



Dynamics of cytotoxic T-lymphocyte exhaustion

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We examine simple mathematical models to investigate the circumstances under which the dynamics of cytotoxic T-lymphocyte (CTL) activation and differentiation may result in the loss of virus specific CD8⁺ cells, a process known as CTL exhaustion. We distinguish between two general classes of viruses: (i) viruses infecting cells that are not involved in the immune response; and (ii) viruses infecting antigen presenting cells (APCs) and helper cells. The models specify host and viral properties that lead to CTL exhaustion and indicate that this phenomenon is only likely to be observed with viruses infecting APCs and helper cells. Moreover, it is found that for such viruses, a high rate of replication and a low degree of cytopathogenicity promote the exhaustion of the CTL response. In addition, a high initial virus load and a low CD4⁺ cell count promote the occurrence of CTL exhaustion. These conclusions are discussed with reference to empirical data on lymphocytic choriomeningitis virus and on human immunodeficiency virus.

Keywords: CTL exhaustion; LCMV; HIV; mathematical models; cell tropism; replication rate

1. INTRODUCTION

Cytotoxic T lymphocytes (CTL) provide an important defence mechanism against intracellular pathogens such as viruses. The T-cell receptor (TCR) recognizes viral peptides which are cleaved from nascent proteins in the cytoplasm and displayed on the cell surface bound to major histocompatibility complex (MHC) class I molecules. In the thymus, a large diversity of TCR specificities is created which is narrowed down by positive and negative selection in order to ensure MHC restriction and avoid autoreactivity. Since the frequency of the naive CTL precursors (CTLp) specific for a given antigen is very low, the naive CTLps enter the recirculation pathway to facilitate contact with the antigen which is required for proliferation. This occurs in the regional lymph nodes and there is some indication that cytokine production by CD4⁺ cells can strongly promote CTLp proliferation at this stage (Tripp *et al.* 1995). A fraction of the expanded CTLp pool may then exit the regional lymph nodes in order to travel to the target organ, where further contact with antigen mediates differentiation into CTL effector activity (Doherty 1993). There is evidence that this final stage of differentiation is independent of CD4⁺ cell help (Tripp *et al.* 1995). The CTL effector cells are short-lived and sensitive to apoptosis which is believed to be triggered directly through the TCR (activation induced cell death or AICD). However, other mechanisms such as growth factor deprivation or action of the Fas molecule may also be important (Fowlkes & Ramsdell 1993; Selin & Welsh 1994).

Although the process of CTL activation and differentiation is successful in eliminating many virus infections from the host, some viruses or virus strains may cause deletion of the antigen specific CD8⁺ cell population, a process

known as CTL exhaustion (Zinkernagel *et al.* 1993). The experimental model for studying this phenomenon has been lymphocytic choriomeningitis virus (LCMV). Although experiments are beginning to unravel some of the viral and host factors responsible for CTL exhaustion (e.g. Moskophidis *et al.* 1995), no general explanation has yet been put forward.

Here, we use simple mathematical models to investigate the circumstances under which the dynamics of the above described CTL activation/differentiation process can result in the loss of specific CD8⁺ cells. For this purpose we distinguish between two general classes of viruses: those infecting cells that are not involved in the immune response, and those infecting cells that are involved in supporting the CTL response to develop, e.g. antigen presenting cells (APCs) or CD4⁺ helper cells. The former class of viruses will be referred to as 'non-lymphocyte infecting', while the latter class will be called 'APC & helper-cell infecting'.

The models identify possible viral properties needed for CTL exhaustion to occur and indicate that these properties may be different for non-lymphocyte and APC & helper cell infecting viruses. In addition to the theoretical studies we present experimental data supporting the conclusions of our models.

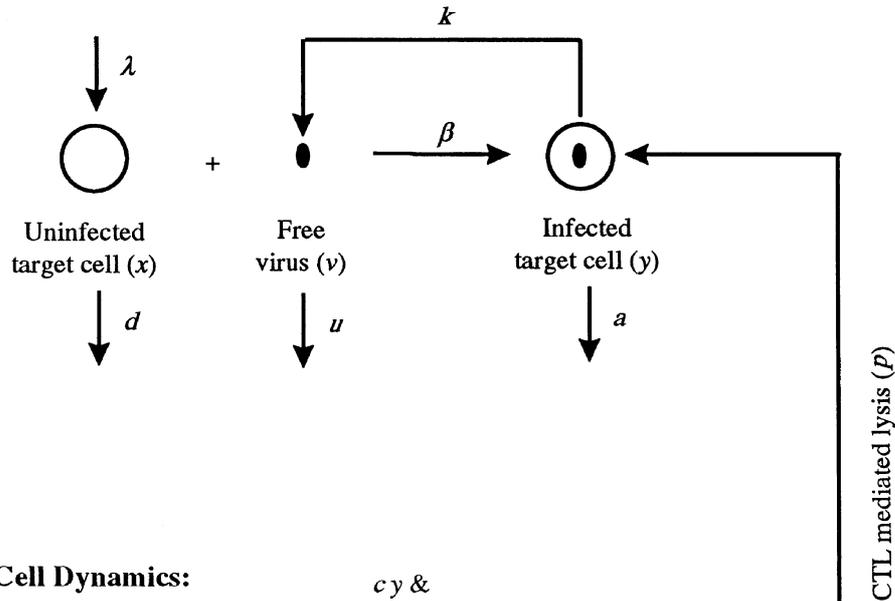
2. NON-LYMPHOCYTE INFECTION

(a) *The model*

To explore the dynamics between virus, non-lymphocyte target cells and CD8⁺ cells, a model by Nowak & Bangham (1996) has been extended, containing the following five variables: uninfected target cells (x), infected target cells (y), free virus particles (v), antigen-specific CTLp (w), and antigen-specific CTL effector cells (z). It is explained graphically in figure 1 and given by the following system of differential equations:

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Virus Dynamics:



CD8+ Cell Dynamics:

