The role of antigen-independent persistence of memory cytotoxic T lymphocytes

Dominik Wodarz, Robert M. May and Martin A. Nowak

Institute for Advanced Study, Olden Lane, Princeton, NJ 08540, USA
Department of Zoology, University of Oxford, South Parks Road, Oxford OX1 3PS, UK

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Abstract

We use mathematical models to analyze the role of a memory cytotoxic T lymphocyte (CTL) response in viral infections. The model predicts that antigen-independent persistence of an elevated number of precursor CTL (CTLp) does not protect the host from clinical symptoms upon re-infection. Instead, we find that antigen-independent long-term persistence of CTLp is required to clear virus infections. This mechanism also applies to infection in hosts that have never experienced the pathogen before. Requirement of antigen for the long-term maintenance of CTLp results in failure to clear the infection, even in hosts characterized by a high CTL responsiveness. We compare the CTL model to a B cell model. In keeping with experimentally established findings, B cells are efficient in protecting against re-infection, but are unlikely to clear viral infections unless the virus is cytopathic. We conclude that the role of ‘memory CTLp’ is different from the role of memory B cells in viral infections: antigen-independent long-term persistence of CTLp is a prerequisite to ensure clearance of infection.

Introduction

A central feature of the immune system is the concept of immunological memory (1–3). Immunological memory can be defined both on a functional and on a cellular level. On a functional level, it describes the observation that hosts are protected against re-infection by a pathogen. On a cellular level, memory describes the persistence of an elevated number of specific immune cells after the resolution of the disease (1–3). Acute viral infections induce both humoral and cellular immunity. Antibodies fight free virus particles and can also prevent viral entry into host cells, whereas CD8-mediated effector mechanisms target the infected cells. Although CD4 cells may in some cases be directly involved in inhibiting viral replication (4), they mainly provide help required for the development of efficient CD8 cell and B cell responses (5). While antibody memory has been shown to be protective against re-infection (6), considerable controversy remains about the nature and protective wisdom of cytotoxic T lymphocyte (CTL) memory, especially about the role of persistent antigen (7–15). While long-term persistence of precursor CTL (CTLp) has been found to be independent of antigen, the protective function of CTL memory seems to require the continuous presence of antigen. The complexity of the dependence of infection on varying the initial numbers of CTLp has been explored by Ehl et al. (16).

The interactions between a virus population, host cells and the immune system are highly non-linear. Thus, the outcome of the infection dynamics are frequently counter-intuitive. In addition to experimental work, mathematical modeling offers a complementary approach to investigate the exact properties of such a complex biological system, and provides a framework upon which to build experiments, interpret empirical data and create new hypotheses. In this paper we use mathematical models to analyze the consequence of antigen-independent maintenance of the CTLp population in viral infections. We compare the CTL model to a B cell model. In particular we investigate the conditions required to clear a viral infection and to protect the host against re-infection.

Results and discussion

Modeling CD8 cell dynamics

There is accumulating experimental evidence that CD8 cell responses are required to resolve viral infections (17–20), and differences in virus load and clinical outcome in persistent infections have been correlated with specific MHC class I genotypes (19–21). After a virus has started replicating in a
host, a vigorous expansion of virus-specific CD8 cells is observed. In a typical infection the rising CTL response induces a decline in virus load. As the infection is resolved, a significant amount of apoptosis occurs in the CD8 cell population until it finally settles at a ‘memory equilibrium’ that is orders of magnitude higher compared to the naive state of the host. The population of memory cells is thought to consist mainly of CTLp and their persistence has been shown to be independent of antigen (9,22,23). However, if antigen does persist, this could induce continuous differentiation to result in low-level effector activity (24). Constant antigenic stimulation might be required for an effective protective function upon re-challenge, since it ensures the presence of effector CTL (CTLe) as well as re-circulation of the CTL population (13,23).

