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Correlates of cytotoxic T-lymphocyte-mediated virus control: implications for immunosuppressive infections and their treatment

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A very important question in immunology is to determine which factors decide whether an immune response can efficiently clear or control a viral infection, and under what circumstances we observe persistent viral replication and pathology. This paper summarizes how mathematical models help us gain new insights into these questions, and explores the relationship between antiviral therapy and long-term immunological control in human immunodeficiency virus (HIV) infection. We find that cytotoxic T-lymphocyte (CTL) memory, defined as antigen-independent persistence of CTL precursors, is necessary for the CTL response to clear an infection. The presence of such a memory response is associated with the coexistence of many CTL clones directed against multiple epitopes. If CTL memory is inefficient, then persistent replication can be established. This outcome is associated with a narrow CTL response directed against only one or a few viral epitopes. If the virus replicates persistently, occurrence of pathology depends on the level of virus load at equilibrium, and this can be determined by the overall efficacy of the CTL response. Mathematical models suggest that controlled replication is reflected by a positive correlation between CTLs and virus load. On the other hand, uncontrolled viral replication results in higher loads and the absence of a correlation between CTLs and virus load. A negative correlation between CTLs and virus load indicates that the virus actively impairs immunity, as observed with HIV. Mathematical models and experimental data suggest that HIV persistence and pathology are caused by the absence of sufficient CTL memory. We show how mathematical models can help us devise therapy regimens that can restore CTL memory in HIV patients and result in long-term immunological control of the virus in the absence of life-long treatment.

Keywords: T lymphocyte; persistent virus infection; immunological memory; immunosuppression; HIV; dynamics

1. INTRODUCTION

There is mounting experimental evidence that cytotoxic T lymphocytes (CTLs) play an important role in limiting viral replication and thus in contributing to virus control or clearance. Both in human immunodeficiency virus (HIV) and human T-cell lymphotropic virus (HTLV) infection, the make-up of the major histocompatibility complex (MHC) class I genes correlates with set-point virus load and with the disease status of the host (Carrington *et al*. 1999; Jeffery *et al*. 1999; Saah *et al*. 1998). In HIV-infected patients, long-term non-progression is associated with high levels of CTLs and low virus load (Harrer *et al*. 1996*a*,*b*; Rosenberg *et al*. 1997), while depletion of CD8 T cells in simian immunodeficiency virus (SIV)-infected macaques results in a rapid increase in viral burden (Jin *et al*. 1999; Schmitz *et al*. 1999).

While these observations generally indicate that the presence of CTLs contributes to virus control, the qualitative properties of CTLs that are required for an efficient response are still unclear. In HIV infection, disease progression can occur despite the presence of CTLs. In HTLV-I infection, vigorous CTL responses are detected. Yet, the virus persists at relatively high levels. In lymphocytic choriomeningitis virus (LCMV) or hepatitis B virus infection, coexistence of virus and CTLs can result in immunopathological effects and consequent death of the host. Understanding the properties of CTL-mediated virus control is not only interesting from an immunological point of view, but it is also crucial for the outcome of antiviral treatment. With viruses such as HIV, drug therapy alone has proven not to be a practical strategy for long-term prevention of disease. The long life span of some infected cells, as well as the presence of latent virus reservoirs renders eradication by drug therapy a difficult goal to achieve (Chun *et al*. 1997; Finzi *et al*. 1997; Perelson *et al*. 1996). In addition, problems regarding compliance of patients and drug resistance limit the use of this approach (Condra *et al*. 1995; Larder *et al*. 1989; Richman 1994). Instead, increased attention has been given to immunotherapy with the aim of achieving long-term immunological control of the infection.

The interactions between virus, target cells and the immune system are highly nonlinear. Thus, the equilibrium outcomes of an infection are frequently counter-intuitive. Mathematical models take us beyond verbal or