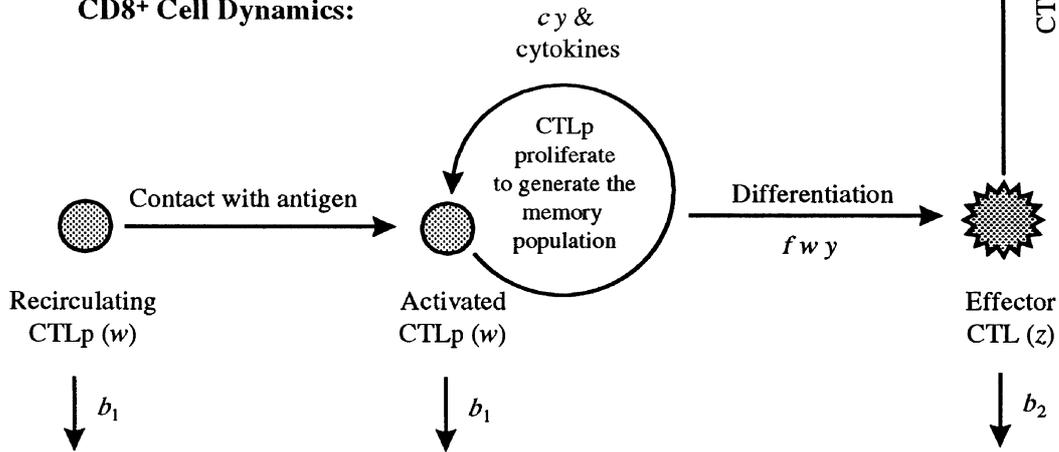


Figure 1. Graphical representation of the dynamics between viruses and CD8⁺ cells underlying the models. Infected cells die at a rate a and produce free virus particles at a rate k . The free virus particles decay at a rate u and enter uninfected target cells at a rate β . Uninfected target cells are produced at a rate λ and die at a rate d . CTL precursor cells suffer a natural death rate of b_1 . Upon encounter of an infected cell, they proliferate at a rate c , and this proliferation is enhanced by cytokines secreted by T-helper cells. On second contact with an infected cell, precursor cells from the expanded CTLp pool differentiate into effector cells at a rate f . The effector cells are characterized by a natural death rate of b_2 which is supposed to be larger than b_1 . Finally, the differentiated effector cells lyse infected target cells at a rate p .

$$\begin{aligned}
 \dot{x} &= \lambda - dx - \beta xv \\
 \dot{y} &= \beta xv - ay - pyz \\
 \dot{v} &= ky - uv \\
 \dot{w} &= cw - fwy - b_1 w \\
 \dot{z} &= fwy - b_2 z
 \end{aligned}
 \tag{1}$$

Uninfected target cells are produced at a rate λ , suffer a natural death rate dx and are infected at a rate βxv . Infected cells are characterized by a death rate of ay and are eliminated by the CTL effector cells at a rate pyz . Virus particles are produced from infected cells at a rate ky and decay at a rate uv . CTLps die at a rate $b_1 w$, and—upon antigenic

challenge—proliferate with a rate cw . A fraction of this expanded CTLp pool then differentiates into CTL effector cells at a rate fwy which in turn die at a rate $b_2 z$.

Without infection ($y=v=w=z=0$), the uninfected target cells (x) attain an equilibrium level of λ/d . The same outcome will be observed when the basic reproductive ratio of the virus ($R_0 = \lambda\beta k/dau$) is less than unity, in which case each infected cell produces on average less than one newly infected cell, making it impossible for the virus population to maintain itself. On the other hand, if $R_0 > 1$, virus abundance may increase initially to high levels and subsequently converge to an equilibrium value. At equilibrium, virus growth can be limited by target cell

availability alone without the immune response being activated, or by a combination of target cell availability and immune response.

In the first case the equilibrium is given by: $x_1^* = a/\beta'$, $y_1^* = (\lambda/a) - (d/\beta')$, $v_1^* = ky_1^*/u$, $w_1^* = 0$, $z_1^* = 0$. We use $\beta' = \beta k/u$. The reason for this outcome is that the equilibrium virus load is too low to provide sufficient antigenic stimulation to maintain an activated CTL response. Therefore, these dynamics should be referred to as 'non-responsiveness' rather than exhaustion.

In the second case the equilibrium values are given by: $x_2^* = [\lambda(c-f)]/[d(c-f) + b_1\beta']$, $y_2^* = b_1/(c-f)$, $v_2^* = (ky_2^*)/u$, $w_2^* = [b_2(c-f)(\beta'x_2^* - a)]/(b_1fp)$.

This time the equilibrium virus load is high enough to maintain an activated CTL response:

$$z_2^* = (\beta'x_2^* - a)p.$$

(b) CTL memory versus non-responsiveness

Whether the CTL response is maintained or disappears depends on the inequality $y_1^*(c-f) > b_1$, and thus

$$\left(\frac{\lambda}{a} - \frac{d}{\beta'}\right)(c-f) > b_1 \quad (2)$$

If this inequality is fulfilled the CTL response will persist. On the other hand, if the inequality is not satisfied, the CTL response will vanish. Since establishment of infection requires the basic reproductive ratio of the virus to be greater than unity, we may assume that $R_0 \gg 1$ and therefore $\lambda/a \gg d/\beta'$. In addition, since a functioning immune system requires c to be bigger than f , we assume that $c \gg f$. With these assumptions the inequality reduces to $\lambda c/a > b_1$.

Among the host properties, the rate of target cell production (λ), the immune responsiveness (c), and the death rate of the CTL precursor cells (b_1) can influence the result of the condition. On the other side, a virus characterized by a high degree of cytopathogenicity may promote the induction of CTL non-responsiveness. However, true CTL exhaustion will not be observed in this model. This point will be discussed below.

3. APC & HELPER CELL INFECTION

(a) The model

Suppose that the virus infects cells that are involved in supporting CTLp proliferation via cytokine production, e.g. APCs such as macrophages and interdigitating dendritic cells or CD4⁺ T-helper cells. The same basic set of equations can be used as in the previous section. The only difference is that now x and y stand for uninfected and infected APCs & helper cells, respectively, and since these cells support CTLp proliferation, the corresponding term is proportional to x and y :

$$\begin{aligned} \dot{x} &= \lambda - dx - \beta xv \\ \dot{y} &= \beta xv - ay - pyz \\ \dot{v} &= ky - uv \\ \dot{w} &= cwxy - fwy - b_1w \\ \dot{z} &= fwy - b_2z. \end{aligned} \quad (3)$$

If $R_0 < 1$, the system behaves in the same way as for non-lymphocyte infection. If $R_0 > 1$ we obtain virus persistence

with the CD8⁺ cell response either being exhausted or persisting as well. In the case of CTL exhaustion, the equilibrium expressions are the same as in the previous model. A time series example is shown in figure 2a.

If the CTL response persists, the equilibrium is given by the solution of a quadratic equation, but only one solution is biologically meaningful:

$$\begin{aligned} y_3^* &= b_1/(cx_3^* - f), v_3^* = (ky_3^*)/u, \\ w_3^* &= [b_2(cx_3^* - f)(\beta'x_3^* - a)]/(b_1fp), z_3^* = (\beta'x_3^* - a)/p, \text{ where} \\ x_3^* &= \left[(\lambda c + fd - b_1\beta') + \sqrt{(\lambda c + fd - b_1\beta')^2 - 4dc\lambda f} \right] / [2dc] \end{aligned}$$

Figure 2b demonstrates how the continuing immune response, consisting of relatively high CTLp and low CTL effector cell levels, keeps the virus population in check.

(b) CTL memory versus exhaustion

The parameter region of this model can be divided into two regions according to the following condition.

$$\left(\frac{\lambda}{a} - \frac{d}{\beta'}\right)\left(c\frac{a}{\beta'} - f\right) > b_1 \quad (4)$$

If this inequality is true, the CTL response will persist. However, if the inequality is not satisfied, the situation is more complicated than in non-lymphocyte infection. Assuming, as before, $\lambda/a \gg d/\beta'$ and $c \gg f$, the above expression can be approximated by $\lambda[(c/\beta') - (f/a)] > b_1$. The rate of target cell production (λ), the immune responsiveness (c) and the death rate of CTL precursor cells (b_1) are again the main host characteristics influencing the result of the inequality. The main viral factor determining the outcome of the condition is β' (the virus replication parameters). The larger the replication rate of a virus (the larger β') the more likely it is that the result will become false. The amount of virus mediated cell killing (a) can also be important. Small values of a , i.e. non-cytopathic viruses, can substantially contribute to the above condition becoming false.