Here we are interested specifically in the long-term antigen-independent persistence of CTLp. Basic virus dynamics can be described by differential equations containing three variables: uninfected cells, $x$, infected cells, $y$, and free virus, $v$. The model is described schematically in Fig. 1(a). In order to analyze the dynamics of CTL, we add two more variables: CTLp, denoted by $w$, and CTLe, $z$ (Fig. 1b). In the following we give a definition of CTLe and CTLp on which the mathematical model is based. We define CTLe as CD8 cells which differentiate from CTLp and have the capacity to inhibit virus replication at a rate $p$. While we assume that this occurs through lysis in this model, including non-lytic CD8 cell activity has similar properties (25). Although there have been speculations that CTL effectors might be longer lived than previously thought, we assume that in accordance with the experimental literature, CTL effectors are short lived in the absence of antigen and do not proliferate significantly (24,26). We define CTLp as CD8 cells that have seen their specific antigen, but lack effector function. They have the capacity to proliferate in response to antigen at a rate $c$. In the absence of antigen, the expanded CTLp population declines at a rate $b$. If the value of $b$ is small, the rate of CTLp decline in the absence of antigen is slow. This corresponds to antigen-independent persistence of CTLp. On the other hand, a large value of $b$ corresponds to a relatively fast rate of CTLp decline in the absence of antigen, i.e. CTLp do not persist at elevated levels in the absence of antigen, and maintenance of the CTLp population depends on constant stimulation.

Note that this is a phenomenological model and is independent of the exact molecular mechanisms responsible for maintaining the population of memory CTLp in the absence of antigen, which is still highly controversial (27). Our analysis is also independent of the exact identity of memory cells, since we define memory CTL as CTLp which persist at a stable level independent of antigen. While significant progress has been made in identifying markers for memory CTL (24,26), this issue is still under investigation. Another controversial area of research is the differentiation pathway that leads to the generation of CTLe and effector cells. This could involve more complicated mechanisms than explicitly captured in the model. However, conclusions drawn from our model should be general, since the ability of the CTLp population to be maintained in the absence of antigen is captured in a single variable.

**Virus clearance versus persistence**

Here we examine the conditions required to result in CTL-mediated clearance of viral infections. Because our model is deterministic, the CTL response cannot reduce virus load, $y$, to exactly 0, although virus load may be reduced to very low levels. Hence we define two thresholds. If $y < y_{\text{min}}$, virus persists, although below the detection limit. If $y < y_{\text{ext}}$, the virus population has gone extinct. Thus, $y_{\text{ext}} < y_{\text{min}}$. Note that these thresholds are arbitrary. In reality they will depend on a complex balance between host and viral parameters as well as stochastic events. In general, the lower the virus load predicted by the model, the higher the chances of viral clearance.

According to the model, two factors promote reduction of virus load below the extinction limit at equilibrium: (i) long-term persistence of CTLp in the absence of antigen (low $b$) and (ii) a high activation rate of CTLp (high $c$). In addition, the model also offers an alternative mechanism of virus elimination. The predicted dynamics of the primary infection are characterized by vigorous oscillations, i.e. the virus population first replicates up to a peak and is then reduced by the rising CTL response (Fig. 2). This initial oscillation may drive virus load to very low levels, below the extinction limit. We call this process ‘dynamic elimination’. Again we find that antigen-independent long-term persistence of CTLp (low $b$) as well as a high activation rate of CTLp (large $c$) maximize the chances of dynamic elimination (Fig. 3a). Even in parameter spaces where equilibrium virus load lies above the extinction threshold, dynamic elimination may still clear the infection. This implies that virus elimination or persistence can depend on the initial conditions or, in other words, on the size of the virus inoculum and the exact route of infection.

