$\tilde{Q}_0(\tilde{\beta}^*) = \frac{c\lambda^2}{4bd(a-\sigma d)}.$$

Furthermore

$$\tilde{Q}_\infty = \lim_{\beta \rightarrow +\infty} \tilde{Q}_0(\beta) = \sigma \frac{c}{b} \left(\frac{\lambda}{a}\right)^2.$$

That is, for this model the immunodeficiency threshold exists when

$$\max\left\{2\sigma d, \lambda\sqrt{\frac{\sigma c}{b}}\right\} < a < \sigma d + \frac{c\lambda^2}{4bd}. \quad (7)$$

The left-hand inequality implies that if the life span of infected cells is sufficiently long (a is small enough) and the infection does not effect the ability of these cells to perform their function (σ is reasonably large), then there is no collapse of immune response, whatever high β is. The right-hand inequality is the necessary condition for activation and existence of the cell-mediated immune response: indeed, if

$$a > \sigma d + \frac{c\lambda^2}{4bd}$$

holds, then immune response is not activated at all (this is attributed to the lack of antigen presenting in a situation where a virus load is very low due to high mortality of infected cells).

We assume now that

$$\max\left\{2\sigma d, \lambda\sqrt{\frac{\sigma c}{b}}\right\} < a$$

holds. The threshold values $\tilde{\beta}_{1,2}$ satisfy the equality

$$\tilde{Q}_0 = \frac{c\lambda\beta - ad\sigma\lambda\beta + a^2 - \sigma ad}{ab\beta} = 1,$$

and hence

$$\tilde{\beta}_{1,2} = \frac{ac\lambda(a-2\sigma d)}{2(ba^2 - \sigma c\lambda^2)} \mp a^2 \frac{\sqrt{c^2\lambda^2 - 4abcd + 4\sigma bcd^2}}{2(ba^2 - \sigma c\lambda^2)}.$$

(Compare with $\beta_{1,2} = (c\lambda \mp \sqrt{c^2\lambda^2 - 4abcd})/2b$ for model (2).) Real-valued threshold values $\tilde{\beta}_{1,2}$ exist if the right-hand inequality in (7) holds.

Condition $2\sigma d \geq a$ implies that an average lifespan of infected CD4⁺T cells is longer than a half of this of healthy cells. For HIV such a situation is unlikely, and we leave it out of consideration. A situation when the cell mediated immune response does not arise at all is also hardly relevant for HIV and is out of our consideration either.

A remarkable feature of this model, compared with model (2), is that its dynamics and, the most importantly, the length of the asymptomatic stage and the existence of the immunodeficiency threshold are extremely sensitive to variation in the system parameters. The principal reason for this sensitivity is that the limit $\tilde{Q}_\infty = \lim_{\beta \rightarrow +\infty} \tilde{Q}_0(\beta)$ is not equal to zero, as it is in the case of model (2), but varies over a range of values depending on the parameters. This limit is the asymptote for the function $\tilde{Q}_0(\beta)$, and hence its slight variation can lead to a very large variation of $\tilde{\beta}_2$ (see Fig. 4). Moreover, when $\tilde{Q}_\infty \rightarrow 1$, $\tilde{\beta}_2$ tends to the infinity at an indefinitely large speed. This extreme sensitivity can be the reason for the diversity of HIV progression. For all examples in Table 2, $\tilde{Q}_\infty < 1$ (see Table 3), and hence the immunodeficiency threshold exists for all these examples. However, situations, where $\tilde{Q}_\infty \approx 1$ or even $\tilde{Q}_\infty \leq 1$, are not impossible in real life and may be accounted for the phenomena of long-term non-progressors and elite controllers.

Another remarkable property of model (6) is that \tilde{Q}_∞ is proportional to λ^2 . This implies that a slightly higher rate of production of naive lymphocytes in the bone marrow and the thymus (which should result in larger λ and c) can lead to a considerably longer (and potentially to a life-long) control of infection. The inverse dependence on a^2 is also noteworthy and may indicate brighter future perspectives: this implies that if the parasite is evolving

towards longer host's survival (in many cases this appears to be a beneficial strategy for a parasite, as it increases parasite's reproduction), then, under the assumption that infected cells are able to partly perform their primary function, the chances for infected individuals to survive grow disproportionately: a 40% increase of the active life of infected cells results in doubling of \tilde{Q}_∞ , which, in turn, can lead to a prolongation of the asymptomatic stage beyond an ordinary human life span.

We denote $\Delta_\beta = \beta_2 - \beta_1$ and $\tilde{\Delta}_\beta = \tilde{\beta}_2 - \tilde{\beta}_1$ the lengths of the cell-mediated immune response (the duration of the asymptomatic stage) with respect to β for model (2) and the present model. Then

$$\tilde{\Delta}_\beta = a^2 \frac{\sqrt{c^2\lambda^2 - 4bcd(a - \sigma d)}}{ba^2 - \sigma c\lambda^2}.$$

Clearly, $\tilde{\Delta}_\beta = \Delta_\beta$ when $\sigma = 0$, and $\tilde{\Delta}_\beta$ grows with the growth of σ ; this is hardly surprising, since model (6) includes the contribution of infected CD4⁺T cells in CTL proliferation. It is also clear that $\tilde{\Delta}_\beta$ grows with the growth of λ and c , and with decrease of a .