If the inequality does not hold, one has to distinguish between two parameter regions. If the CTL persistence equilibrium expressions are complex ($[\lambda c + fd - b_1\beta']^2 > 4dc\lambda f$), or negative ($x_3^* < f/c$ or $x_3^* < a/\beta'$), the CTL persistence equilibrium becomes unstable and CTL exhaustion will be observed. If, on the other hand, the CTL persistence equilibrium expressions are real ($[\lambda c + fd - b_1\beta']^2 > 4dc\lambda f$), and positive ($x_3^* > f/c$, and $x_3^* > a/\beta'$), both the CTL persistence and the CTL exhaustion equilibria are stable. Now the outcome of the system depends on the initial conditions (figure 3). Assuming the host to be naive (small w) a small initial virus load promotes CTL persistence, while a high initial virus load promotes exhaustion of the CTL response. The initial number of CD4⁺ cells is also significant with a low initial CD4⁺ cell count driving the system towards CTL exhaustion. A high initial level of CTL precursors counters the deletion of the CTL response. This behaviour is explained schematically in figure 4.

In summary, fast replicating and predominantly non-cytopathic viruses will be most efficient at causing CTL exhaustion. Note that these are also the two viral properties needed to depress the uninfected APCs & helper cells in the absence of a CTL response (x_1^*) to a relatively low equilibrium level.

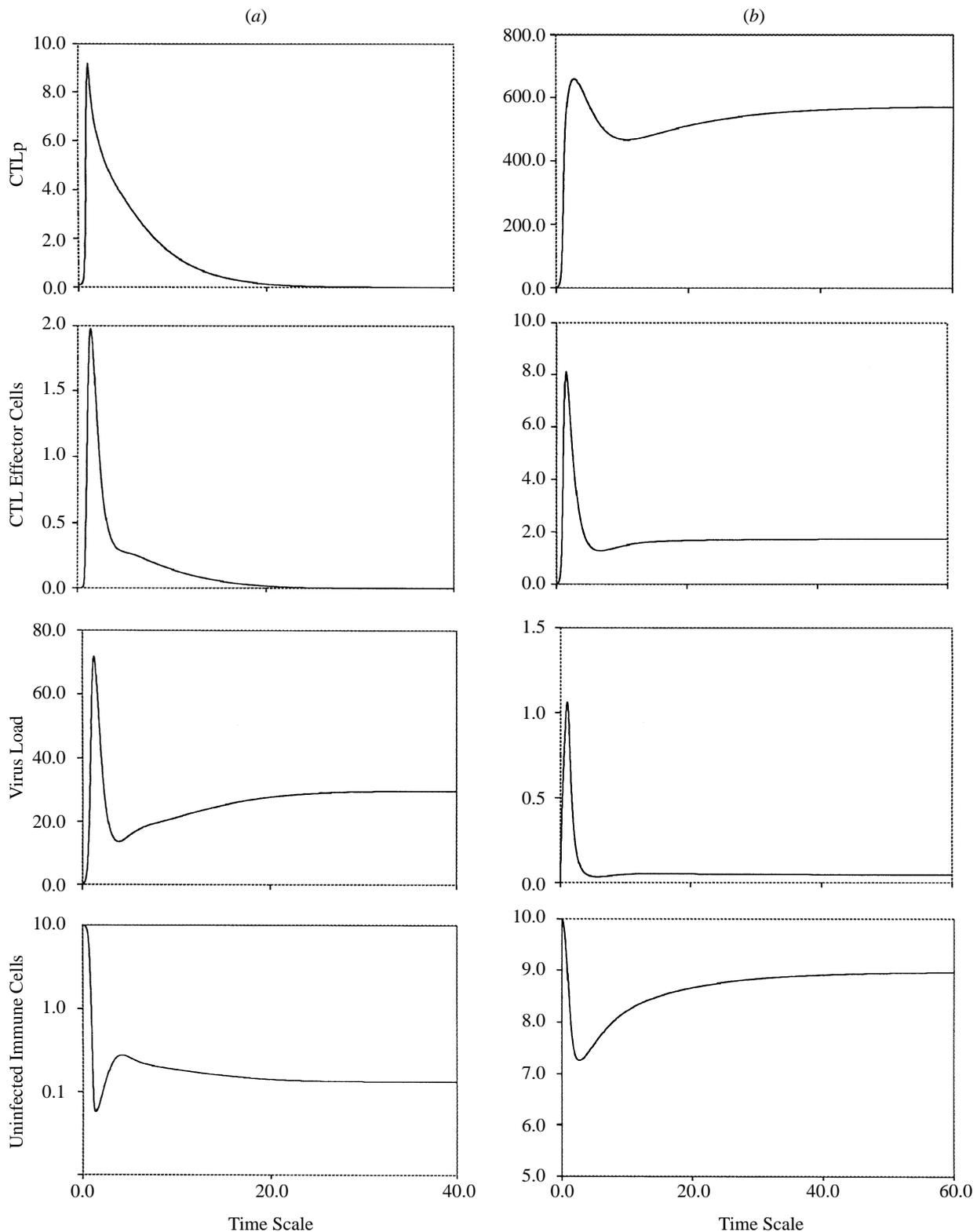


Figure 2. (a) Dynamics of CTL exhaustion for viruses infecting APCs and helper cells. See text for details. Parameter values: $b_1=0.2$, $b_2=1.5$, $c=0.5$, $f=0.1$, $\lambda=1$, $d=0.1$, $a=0.5$, $p=1$, $\beta'=3.75$. (b) Dynamics of CTL persistence for viruses infecting APCs and helper cells. See text for details. Parameter values as in (a), except that $\beta'=0.25$.

4. APPLICATION AND DISCUSSION

Our models have specified the circumstances under which CTL exhaustion might occur. They depended both on immunological and viral characteristics and there was

a basic difference between non-lymphocyte and APC & helper cell infecting viruses. Concerning host factors, the models identified mainly the amount of CTLp proliferation in response to antigenic challenge, the rate of target cell production, and the rate of CTL precursor cell death