In biological terms, this can be interpreted as follows. The result that a high activation rate of CTLp, or a high CTL responsiveness, $c$, is required to control viral infections is in accord with a variety of previous theoretical results analyzing the dynamics between CTL and virus replication (28,29). It also keeps with experimental results showing that differences in the genetic makeup of the MHC can determine pathogenesis in persistent infections (19–21). However, our current modeling makes the novel prediction that this is not sufficient to clear the infection: persistence of the CTLp population in the absence of antigen–traditionally considered only in the context of protection against re-infection–is a prerequisite to clear the virus. This also applies to hosts which have never experienced the pathogen before. Hence, our model suggests that virus clearance results from antigen-independent persistence of CTLp. If long-term persistence of CTLp is antigen dependent (higher values of $b$), the model predicts continuous virus replication, with the levels of virus load depending on the overall efficacy of the immune response. This can be understood intuitively as follows. If long-term maintenance of the CTL population requires persistent antigen, the CTLp population declines to low levels as virus load drops following CD8-mediated lytic activity. This decline in the CTL population enables the virus to regain a positive growth rate and therefore to settle at a stable equilibrium where the virus is replicating persistently in the presence of the CTL response. In contrast, with antigen-independent persistence of CTLp, the CTL popu-
Fig. 1. Schematic explanation of mathematical models. For details see Methods. (i) Basic model of virus replication. (ii) CD8⁺ T cell dynamics. Note that this is a simplified model and the results do not depend on the specific pathway underlying CTL differentiation which is still controversial. Moreover, although we model a lytic CTL response, the conclusions reached from this model also hold true for non-lytic mechanisms of CD8⁺ T cell activity (25). (iii) B cell dynamics. The differentiation pathway of virus-specific B cells is complex and involves T cell-dependent activation, proliferation, isotype switching and germinal center reactions. However, the net result of this differentiation pathway is the production of neutralizing antibodies and this production may continue for a long time after the infection has been resolved. We consider a simple phenomenological model, assuming that antibodies are produced in response to virus and that antibody production diminishes at a certain rate.
Fig. 2. Antigen-independent long-term persistence of CTLp contributes to virus clearance. (i) Virus clearance. If the CTLp response is long lived in the absence of antigen, virus load is driven below the extinction threshold. After virus clearance, the level of CTLp remains high while the effector response quickly diminishes. Parameters were chosen as follows: $\lambda = 10; \beta = 0.01; a = 0.5; p = 1; c = 0.1; b = 0.001; q = 0.1; h = 0.1$. (ii) Virus persistence. A shorter life-span of the CTLp response in the absence of antigen can lead to virus persistence in the presence of the CTL response. In this simulation the CTLp response is still relatively strong and keeps the replicating virus below the detection limit. After the virus has been controlled, an elevated number of CTLp remains due to a combination of constant antigenic stimulation and antigen-independent persistence of CTL. Since the virus has not been cleared, the effector response also persists at low levels. If the life-span of CTLp in the absence of antigen is even shorter, virus load may remain above detection limit which in turn may lead to clinical symptoms. Parameters were chosen as follows: $\lambda = 10; d = 0.1; \beta = 0.01; a = 0.5; p = 1; c = 0.063; b = 0.0015; q = 0.1; h = 0.1$. Note that in the model and the simulation, we concentrate exclusively on ‘memory CTLp’. Other CTL clones that may proliferate in response to the pathogen and subsequently undergo apoptotic cell death are not taken into consideration. Hence the lack of a marked peak and sharp fall in the number of CTLp before reaching the ‘memory equilibrium’ is not seen in this figure.

Antigen-independent long-term persistence of CTLp contributes to virus clearance. This also applies to primary infection. Hence, beside a possible protective role upon re-challenge, we suggest that persistence of CTLp in the absence of antigen has evolved in order to achieve virus clearance in the first place. Note that we are not making the trivial statement that an effective CTL response is required to control viral infections. Even if the CTL responsiveness is high, requirement of antigen to maintain the CTLp population as well as stochastic factors determining extinction events.

If memory CTLp do have the capacity to persist in the absence of antigen (small b), the model is characterized by the presence of a quasi-equilibrium (see Materials and Methods). Virus load at the quasi-equilibrium has the same properties as virus load at the true equilibrium, but is slightly higher. At the quasi-equilibrium, virus load decays only at a very slow rate and reaches the true equilibrium after a timescale of $\sim 1/b$. In biological terms, this means that even if the CTL response has the capacity to clear the infection, we can expect the virus population to remain decaying at a slow rate at very low levels for a prolonged period of time before going extinct.

Predictions and experimental data

The basic result of our model so far is that antigen-independent persistence of CTLp is necessary to result in CD8-mediated viral clearance. This also applies to primary infection. Hence, beside a possible protective role upon re-challenge, we suggest that persistence of CTLp in the absence of antigen has evolved in order to achieve virus clearance in the first place. Note that we are not making the trivial statement that an effective CTL response is required to control viral infections. Even if the CTL responsiveness is high, requirement of antigen to maintain the CTLp population in the long term will lead to failure of virus clearance.