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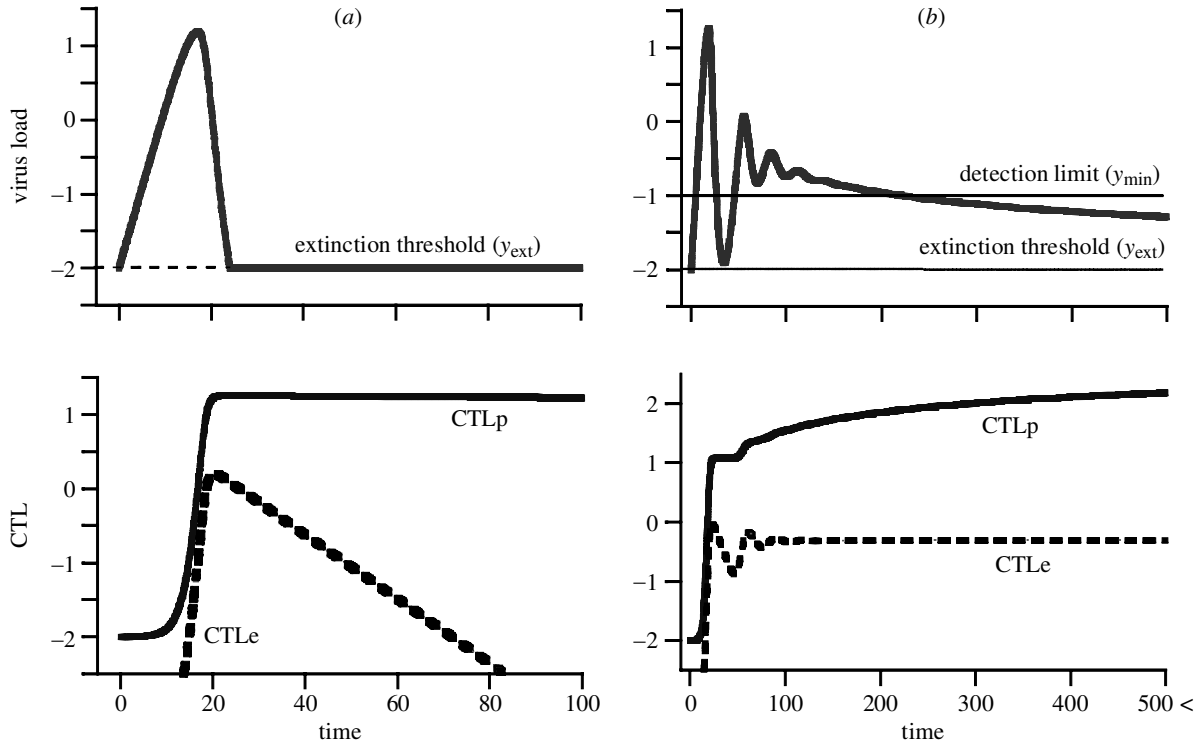

Figure 1. Antigen-independent long-term persistence of CTL precursors (CTLp) contributes to virus clearance. (*a*) Virus clearance. If the CTLp response is long lived in the absence of antigen, virus load is driven below the extinction threshold. After virus clearance, the level of CTLp remains high while the effector response quickly diminishes. (*b*) 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 CTLs. 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 the detection limit, which in turn may lead to clinical symptoms.

graphical reasoning and provide a solid framework upon which to build experiments and generate hypotheses. In this review we summarize recent insights gained by mathematical modelling concerning correlates of CTL-mediated virus control. These findings improve our understanding of possible mechanisms underlying virus-induced immune suppression and suggest treatment regimens that can overcome immune impairment and result in long-term control of the infection.

2. THE CORRELATION BETWEEN CTLs AND VIRUS LOAD

A basic immunological measure is the correlation between CTLs and virus load in persistent infections. Indeed, this measure has been used to make inferences about the role of the CTLs in controlling the virus (Ogg *et al*. 1998; Wodarz *et al*. 2000). Among HIV-infected patients, a negative correlation between CTLs and virus load was found (Ogg *et al*. 1998). This negative correlation has been interpreted as evidence for a strong CTL response controlling the infection, since high levels of CD8 T cells were associated with low virus load. However, among people infected with the related virus HTLV-I, a positive correlation was found between provirus load and specific CTLs in asymptomatic carriers, while the correlation was zero in symptomatic patients (Wodarz *et al*. 2000). At the same time there is genetic evidence that virus control in asymptomatic carriers is due to an efficient CTL response (Jeffery *et al*. 1999).

Both the data on HIV-1 and HTLV-I are in agreement with basic mathematical models of virus infections (Wodarz *et al*. 2000). The models predict that efficient CTL-mediated virus control results in a positive correlation between virus load and CTLs in cross-sectional studies, while the correlation is zero if CTLs are ineffective and do not significantly limit viral replication. The positive correlation among strong CTL responders is a general principle underlying predator–prey type interactions: the more efficient the CTLs, the lower virus load and the weaker the stimulus to maintain high numbers of CTLs. In the light of these findings, how can the negative correlation between CTL and virus load among HIV-infected patients be explained? Mathematical models (Wodarz *et al*. 2000) suggest that the negative correlation is the result of extensive virus-induced immune impairment, and not the result of efficient virus control. The higher the virus load, the more the virus population gains the upper hand and the fewer the number of CTLs.