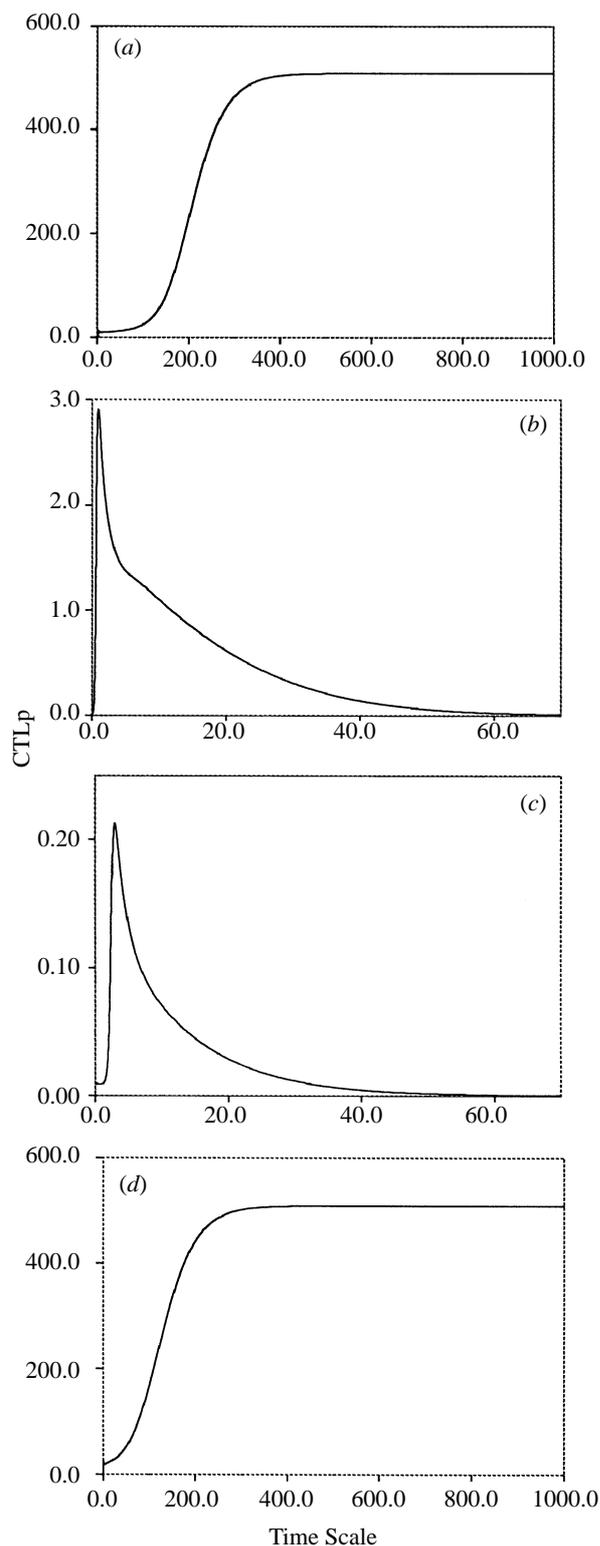


Figure 3. Dependence of the CTL dynamics on the initial conditions. (a) Control time series, $w_0=0.01$, $x_0=10$, $v_0=0.01$. (b) A relatively high initial virus load leads to CTL exhaustion, $w_0=0.01$, $x_0=10$, $v_0=1$. (c) A reduced initial CD4⁺ T cell count also results in CTL exhaustion, $w_0=0.01$, $x_0=5$, $v_0=0.01$. (d) Increased initial numbers of CTL precursors (CTL memory) leads to CTL persistence even with a relatively high initial virus load, $w_0=0.1$, $x_0=10$, $v_0=1$. Parameters were chosen as follows: $b_1=0.2$, $b_2=1.5$, $c=0.5$, $f=0.1$, $\lambda=1$, $d=0.1$, $a=0.5$, $p=1$, $\beta'=1.6$.

as being important in determining whether the CTL response persists.

For non-lymphocyte infecting viruses, the main viral characteristic contributing to the disappearance of the CTL population was a high degree of cytopathogenicity. The initial conditions never influenced the outcome of the infection. However, because the specific CTLs will only vanish if the virus load is below a threshold value necessary for the CTL response to become activated, this phenomenon should not be referred to as CTL exhaustion but rather as CTL non-responsiveness. Moreover, this is an unlikely strategy for viral persistence, since the host might die upon persistent infection with a cytopathic virus.

In contrast, for lymphocyte infecting viruses a high replication rate was the main viral property needed for CTL exhaustion to occur, although a low degree of target cell killing could also make a substantial contribution. Moreover, there was a parameter region in which the initial conditions could influence the course of infection with a high initial virus load, a low CD4⁺ cell count and the naive state of the host promoting the exhaustion of the CTL response. In contrast to the case of non-lymphocyte infection, this phenomenon can be referred to as CTL exhaustion and represents a feasible strategy for viral persistence in the host, since it is now induced by immunosuppression.

(a) CTL exhaustion in LCMV infection

Experiments with LCMV are in agreement with the predictions of our model. LCMV is thought to be non- or poorly cytopathic (Lehmann-Grube 1971; Moskophidis *et al.* 1993a), and when mice become neonatally or transplacentally infected, the virus persists for life (Hotchin 1971). However, when adult mice are challenged, the phenotype of the infection can vary between two extremes depending on the LCMV strain used and the dose given (figure 5). On the one hand, the virus may induce an efficient CTL response leading to its clearance or decline to low or undetectable levels. On the other hand, there are LCMV strains which may cause CTL exhaustion resulting in viral persistence. In between, there are situations where CTL and virus coexist, leading to immune mediated pathology. The *in vivo* dynamics of CTL exhaustion and persistence in LCMV infected mice is shown in figure 6.

In accordance with our model, Moskophidis *et al.* (1995) pointed out that the outcome of LCMV infection depends both on host and viral characteristics. Among the host factors, they identified the kinetics and magnitude of the anti-viral CD8⁺ cell response as being significant, and this was also indicated by our theory.

Because of the non-cytopathic nature of LCMV, our models predict that the occurrence of CTL exhaustion should depend on (i) the ability of the virus to infect APCs and helper cells, and (ii) on its replication kinetics. Moreover, the course of infection may also be influenced by (iii) the initial conditions, with a high dose of infection, a low CD4⁺ cell count, and the naive state of the host promoting the occurrence of CTL exhaustion.

These predictions agree with experimental results, as shown in the following sections.