One way to test this hypothesis is to analyze a situation where the primary CTL response to a viral infection is intact, while the CTLp response at low levels of antigen is short lived. Such a situation is given in MHC class II$^+$ and CD40L$^+$ knockout mice infected with lymphocytic choriomeningitis virus (LCMV) (30–32). During the early phase of the infection, both wild-type and class II-deficient mice reduce virus load to undetectable levels. However, in contrast to wild-type mice, virus load re-emerges to high levels in class II-deficient mice $\sim$1 month after infection. Resurgence of virus load is associated with lack of a significant memory CTL response in class II-deficient mice. The absence of CD4$^+$ T cell help interferes with the generation and/or maintenance of CTL memory (30,33,34). Similar results are obtained from CD40L$^-$ mice infected with LCMV (31).
initial activation and differentiation of primary effector cells, they are essential for the generation of memory cells (31,37,38). Interpretation of these data strongly supports our theory that antigen-independent persistence of CTLp may be necessary to successfully resolve the infection in the first place, although caution is required because the knockout mice also lack efficient B cell responses which may contribute to the overall immunological control of LCMV (32,39). The role of B cell responses for the control of viral infections is addressed below.

The relationship between CTL dynamics and viral clearance has recently been studied in more detail (40,41). Although infection with LCMV resulted in generation of significant levels of CTLp that could be maintained in the absence of antigen and in resolution of the infection, the LCMV population was not extinct. It remained at very low levels for an extended period of time. LCMV was detected at least until day 30 and with more sensitive assays it was found for even longer periods of time (40,41). These observations indicate that although LCMV was not driven extinct immediately and persisted at low levels, the virus population decayed at a slow rate. This behavior is similar to the dynamics of virus load at the quasi-equilibrium in our model, where virus load decays at a very slow rate before reaching the true equilibrium. However, while this observation does not contradict our model, it also highlights a problem when analyzing properties of the CTL response required for virus clearance. Even if the CTL response is efficient and has all properties needed to clear an infection, the virus population could still persist if it is very invasive, especially when replicating in sites that are not easily accessible to the CTL. Hence, in practical terms, it is difficult to make a distinction between true virus clearance and virus persistence at very low levels, below the limit of detection. In addition, in the LCMV case, it is not clear whether CTL-induced killing of infected antigen-presenting cells could have induced minimal impairment of helper function, accounting for the prolonged persistence of virus load at low levels.

**CTL-mediated protection against re-challenge**

The role of CTL memory for protection against re-infection has to be considered separately for the cases when the virus is eliminated and when it persists. If the virus is driven below the extinction threshold during the primary challenge, only CTLp remain present at elevated numbers since the CTL response is assumed to be short lived. Because it is the CTL that confer anti-viral activity, the initial growth rate of the virus is always positive upon re-challenge. Figure 3(b) shows the effect of increased CTLp abundance on the size of peak virus load on secondary challenge, assuming different rates of effector cell production (cq). Parameters were chosen as follows: $\lambda = 10; d = 0.1; b = 0.01; a = 0.5; c = 0.1; b = 0.001; g = 0.1; h = 0.1$.

### Parameters
Traub in wild-type and mutant mice is similar. However, CD40L$^-$ mice lack CTL memory. While virus load is suppressed to low or undetectable levels in wild-type mice, levels of LCMV remain high 28 and 80 days post-infection in CD40L$^-$ mice. A possible mechanism for the lack of CTL memory in CD40L$^-$ mice is that the interaction between Th cells, antigen-presenting cells and CD8$^+$ T cells is impaired (35,36). Although these interactions may not be required for...
CTLp. This is in agreement with experimental findings on CTL memory in LCMV and vesicular stomatitis virus (VSV) (13,42).

On the other hand, if virus persists during primary challenge (below or above detection limit), secondary infection represents a disturbance of a stable equilibrium. Hence, the virus population cannot grow and the host is protected. However, this protection may be compromised if re-infection occurs with a virus strain that has mutated to become competitively superior to the immunizing strain, e.g. due to a faster rate of replication. In this case, the invading virus variant will have a positive initial growth rate and can therefore replicate up to a peak, the size of which is determined by the competitive superiority of the mutant over the wild-type.

In summary, the model predicts that persistence of elevated numbers of CTLp after the resolution of the infection is unlikely to protect the host against re-challenge. Protection is most likely if the virus has not been eliminated in the first place or if viral antigen remains in certain reservoirs.