5. Conclusion

There is a plausible hypothesis that rapid and continuous evolution enables HIV to avoid immune control and eventually win its prolonged struggle with immune system. However, the mechanism of this escape and the link between evolution and the development of AIDS remain mysteries. An objective of this paper is to propose a possible mechanism of the ultimate collapse of immune system. In order to demonstrate this mechanism, we consider two simplistic conceptual mathematical models of HIV infection, which describe the interactions between CD4⁺T cells, HIV and CTL, and which differ from earlier introduced models in that these models explicitly include the function of HIV target cells, CD4⁺T cells, in activating immune response. We combine these models with the assumption that HIV is continuously evolving towards higher replication rates. We intentionally used as simple models as possible, preserving only the elements, which are crucial for the proposed mechanism of escape. Despite their simplicity, the models brought an insight into HIV progression, and in particular in how evolution makes HIV capable to avoiding immune control. The analysis shows that the model dynamics is entirely determined by two numbers, namely the basic reproduction numbers of virus, R_0 , and that of CTL, Q_0 (or \tilde{R}_0 and \tilde{Q}_0 for the second model); both these numbers and consequently the overall dynamics depend on the virus replication rate (which is assumed to be gradually increasing).

Depending on a value of the reproduction rate β , both models exhibit four remarkably different behaviors and three thresholds. The first of these thresholds, at $R_0=1$, is for the virus ability to persist; for R_0 below this threshold virus is unable to invade and persist within a host. (We have to point out, however, that in these models the reproduction number R_0 exclusively refer to the ability of HIV to infect CD4⁺T cells, whereas HIV also targets a variety of other cells, such as macrophages or dendritic cells; all these cell types can serve as a bridgehead for the invading virus, while specific R_0 with respect to CD4⁺T cells may be below 1.) Two other thresholds, the immune activation threshold and the immunodeficiency threshold, describe the capabilities of immune system. The first threshold, at $R_0=1$, describes the fitness of virus, and as such is an essential feature of any host–parasite system. The immune activation threshold, which implies that a critical level of antigen-presenting cells is needed for activating specific immune response, is an attribute of any model with immune response. The third threshold, namely the

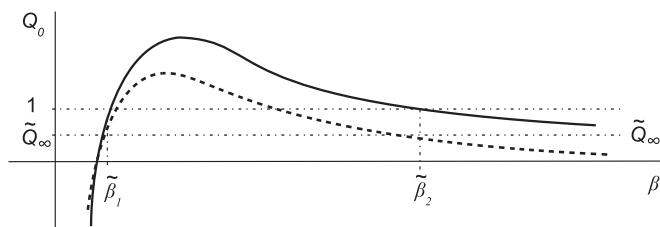


Fig. 4. Schematic relative positioning of curves $Q_0(\beta)$ (dashed line) and $\tilde{Q}_0(\beta)$ (solid line). Here, $Q_0(\beta^*), \tilde{Q}_0(\beta^*) > 1$ and $\tilde{Q}_\infty < 1$. Note the dependence of $\tilde{\beta}_2$ on \tilde{Q}_∞ .

Table 3

The values of the immune activation and the immunodeficiency thresholds and the durations of asymptomatic stage for three patients for model (6) for $\sigma = 1$ and 0.5.

Parameter	#1	#2	#3
$\sigma = 1$			
Limit \tilde{Q}_∞	0.85	0.94	0.91
The immune activation threshold $\tilde{\beta}_1$	0.042	0.082	0.044
The immunodeficiency threshold $\tilde{\beta}_2$	6.232	45.52	29.28
Relative duration of the asymptomatic stage	148.6	552.4	668.4
Estimated duration of the asymptomatic stage (years)	20.0	74.34	89.98
$\sigma = 0.5$			
Limit \tilde{Q}_∞	0.423	0.47	0.457
The immune activation threshold $\tilde{\beta}_1$	0.042	0.082	0.044
The immunodeficiency threshold $\tilde{\beta}_2$	1.701	5.424	4.672
Relative duration of the asymptomatic stage	39.81	64.94	105.8
Estimated duration of the asymptomatic stage (years)	5.36	8.742	14.25

immunodeficiency threshold, is specific for HIV dynamics. Due to the lack of antigen-presenting cells, specific immune response cannot be activated when the virus reproduction rate is below the immune absence threshold. Immune system is persistent and effective in virus control when the replication rate is between the immune activation and the immunodeficiency thresholds. However, once the immunodeficiency threshold is exceeded, specific cell-mediated immune response vanishes due to the depletion of CD4⁺T cells. This dynamics has a transparent biological interpretation and is in an agreement with clinical observations.