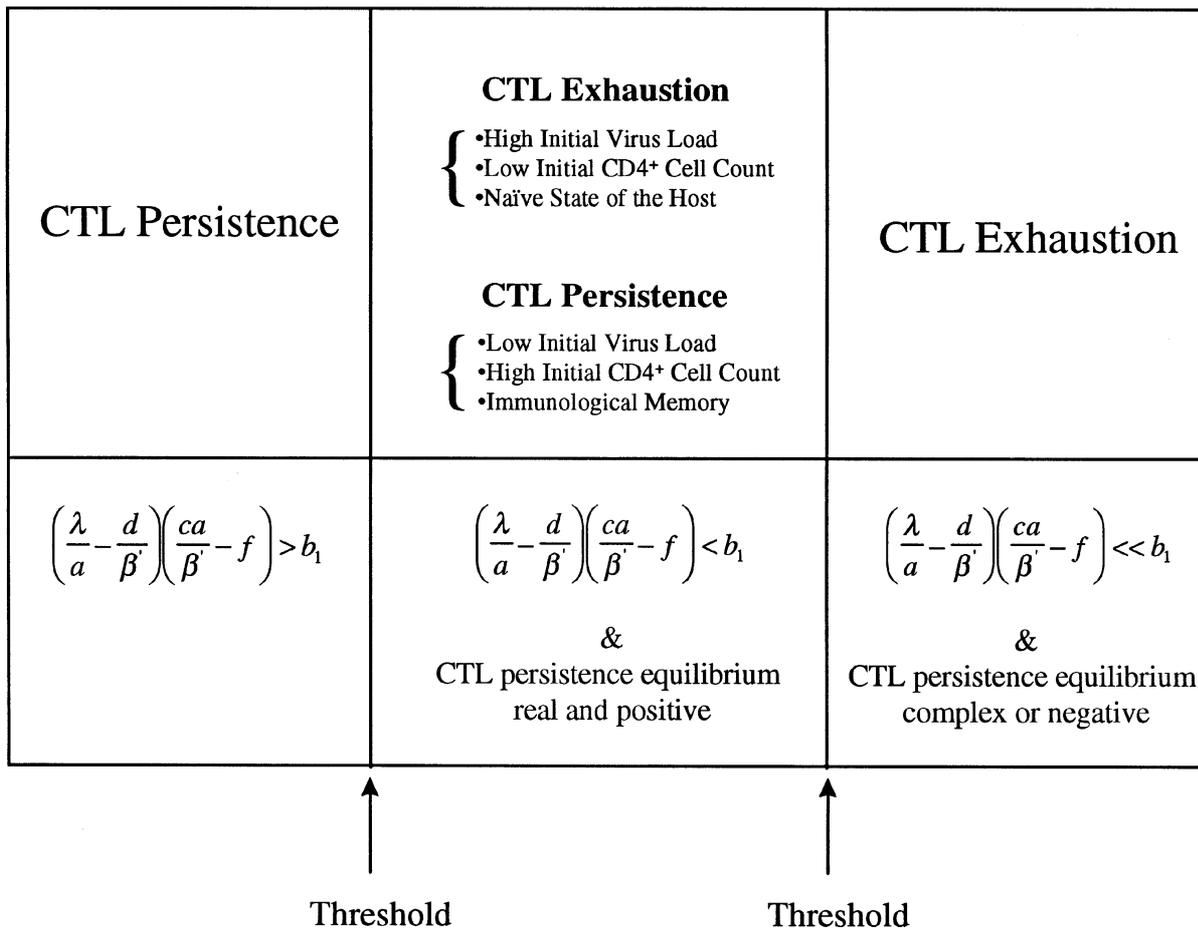


Figure 4. Schematic representation of the possible outcomes of APC and helper cell infection. The system is characterized by three parameter regions. If inequality (4) holds, the CTL response always persists. In the opposite case, the CTL response may either persist or be exhausted. If the CTL persistence equilibrium is real and positive, the outcome depends on the initial conditions, with a high initial virus load, a low CD4⁺ cell count, and the naïve state of the host promoting the exhaustion of the CTL response. On the other hand, if the CTL persistence equilibrium is complex or negative, CTL exhaustion is observed.

(i) *Cell tropism*

Ahmed & Oldstone (1988) demonstrated that virus strains differing in cell tropism may arise by organ-specific selection. When mice are infected neonatally with a predominantly neutrotropic strain such as 'Armstrong', lymphotropic variants, such as C13 or Docile, can be recovered from the spleen later in the course of infection. In contrast to Armstrong, these lymphotropic variants may induce CTL exhaustion when infected into adult mice. They not only replicate in CD4⁺ T cells and macrophages, but also in interdigitating dendritic cells thereby compromising antigen presentation and thus CD4⁺ cell function. Several authors have speculated that these properties might be required for the virus to induce deletion of the CTL response (e.g. Ahmed & Oldstone 1988; Oldstone *et al.* 1988; King *et al.* 1990; Villarette *et al.* 1994; Borrow *et al.* 1995). A single amino acid change from phenylalanine to leucine at position 260 of the viral glycoprotein has been correlated with the lymphotropic property and also with the ability to induce CTL exhaustion (Evans *et al.* 1994; Villarette *et al.* 1994; Dockter *et al.* 1996). Whether infection of CD4⁺ T cells or APCs is more important in contributing to the occurrence of CTL exhaustion is hard to distinguish. Moskophidis *et*

al. (1993a, 1995), concluded that antigen presentation is not a limiting factor since delayed adoptive transfer of T cells at a time when all endogenous CTL precursors have already vanished still results in vigorous expansion. However, looking at their data (Moskophidis *et al.* 1993a), it is clear that the transgenic TCR⁺ T cells are not stimulated to the same extent with delayed transfer, indicating a possible significance for functional APCs in maintaining a CTL response. This is further supported by the findings that the absence of macrophages leads to increased propensity to exhaustion (Karrer *et al.* 1997; Seiler & Aichele 1998). On the other side, it has been pointed out that only few CD4⁺ T cells are infected with LCMV (Tishon *et al.* 1988; Borrow *et al.* 1991; Zinkernagel *et al.* 1993), and that this may argue against the importance of CD4⁺ T-cell infection in promoting CTL exhaustion. However, the fact that CD4⁺ T-cell function is significant for preventing CTL exhaustion has been shown by several authors (e.g. Battagay *et al.* 1994, 1996; Thomsen *et al.* 1996), and with human immunodeficiency virus (HIV), infection of T-helper cells is thought to be a relevant factor contributing to disease progression even if in this case as well, only a small proportion of CD4⁺ T cells is infected (Wei *et al.* 1995; Ho *et al.* 1995).

In conclusion, these patterns fit in well with our model and with the idea that virus persistence *in vivo* is usually associated with immunocyte infection (McChesney & Oldstone 1987).

(ii) *Replication kinetics*

Concerning the relationship between the replication kinetics of the virus and its ability to induce exhaustion of the CTL response, experiments by Moskophidis *et al.* (1993a, 1995) are of interest with respect to our models. In accordance with the theory presented here, they showed that faster replication kinetics of LCMV strains were associated with a higher efficiency of inducing CTL exhaustion. Moreover, our experiments have demonstrated that it is not necessarily the *in vitro*, but the *in vivo* growth characteristics of the virus isolate that correlate with its ability to induce the exhaustion of the CTL response in mice (figure 5). Whereas the *in vitro* growth rates of LCMV Armstrong and Docile were similar, the *in vivo* replication kinetics were clearly faster for Docile which induced the depletion of the virus-specific CTLs.

Not only were the viral replication parameters found to be important, but also the route of infection (Moskophidis *et al.* 1995). Subcutaneous infection was less efficient at causing the depletion of the CTL response than intravenous infection. The authors argued that subcutaneous infection considerably slowed down the spread of the virus, and thus the overall replication kinetics, compared to intravenous infection.

Moreover, it has been found that branches of the immune system inhibiting the replicative capacity of the virus may be necessary to maintain a strong CTL response against LCMV. Thus, mice lacking type I, or type I and type II, interferon (IFN) receptors showed CTL exhaustion even upon challenge of slower replicating strains of LCMV which are normally cleared in wild-type mice (Van den Broek *et al.* 1995a,b). In addition, Moskophidis *et al.* (1994a) found a correlation between resistance of LCMV strains to interferon (enhancing the replication rate of the virus) and CTL exhaustion accompanied by viral persistence. Along similar lines, antibody deficient mice, increasing the overall replication kinetics of the virus, were shown to promote the depletion of the CTL response (Thomsen *et al.* 1996).