CTL memory and the antigen persistence debate
There has been considerable controversy about the exact nature and protectiveness of CTL memory. In particular, the role of persisting antigen for maintaining CTL memory and ensuring an efficient secondary response is under intense debate (7–9,12,13,22,23,43–45). Recent results indicate that maintenance of CTL precursors is antigen independent and that the efficacy of the secondary response may or may not require persistent antigen, depending on the kinetics of effector cell production (13,23,42). This has been shown with LCMV and VSV infection in mice. If the secondary infection is i.v., then protection seems to be independent from the persistence of antigen (13,23). In this case, the virus population directly encounters the memory CTLp which leads to instant CTL activation and thus termination of the infection before the appearance of clinical symptoms. On the other hand, protection against peripheral infection appears to be dependent on the persistence of antigen (13,23). This is because antigen persistence induces the expression of relevant markers on the surface of CTLp, such as LFA-1 and VLA-4 (27,46). This ensures constant recirculation through non-lymphoid tissues which is required to recognize the invading virus before it has already replicated to high levels. Recently, the effectiveness of protection against secondary challenge was compared directly in an experimental system, immunizing mice both with LCMV and recombinant Listeria monocytogenes expressing the nucleoprotein of LCMV (40). In contrast to recombinant Listeria immunization, antigen was reported to persist at low levels following LCMV immunization. At comparable levels of memory CTLp, protection against secondary LCMV challenge was significantly more efficient for LCMV-induced memory CTL compared to recombinant Listeria-induced memory CTL. Thus, in agreement with our theoretical results, experiments show that CTL memory is only protective if the time window between invasion of the pathogen and induction of anti-viral CTL-effector activity is short.

However, in general it is not clear whether antigen persists after resolution of the infection, and if it does, how it persists and in what form. If antigen persists without low-level viral replication, it is likely that this antigen depot will be depleted after a while, rendering the elevated numbers of CTLp unprotective. On the other hand, if antigen persists because of low-level viral replication, there is a chance that the host eventually loses control, resulting in re-emergence of the disease. This is especially likely if a new CTL response to heterologous antigen reduces the old CTL memory population, as demonstrated by Selin et al. (47,48).

Our model predicts that the antigen-independent survival of CTLp is a requirement for CD8-mediated clearance, also in primary infection. With this result in mind, it makes sense that long-term persistence of CTLp is independent of antigen, but that recirculation of the CTL requires constant stimulation. Although CD8-mediated virus clearance depends on the long life-span of CTLp in the absence of antigen, there is no need for them to continue re-circulating once their job is done and the virus has been driven extinct. Hence, they are ‘parked’ in the lymphoid tissue and can potentially be eliminated if an infection with a heterologous virus occurs at a later time point (47,48).

A comparison with B cell responses
Here we consider neutralizing antibody responses. Virus-specific antibodies are created by a complex differentiation pathway that includes B cell proliferation, isotype switching, germinal center formation and affinity maturation (49–53). The net result is the presence of specific antibody-secreting plasma cells and antibody production tends to persist long after the resolution of the infection. Hence we can simply model B cell responses by assuming that antibodies \( z \) are produced in response to free virus and that antibody production diminishes at a certain rate. The model is explained in Fig. 1(c).

Antibody memory occurs at the level of effectors. In this model it is defined by persistence of antibody production after the resolution of the infection (low value of \( b \)). Note that this definition of memory is phenomenological and is independent of the exact mechanism underlying the persistence of antibodies. The mechanism may include continuous re-stimulation by antigen remaining on antigen-presenting cells (54–56) or long-lived plasma cells (6). According to the model, parameters associated with memory (low \( b \)) reduce free virus particles to very low levels. However, this does not imply virus extinction, since infected cells may persist at a high abundance for prolonged periods of time, unless the virus is cytopathic and kills infected cells at a fast rate (Fig. 4). This is also reflected by the fact that the model describing antibody memory is characterized by much more stable dynamics than the CTL memory model. Hence dynamic elimination of the virus is unlikely to occur (Fig. 4). These notions have also been observed experimentally: non-lytic antiviral activity alone is not sufficient to control an infection with a non-cytopathic virus, such as LCMV (57,58).

Since antibody memory lies at the level of effectors (neutralizing antibodies remain after clearance of infection), our model predicts that it is highly effective at protecting against secondary challenge by the virus. The virus fails to grow in the host upon re-infection if \( pz^* > \beta \lambda k \delta a - u \), where \( z^* \) represents the level of memory antibodies. Thus, non-cytopathic and/or fast replicating viruses require more efficient antibody memory for protection.
responses are unlikely to result in virus clearance unless Uninfected target cells are produced at a rate \( \lambda \) at protecting the host against re-infection, while B cell have primarily evolved in order to clear viral infections. established if the basic reproductive ratio of the virus, \( R \). 