3. PROPERTIES OF VIRUS CONTROL

(a) *Virus clearance*

To understand how specific virus infections can result in suppression of immunity we have to know the factors

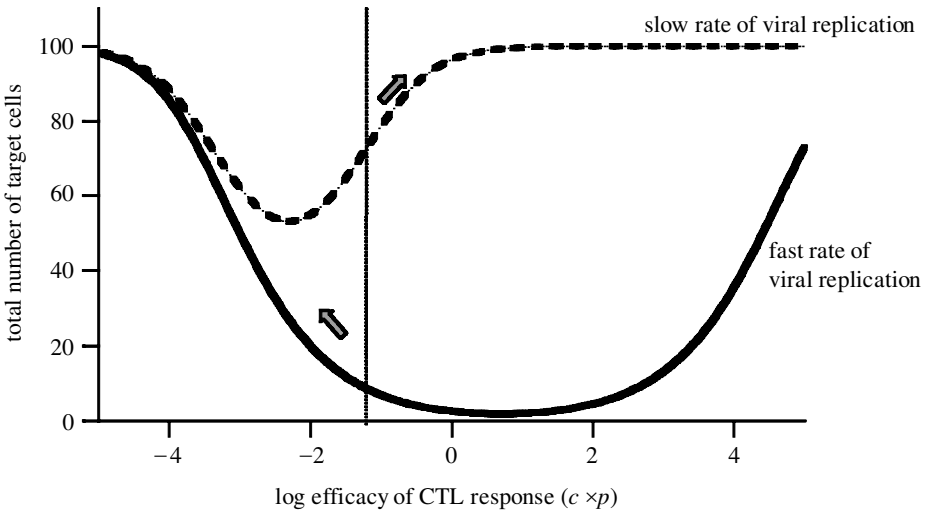

Figure 2. Basic properties of CTL-induced pathology, as predicted by a simple mathematical model describing the basic dynamics between a virus population, its target cells and a lytic CTL response. On the horizontal axis, c denotes the rate of proliferation of specific CTL per unit of viral antigen, and p denotes the rate of lysis of virus-infected cells by the CTLs. We plot the total number of target cells (uninfected + infected) at equilibrium, in dependence of the efficacy of cell-mediated immunity and the replication rate of the virus. We assume that the virus is non-cytopathic. We define immunopathology by a reduction of the total number of target cells in the presence of CTLs, compared with the absence of CTLs. CTL-induced pathology is most likely to occur at a low or intermediate efficacy of the CTL response. In addition, the replication rate of the virus plays an important role. The faster the replication kinetics of the virus, the more severe the degree of pathology observed. If the virus replicates at a fast rate, a significant reduction in the total number of target cells will be observed even in the presence of a relatively strong CTL response. If the virus replicates slowly, any degree of immunopathology is observed only in the presence of inefficient CTL. Thus, for slowly replicating viruses, an increase in the CTL responsiveness is likely to benefit the host, while for faster replicating strains, the opposite applies (see vertical dashed line and arrows).

that are required for efficient CTL-mediated control of viral replication, or virus clearance. Mathematical models have identified two parameters (Nowak & Bangham 1996; Wodarz *et al*. 1999). First, the rate of CTL activation–proliferation in response to antigen (Nowak & Bangham 1996) is important for limiting virus load, and this has been shown in persistent infections such as HIV and HTLV (Carrington *et al*. 1999; Jeffery *et al*. 1999; Saah *et al*. 1998). However, in addition, virus clearance, or efficient long-term CTL-mediated control, also requires antigen-independent long-term persistence of memory CTL precursors (CTLp) (Wodarz *et al*. 1999). If the life span of the CTLp in the absence of antigen is short, the virus can establish a persistent infection (figure 1). This can be understood intuitively as follows. When the virus population declines because of CD8-mediated activity, the CTLp population also declines, which in turn reduces the immunological pressure on the virus population and enables the virus to settle at a stable equilibrium. In contrast, with antigen-independent persistence of CTLp, the CTL population reaches a stable level while virus load drops. This maintains continuous pressure on the declining virus population, and will drive the virus population to extinction (or to an extremely low equilibrium abundance). Thus, antigen-independent persistence of memory CTLp might be required for long-term virus control or clearance.