(iii) *Dependence on initial conditions*

In accordance with our theory, Moskophidis *et al.* (1993a, 1995) found that the initial conditions were significant in determining the course of the infection. A higher virus load correlated with a higher efficiency of exhausting the CTL response, and the faster replicating a strain was, the lower was the initial dose necessary to induce a decline of the specific CTLs. The dependence of the course of infection on the initial virus load was also shown by our data (figure 5). While LCMV Docile failed to induce the exhaustion of the CTL response at an initial dose of 2×10^2 pfu, the specific CTLs vanished when mice were infected with 2×10^6 pfu.

Among the initial conditions, the models also identified the CD4⁺ cell count and the immunity of the host as being significant factors influencing the course of LCMV infection. In support of this notion, it has been found that CD4⁺ cell depletion promoted CTL exhaustion in mice

(Battegay *et al.* 1994; Matlobian *et al.* 1994; Thomsen *et al.* 1996). On the other hand, high-dose infection with LCMV Docile under conditions where there are elevated antiviral precursors (i.e. in a previously exposed mouse) may not induce exhaustion (figure 5c).

The close agreement between theoretical and empirical results indicates that exhaustion of the virus specific CTL response is a direct consequence of the viral dynamics and distribution.

(b) *Implications for HIV infection*

It is important to extrapolate these ideas from LCMV to human immunodeficiency virus (HIV) infection. Although the phylogeny of the viruses is very different, both infections are characterized by an early and dominant CTL response, and in both the fine balance between the two may determine the ultimate outcome (Zinkernagel & Hengartner 1994; Klenerman *et al.* 1996a).

CTL exhaustion has been proposed as a mechanism for the eventual breakdown of the immune system upon progression to AIDS (e.g. Doherty 1993; Moss *et al.* 1995; Rinaldo *et al.* 1995a). Given that HIV infects cells that are involved in helping the CD8⁺ cell response to develop (i.e. CD4⁺ T cells and APCs), there should be a replication rate threshold beyond which the CTL response vanishes. Assuming HIV to evolve towards higher replication rates during the asymptomatic period of the infection, our model suggests a pattern of disease progression similar to that seen in HIV patients (figure 7). As long as the CTLs are still present, virus load remains at relatively low levels. When the replication rate of the virus evolves beyond the threshold for CTL exhaustion to occur, virus levels can shoot up to high abundances, uninhibited by any efficient immune response. This theory is supported by the literature. Connor & Ho (1994) analysed sequential HIV-1 isolates from a patient who progressed to AIDS in five years. Shortly after seroconversion, they only isolated slowly replicating variants. However, during the asymptomatic period of the infection, the replication kinetics of the isolates increased steadily and strongly until the onset of AIDS. They also reported that long-term non-progressors harboured only relatively slowly replicating HIV variants. The notion, that differences in replication rates may be important for understanding progression to AIDS has also been formulated by others (e.g. Asjo *et al.* 1986; Fenyo *et al.* 1988; Tersmette *et al.* 1989; Gruters *et al.* 1991; Ferbas *et al.* 1996). Mathematical models describing the increasing abundance of faster replicating strains during disease progression are discussed by Nowak & May (1991, 1992), deBoer & Boerlijst (1994), and Schenzle (1994).

Two points are worth noting in figure 7. First, before the replication threshold is reached, i.e. in the asymptomatic period, there is a slow but steady increase in virus load given that the virus continuously evolves towards higher replication rates. This notion is supported by recent data analysed by Rinaldo *et al.* (1997), who performed longitudinal studies on 14 men from the Multicenter AIDS Cohort Study (MACS). They found that, after the initial viraemia had been resolved, there was a small but sustained exponential increase in virus load before the T-cell inflection point was reached, thus supporting the predictions of our model.