Using mathematical models, we have shown that the role of memory CTLp is different from the role of memory B cells in viral infections. In keeping with experimental observations, theory predicts that B cell memory is efficient at protecting the host against re-infection, while B cell responses are unlikely to result in virus clearance unless the virus is cytopathic. On the other hand, our models suggest that contrary to previous thinking, persistence of readily activated CTLp in the absence of antigen might have primarily evolved in order to clear viral infections. This mechanism also applies to hosts which have never experienced the pathogen before. Requirement of antigen for the long-term maintenance of CTLp results in failure to clear the infection, even in hosts characterized by a high CTL responsiveness. CTL-mediated protection from re-infection could be a carry-over effect resulting from the long-term persistence of CTLp, accounting for the controversial debate about this issue. This insight provides a new perspective in which to interpret long-term antigen-independent persistence of CTLp.

Our findings also have implications for improving our understanding of the dynamics between HIV and the immune system. Development of CTLp that are long lived in the absence of antigen has been shown to depend on CD4 T cell help (30,31,37,38). During acute HIV infection, virus replication to high loads results in impairment of specific T_h cell responses (18,59–61). Absence of CD4 T cell help could result in the reduction or absence of CTLp that are long lived in the absence of antigen, and this could be a reason for persistent HIV replication and eventual pathogenesis. This is supported by data from HIV-infected patients starting on anti-retroviral drugs (62,63). In these patients, the CTLp response has been observed to decline at a relatively fast rate when virus load is reduced by drug therapy, indicating that CTLp are short lived in the absence of antigen and that they are maintained during the asymptomatic phase mainly by active viral replication. Such a response is expected to lose control of the virus in the long term. Specific application of our model to HIV infection offers a theoretical framework in which to interpret and analyze experimental data showing improved virus control following therapy during primary infection as well as intermittent treatment regimes (64–66). The effect of such treatment regimes could be to provide the immune response with an antigenic boost while preventing significant amounts of immune impairment. This results in sufficient T cell help for the development of CTLp that are long lived in the absence of antigen, capable of controlling HIV in the long-term, absent continuous therapy (66). These insights also have implications for developing vaccination strategies aiming at preventing establishment of HIV infection, or at improving the degree of immunological control achieved.

**Methods**

**Basic virus infection model**

Denoting uninfected cells by \( x \), infected cells by \( y \) and free virus by \( v \), the model is given as follows:

\[
\begin{align*}
\dot{x} &= \lambda - \beta xv - dx \\
\dot{y} &= \beta xv - ay \\
\dot{v} &= ky - uv 
\end{align*}
\]

Uninfected target cells are produced at a rate \( \lambda \), die at a rate \( dx \) and become infected at a rate \( \beta xv \). Infected cells die at a rate \( ay \) and produce new virus at a rate \( ky \). Virus particles decay at a rate \( uv \). A persistent infection is established if the basic reproductive ratio of the virus, \( R_0 = \frac{\beta k}{dau} \), is greater than unity. This corresponds to the situation when one infected cell on average gives rise to more than one newly infected cell at the beginning of the infection. Persistent virus replication is described by

**Conclusion**

Using mathematical models, we have shown that the role of memory CTLp is different from the role of memory B cells in viral infections. This is true for both virus extinction at equilibrium and dynamic elimination of the virus. While a strong antibody response may reduce the equilibrium number of free virus particles to low levels, the equilibrium number of infected cells may be much higher. In addition, especially for non-cytopathic viruses, the number of infected cells decays only slowly. If the virus replicates relatively efficiently, the presence of high levels of infected cells also slows down the rate of free virus decay, since replication counters the effect of antibodies on the virus population. \( \lambda = 10; d = 0.1; \beta = 1; a = 0.1; p = 1; c = 0.1; b = 0.001; k = 0.1; u = 2. \)
Virus replication controlled by a CTL response is described by $\ln(\gamma)$ parameter ranges, $\gamma$. In addition, contact with antigen induces differentiation into CTLe at a rate $\gamma\lambda$. CTLe lyse infected cells at a rate $\gamma\lambda$. The model is given by:

$$
\begin{align*}
\dot{x} &= \alpha (1-q) - b \beta y, \\
\dot{y} &= b - \frac{\alpha (1-q)}{\gamma}, \\
\dot{v} &= ky - uv, \\
\dot{w} &= cyw(1-q) - bw, \\
\dot{z} &= cyw - hz.
\end{align*}
$$