We postulate that virus is continuously evolving towards higher replication rates. In process of its evolution, virus gradually goes through the whole range of replication rates, and across all three thresholds (when the third threshold exists), which divide the range of β into four intervals corresponding to the stages of HIV progression. Thus, the interval between the immune activation threshold and the immunodeficiency threshold roughly corresponds to the asymptomatic stage of infection, while exceeding the immunodeficiency threshold means that the patient enters AIDS stage. Moreover, the relative lengths of these intervals are in an agreement with the observed durations of HIV infection stages.

It is remarkable that for the model where infected CD4⁺T cells are participating in CTL proliferation the length of the asymptomatic stage is extremely sensitive to variation in parameters value, and the most importantly, to variation of the production rate of CD4⁺T cells λ . This implies that a comparatively slight increase of λ (the productivity of naive lymphocytes in the bone marrow and the thymus) can lead to a disproportional prolongation of the asymptomatic period and potentially to life-long control of the infection. This high sensitivity explains the diversity in disease progression and may be accounted for the phenomena of long-term non-progressors and elite controllers.

In order to keep the models as simple as possible, we intentionally disregard details of many real-life processes. Thus, we disregard the detail of immune response activation and development. For the sake of simplicity, in this model we include only one function of CD4⁺T cells, namely their role in assistance of the activation. However, CD4⁺T cells also serve as amplifier for immune response; that is the killing rate of infected cells depends on CD4⁺T cells levels as well. This function can be straightforwardly incorporated into the system as well; it suffices to assume that the killing rate is proportional (or directly depends in another way) to the level of CD4⁺T cells. Then the second equation of system (2) takes form

$$\dot{y}(t) = \beta xy - ay - \tilde{p}(x + \varepsilon y)yz \quad (8)$$

or

$$\dot{y}(t) = \beta xy - ay - p(1 + \delta(x + \varepsilon y))yz. \quad (9)$$

(Here ε reflects the capability of infected cells to perform.) In this paper, however, we prefer to disregard the amplifying function of CD4⁺T cells, because replacing the second equation of system (2) with Eq. (8) or (9) does not change principal dynamic properties of the model. The model with T helper cells, which amplify the killing, produces a slightly shorter asymptomatic stage and lower levels of virus load (and higher levels of CD4⁺T cells) in the asymptomatic stage and hence a relatively higher increment of the virus load at the end of this stage (and in this way it even is in a better agreement with clinical observations). The principal features of the model behavior, such as the presence of three thresholds separating four stages, are, however, preserved in this model.

In this paper we also completely disregard humoral immune response. The humoral response depends on CD4⁺T cells assistance even more than the cell-mediated response does, and hence the depletion of the CD4⁺T cells level should affect humoral response

in the same way. The humoral response can be included into the model using an assumption that the humoral response inhibits the spread of infection. One can assume that the incidence rate inversely depends on the strength of the immune response; a form $\beta xy/(1 + qz)$ (where q is a parameter) suggested by Wodarz et al. (2002) (see also Vargas-De-León and Korobeinikov, 2011) can be used as the simplest case. Such a model exhibits the immune absence and the immune deficiency thresholds as well.

The most important conclusion that follows from our analysis is that the continuing succession of mutations and evolution is indeed the critical factor, which brings HIV out of the control of immune system.

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Appendix: Stability of the equilibria

The Jacobian of (2) at a point $E(x, y, z)$ is

$$J = \begin{pmatrix} -d - \beta y & -\beta x & 0 \\ \beta y & \beta x - a - pz & -py \\ cyz & cxz & cxy - b \end{pmatrix}.$$

At the infection free equilibrium state E_0 , the characteristic equation is

$$(r + b)(r + d)(r + a(1 - R_0)) = 0.$$

Roots of this equation are $r_1 = -b$, $r_2 = -d$ and $r_3 = a(R_0 - 1)$, and hence $R_0 < 1$ is necessary and sufficient to ensure the asymptotic stability of E_0 .

At the point E_1 , the characteristic equation is

$$(r + b - cx_1y_1)(r^2 + (d + \beta y_1)r + \beta^2 x_1y_1) = 0.$$

Here root $r_1 = cx_1y_1 - b = b(Q_0 - 1)$ is negative when $Q_0 < 1$, and positive when $Q_0 > 1$. Furthermore, when E_1 exists, all coefficients of quadratic equation

$$r^2 + (d + \beta y_1)r + \beta^2 x_1y_1 = 0$$

are positive, and hence both its roots have negative real parts. That is, $Q_0 < 1$ ensures that all eigenvalues of the Jacobian have negative real parts and hence that the equilibrium state E_1 is asymptotically stable.

The characteristic equation at E^* is

$$r^3 + (d + \beta y^*)r^2 + (\beta^2 + cpz^*)x^*y^*r + cdp x^*y^*z^* = 0.$$

It is easy to verify that $a_1 = d + \beta y^* > 0$, $a_2 = (\beta^2 + cpz^*)x^*y^* > 0$, $a_3 = cdp x^*y^*z^* > 0$ and $a_1 a_2 - a_3 > 0$. Hence, by the Routh–Hurwitz criterion, all the eigenvalues of the Jacobian have negative real parts, and E^* is asymptotically stable when it exists.

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