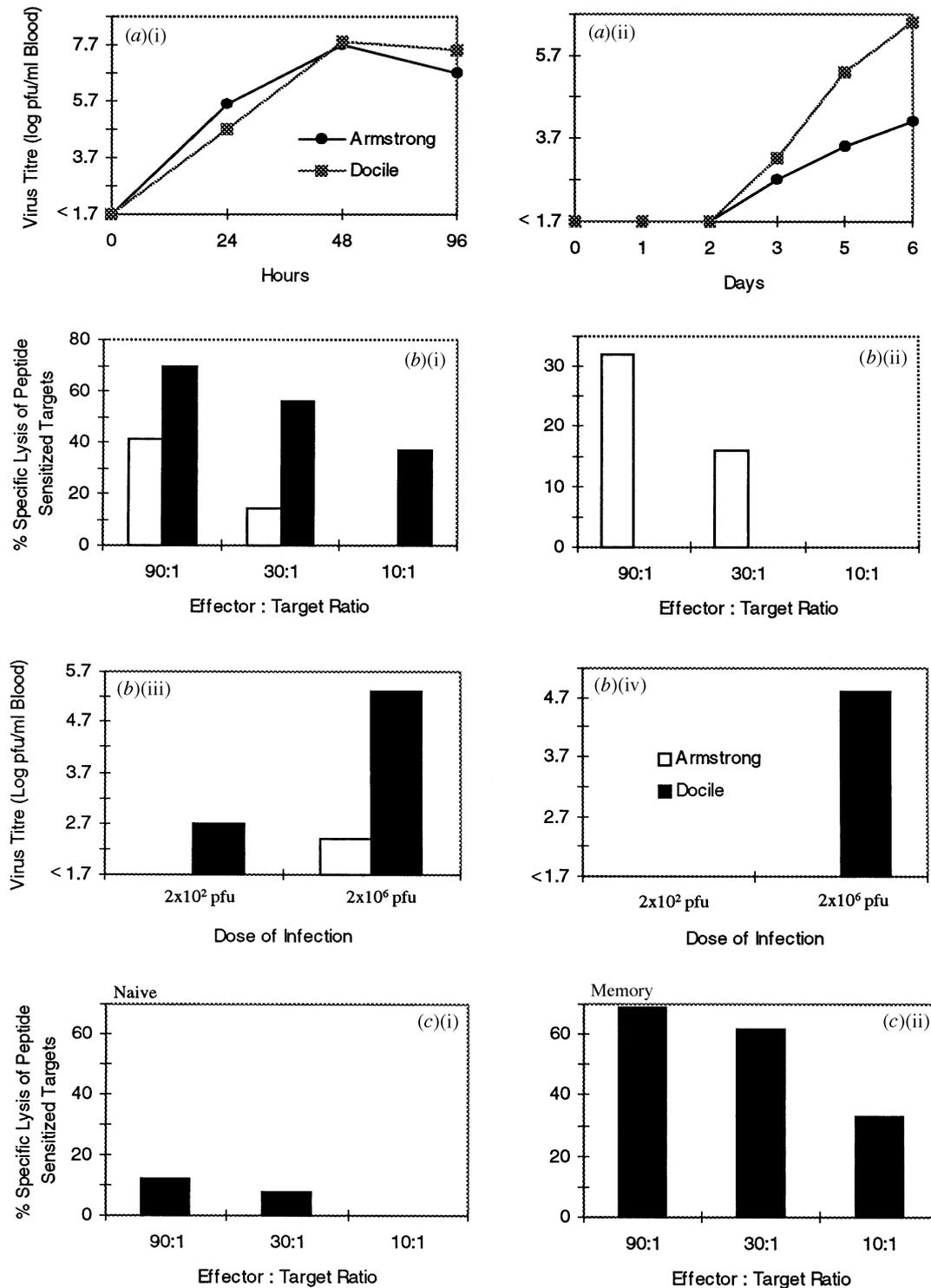


Figure 5. New experimental data on CTL persistence versus exhaustion in mice infected with LCMV Armstrong and Docile. (a) (i) *In vitro* and (ii) *in vivo* growth rates of the two strains. To measure *in vitro* growth, MC57 murine fibroblasts were infected with LCMV strains Armstrong and Docile at a multiplicity of infection of 0.01. Cells were maintained in culture (minimal essential medium plus 5% foetal calf serum), and supernatants taken for assay of viral titres at the time points shown. To measure *in vivo* growth, AG129 mice deficient in both alpha/beta and gamma interferon receptors, and highly susceptible to infection with LCMV (Van den Broek *et al.* 1995), were infected with 1 pfu Docile or Armstrong strain and blood viral titres determined as shown. The graphs demonstrate that while LCMV Docile and Armstrong do not differ significantly in their *in vitro* growth rates, Docile clearly grows at a significantly faster rate than Armstrong *in vivo*. (b) CTL persistence versus exhaustion. C57BL/6 mice were infected intravenously with LCMV (strains Docile or Armstrong and doses as indicated). Direct *ex vivo* antiviral lytic activity was determined exactly as previously using chromium release assays in which effectors were splenocytes taken from mice eight days after infection and targets were MHC Class I matched fibroblasts (Lehmann-Grube *et al.* 1985; Aebischer *et al.* 1991; Althage *et al.* 1992; Moskophidis *et al.* 1992, 1993*a,b*, 1994*b*, 1995; Battagay *et al.* 1993, 1994; Zinkernagel *et al.* 1993). Virus titres in blood and organs were determined by immunological focus assay (Battagay *et al.* 1991) at day 8 (iii) and day 30 (iv). With low dose infection ((i):

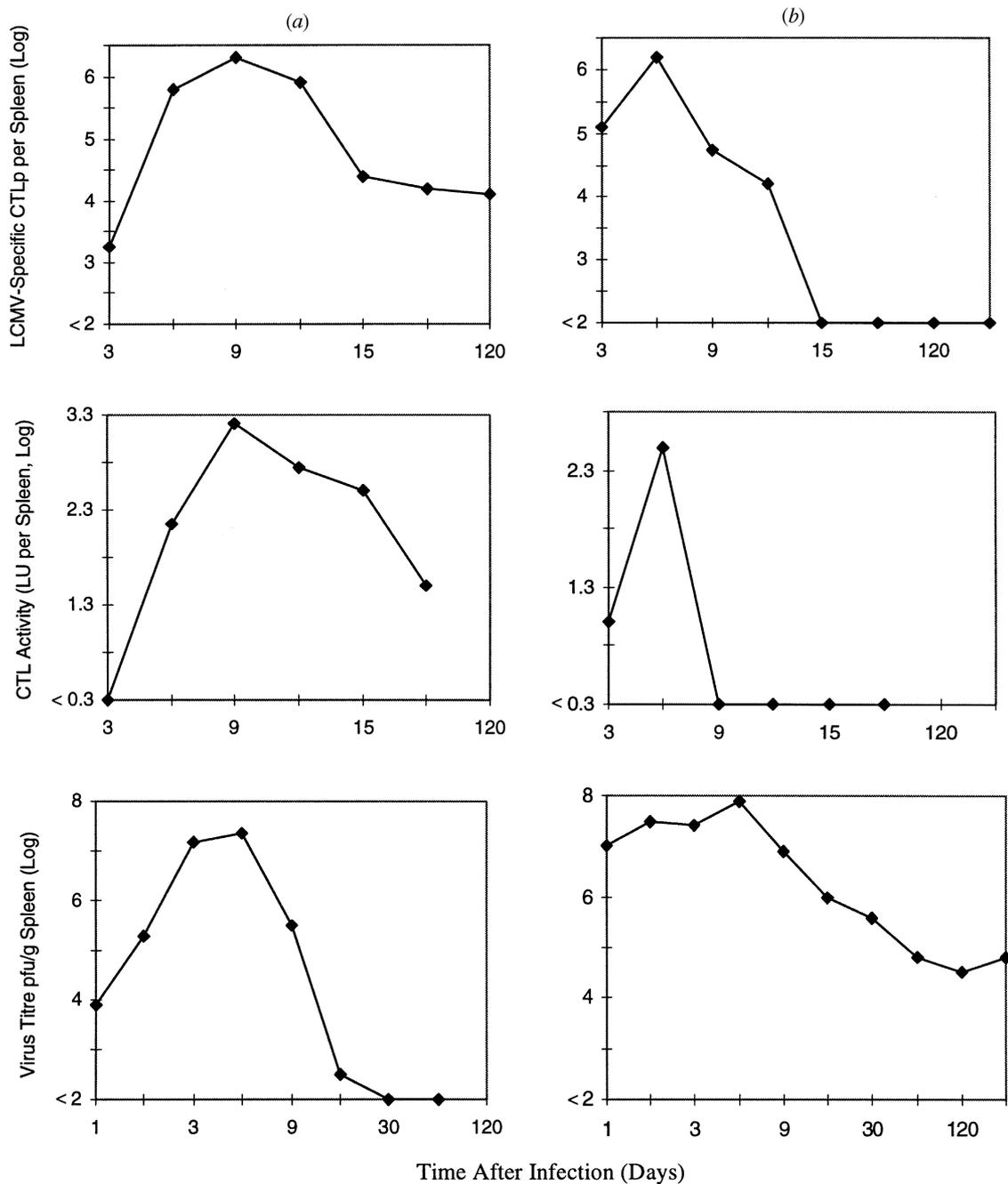


Figure 6. *In vivo* dynamics of the CTL response in mice infected with LCMV-Docile at initial doses of (a) 10^2 pfu and (b) 10^7 pfu. These time-series qualitatively match with the simulation results presented in figure 2. Data were taken from Moskophidis *et al.* (1993a). With low-dose infection the CTL precursor population grows and stays at a relatively high memory level. With high dose infection, the CTLp population first rises to a peak followed by a decline to undetectable levels. Persistence versus exhaustion of the CTL response is also reflected in the values of CTL activity measured. In the case of CTL persistence, virus levels drop to low or undetectable levels, whereas with CTL exhaustion, the virus population may grow without the inhibition by the CTL response to relatively high levels.