The CTL response becomes established if $\gamma(1-q) > b$. Virus replication controlled by a CTL response is described by equilibrium $E_2$:

$$
\begin{align*}
\dot{x}^{(2)} &= \frac{\lambda(1-q)}{c(1-q) + b\beta'}, \\
\dot{y}^{(2)} &= \frac{b}{c(1-q)}, \\
\dot{v}^{(2)} &= ky^{(2)}/u, \\
\dot{w}^{(2)} &= \frac{z^{(2)}h(1-q)}{bq}, \\
\dot{z}^{(2)} &= \frac{\beta'x^{(2)} - \alpha}{\rho}.
\end{align*}
$$

The dynamics of system (2) are characterized by the presence of a quasi-equilibrium, $\gamma$, for relatively low values of $b$, at which virus load decays only very slowly towards its true equilibrium $\gamma^{(2)}$. It can be shown that over realistic parameter ranges, $\gamma$ and $\gamma^{(2)}$ are very close, and that the parameters $b$ and $c$ have identical effects $\gamma$ and $\gamma^{(2)}$. Only for very low values of $b$, tending towards 0, do we observe an increasing discrepancy between $\gamma$ and $\gamma^{(2)}$.

The detailed dynamics of system (2) can be understood by re-scaling the equations. Defining $X = dx/k (X_0 = 1)$, $Y = \beta'y/d (Y_0 = \beta'y_0/d)$, $Z = pz/a (Z_0 = 0)$, $W = capdw/ha''t^1$ ($W_0 = capdw/ha''t^1$) and assuming a quasi-steady state for $\gamma$, the model can be re-written as follows:

$$
\begin{align*}
\dot{X} &= \gamma(1 - X(1 + Y)) \\
\dot{Y} &= aY[R_0X - 1 - Z] \\
\dot{W} &= W[Y - b] \\
\dot{Z} &= h(WY - Z)
\end{align*}
$$

where $\gamma = \alpha(1-q)/\beta'$. The initial dynamics between the virus and the CTL response depend on the rate at which the CTL response is mobilized. If the immune response is sufficiently strong, peak virus load is determined by the CTL. In contrast, if CTL are mobilized at a slower rate, peak virus load is determined by target cell limitation and will therefore be higher.

If CTL limit initial virus growth, peak viraemia will be relatively small and we can make the assumption $X = 1$. Also assuming $Z = WY$ (corresponding to a high CTLp death rate and large $h$), we can reduce the model to two differential equations: $\gamma = aY(\alpha - WY)$; $W = W[Y - b]$, where $\alpha = R_0 - 1$. Such CTL-limited initial virus growth will be observed if $W_0 > y/a \exp(-\gamma/b)$, i.e. if the initial number of CTLp lies above a threshold determined by the rate of CTLP activation. For the following analysis we assume effectively $b \to 0$, compared with other relevant rates. Initial virus growth (seen in the early phase of Fig. 2ii) can be described by:

$$
Y(t) = Y_0 e^{axt},
$$

$$
W(t) = W_0 \exp \left[ \frac{\gamma Y_0}{ax} \left( e^{axt} - 1 \right) \right].
$$

This phase of the dynamics continues until $Y = Y_{\max}$ and $t = t_{\max}$ (Fig. 2ii), where:

$$
Y_{\max} = \left( \frac{ax}{\gamma} \right) \ln \left( \frac{\gamma}{\alpha W_0} \right),
$$

$$
t_{\max} = \frac{\ln(Y_{\max}/Y_0)}{ax}.
$$

For $t > t_{\max}$, virus load declines due to CTL-mediated suppression. If $b \to 0$, the system asymptotically approaches a quasi-equilibrium (Fig. 2ii, late phase), given by:

$$
Y(t) \to 1/\gamma \left[ 1 + \frac{\ln(ax/t)}{ax} + \ldots \right],
$$

$$
W(t) \to ax \left[ 1 + \frac{\ln(t)}{ax} + \ldots \right].
$$

For $b \neq 0$, the system eventually reaches its true equilibrium, $Y^{(2)}$, at $t >> 1/b$, once $t$ significantly exceeds $1/b$. Thus, for relatively low values of $b$, the system remains near the quasi-equilibrium for a prolonged period of time. Although the quasi-equilibrium is higher than the true equilibrium, virus load at the quasi-equilibrium has qualitatively similar properties compared to virus load at the true equilibrium.

On the other hand, if $W_0 < y/a \exp(-\gamma/d)$, the rate of CTL activation is not sufficiently fast to reduce initial virus growth which is now limited by target cell availability. In this case, the dynamics are more complicated compared to CTL-limited virus growth. The virus population first rises to a peak, $Y_{\max}$ and then sharply falls to a minimum value, $Y_{\min}$ (Fig. 2), as the CTL response rises. Subsequently the virus attains a positive growth rate and, after initial oscillations, approaches the quasi-equilibrium and the true equilibrium as in the previous case. We will concentrate on the fall of virus load to $Y_{\min}$. During initial virus growth the number of uninfected cells, $x$, will drop significantly because of the slow mobilization rate of the CTL response. Therefore we have to take into account the dynamics of uninfected cells. Initial virus growth is described by $\dot{X} = \alpha(1 - X(1 + Y))$, $\dot{Y} = aY[R_0X - 1 - WY]$. Peak virus load is given by $Y_{\max} \approx ax/d$ and is attained at
time $t_{\text{max}} \approx \ln(\alpha dY(t)/\alpha)$. At $t_{\text{max}}$, $R_0 X \rightarrow 1$ with the CTL response still only making a small contribution to the dynamics. Therefore for a time scale $\sim 1/\alpha$, fall of virus load is described by $Y(t) = Y_{\text{max}} e^{-\alpha t}$. During this time scale, the increase in the CTL response is given by:

$$W(t) = W_{\text{max}} \exp \left[ \frac{Y_{\text{max}}}{a} \left( 1 - e^{at} \right) - bt \right],$$

where $W_{\text{max}}$ denotes the value of $W$ at $t_{\text{max}}$. For $t_{\text{max}} + 1/\alpha < t < t_{\text{min}}$, $Y(t)$ has decreased to the point where, on route to $Y_{\text{min}}$, it will make negligible further contribution to $W(t)$. Thus for small $b$, $W(t) = W_0 \exp[\gamma R(t)/d]$, or $W(t) = \gamma$ if $Y_{\text{max}}$. Hence for $t_{\text{max}} + 1/\alpha < t < t_{\text{min}}$ the dynamics of virus load are given by $Y = aY[1 + \Gamma Y]$. The expression for $Y_{\text{min}}$ depends on the initial number of CTLp. If $W_0$ is so small that $\Gamma Y_{\text{max}} < 1$ then $\ln(Y_{\text{min}}) = -\ln(W_0)$, and the minimum virus load is attained at time $t_{\text{min}} = \ln(Y_{\text{max}}/Y_{\text{min}})$. Hence, $\ln(Y_{\text{min}})$ and $\ln(W_0)$ are related by a negative slope of $-1$. On the other hand, if $W_0$ is larger, so that:

$$\Gamma Y_{\text{max}} > 1, Y_{\text{min}} = \frac{e^{\gamma R_0/d}}{W_0} \left( \frac{1}{e^{at_{\text{min}}} - 1} \right),$$

where $t_{\text{min}} = 1/(dR_0)$. Thus, the relationship between $Y_{\text{min}}$ and $W_0$ is similar and is given by $\ln(Y_{\text{min}}) = -\ln(W_0) + C$.

**Antibody responses**

Neutralizing antibody ($x$) production occurs in response to free virus particles at a rate $cvz$ and diminishes at a rate $b$. Antibodies neutralize free virus particles at a rate $pvz$. The model is given by:

$$\begin{align*}
\dot{x} &= \lambda - \beta xv \\
\dot{y} &= \beta xv - ay \\
\dot{v} &= ky - uv - pvz \\
\dot{z} &= cvz - bz
\end{align*}$$

(4)

The antibody response becomes established if $cv(t) > b$. Virus replication controlled by an antibody response is described by $E_3$:

$$\begin{align*}
\dot{x}^{(3)} &= \frac{\lambda c}{dc + b\beta}, \quad y^{(3)} = \frac{\beta \lambda b}{a(dc + b\beta)}, \\
v^{(3)} &= \frac{b}{c}, \quad z^{(3)} = \frac{1}{\rho} \left( \frac{ky^{(3)}}{v^{(3)}} - u \right).
\end{align*}$$

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**References**


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