Figure 5 (continued) 2×10^2 pfu), the CTL response against both strains persists with higher levels of lysis observed for LCMV Docile infected mice. Virus titres are kept at low or undetectable levels. With high dose infection ((ii): 2×10^6 pfu) the CTL response against LCMV Armstrong persists while it is exhausted with LCMV Docile infection. Accordingly, virus titres in LCMV Docile infected mice persist at relatively high levels. (c) (i) (naive) Primary challenge versus (ii) (memory) Re-challenge. C57BL/6 mice were infected intravenously with low-dose LCMV WE (2×10^2 pfu) or remained uninfected. After 30 days, they were infected intravenously with 2×10^6 pfu LCMV Docile and *ex vivo* lytic activity tested as above. CTL mediated lysis remained much higher in memory compared to naive mice. This may be due to the presence of an elevated number of CTL precursor cells in the memory mice, although a secondary antibody response may also contribute to this effect.

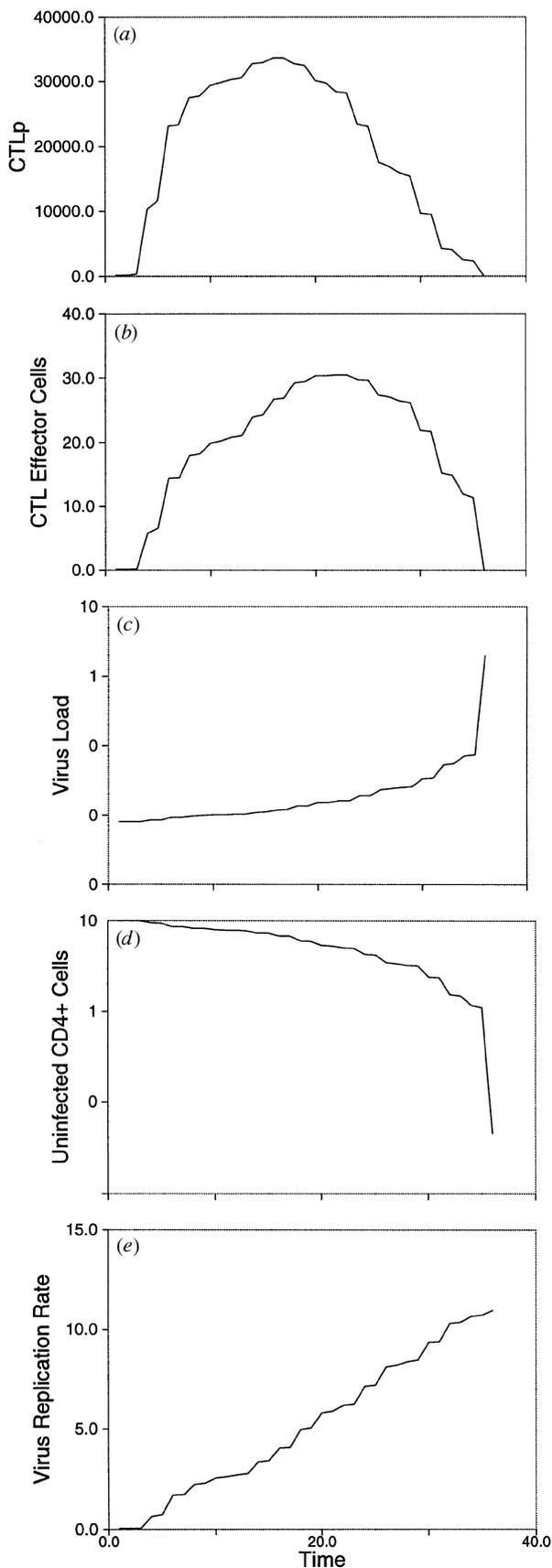


Figure 7. Implications of CTL exhaustion for HIV disease progression. The graphs show the effect of virus evolution towards higher replication kinetics on the number of (a) CTL precursor cells, (b) CTL effector cells, (c) virus load and (d) the number of uninfected target cells. At each time interval the viral replication rate (β') is increased by a random amount and the equilibrium values of the respective variables calculated.

The second point to be made concerns the decline of $CD8^+$ cells. Although complete exhaustion occurs at the same time for both CTL populations, the precursor cell population starts to decline earlier and declines at a faster rate than the CTL effector cell population. Thus, when the CTLp pool has largely vanished, one might still expect to see considerable CTL effector activity. This has also been observed in experimental data. Rinaldo *et al.* (1995*a,b*) found that while the level of CTL precursors was correlated with disease progression, this was not observed for CTL effector cells. In advanced progressors a significant CTL effector response was still evident, probably arising from residual memory. There was only a correlation between CTL effector activity and time, leading to the hypothesis that the effector response strongly declines only at the end-stage of the disease (Rinaldo *et al.* 1995*b*). This is supported by our model.

Another way HIV may evolve to faster replication is to mutate CTL epitopes (Phillips *et al.* 1991; Nowak *et al.* 1995; Borrow *et al.* 1997; Goulder *et al.* 1997). They may either escape recognition altogether or alternatively represent a class of altered peptide ligands which partially activate T cells. The presence of populations of viruses bearing variant ligands in the evolving quasispecies may allow effectively faster viral replication by acting as TCR antagonists (Jameson *et al.* 1993; Bertoletti *et al.* 1994; Klenerman *et al.* 1994; Meier *et al.* 1995), or promoting responses with inappropriate specificities (Jameson & Bevan 1995; Kalams & Walker 1995; Klenerman *et al.* 1995, 1996*b*; McAdam *et al.* 1995; Borrow *et al.* 1997), in this way contributing to the exhaustive process. Virus evolution and escape from the CTL response as a mechanism of disease progression has been modelled by Nowak *et al.* (1991, 1995).

Progression towards the threshold for CTL exhaustion may not only be achieved by the virus evolving towards higher replication kinetics, but also by a loss of efficiency of those branches of the immune system that limit the overall replication kinetics of the virus. Thus, a change in the pattern of cytokine production (Levy *et al.* 1996), or a decline in the levels of neutralizing antibodies may increase the viral replication kinetics, driving the system towards CTL exhaustion. Low levels of neutralizing antibodies have been correlated with faster progression to AIDS (e.g. Fenouillet *et al.* 1995; Pantaleo *et al.* 1995; Fenyo & Putkonen 1996), and Scarlatti *et al.* (1996) found that the presence of neutralizing antibodies to

Before the CTL exhaustion threshold is reached, virus load increases and the number of uninfected target cells decreases at a relatively slow rate. Once the CTL exhaustion threshold is reached, virus load can shoot up to high levels, not being inhibited by any efficient immune response. Consequently, the number of uninfected target cells drops to low levels. Note that the CTL precursor population starts to decline earlier and declines at a faster rate than the CTL effector population. For the parameter values used, the CTLp population starts to decline at a viral replication rate β' ca. 4.2, whereas the CTL effector cells start to decline at β' ca. 6.2. Parameters were chosen as follows: $\lambda=1$, $d=0.1$, $c=2.5$, $f=0.1$, $a=0.5$, $b_1=0.2$, $b_2=1.5$, $p=1$.

autologous virus as well as a slow replicative capacity of the virus were associated with slow progression to AIDS.

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