(b) *Virus persistence and pathology*

According to the model, failure to generate efficient memory CTLp results in virus persistence. The level of virus load in such a scenario depends on the efficacy of the CTL response, or the CTL responsiveness. The weaker the CTL responsiveness, the higher the virus load and the higher the chances of developing clinical symptoms with cytopathic infections. With non-cytopathic viruses, high virus load does not result in disease (Zinkernagel 1996). However, in this case, coexistence of CTLs and virus has been shown to result in immunopathology, defined as a reduction of the overall number of target cells in the presence, compared with the absence, of CTLs (Zinkernagel 1993, 1996). It has been argued that with such infections, CTLs play a detrimental role and that therapy should be aimed at removing the CTL responses. Mathematical models can be used to define the conditions under which a persistent infection with a non-cytopathic virus results in CTL-induced pathology (Krakauer & Nowak 1999; Wodarz & Krakauer 1999). CTL-induced pathology occurs if the rate of viral replication is fast relative to the CTL responsiveness of the host (Wodarz & Krakauer 1999) (figure 2). In this scenario, viral replication depletes the number of uninfected cells, while the number of infected cells is limited by the CTL response. For slowly replicating viruses CTL-induced pathology will be observed if the CTL response is very weak (figure 2). For fast-replicating viruses, CTL-induced pathology occurs if the CTL response is weak or intermediate. The presence of an efficient and strong CTL response results in virus control and never in CTL-induced pathology. Hence, similar to more cytopathic viruses, pathology with non-cytopathic infections can be the result of an inefficient CTL response.

(c) *Clonal composition of CTLs*

The degree of virus control achieved is also reflected in the clonal composition of the CTL responses. This can be

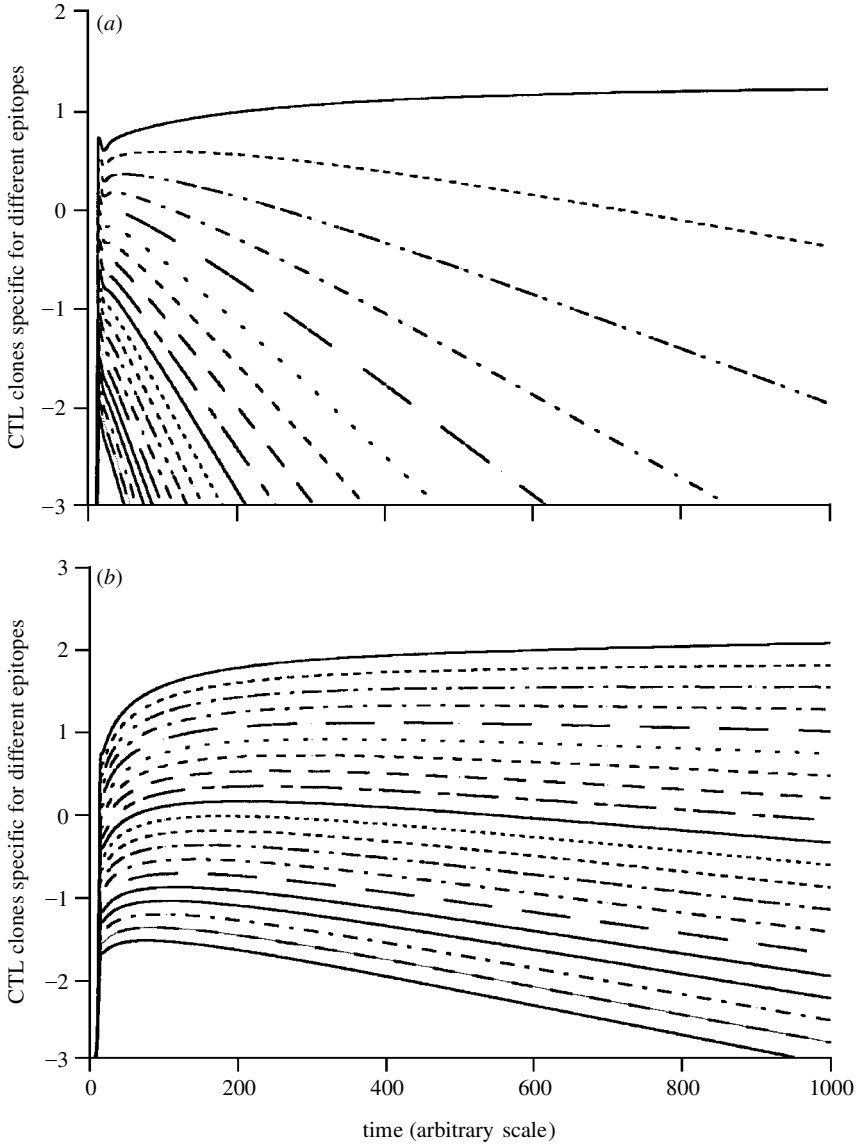

Figure 3. Relationship between the longevity of the CTL response in the absence of antigen and the clonal composition of the CTL response. (*a*) If CTLp decay at a fast rate in the absence of antigen, most CTL clones are out-competed by the most immunogenic clone, resulting in immunodominance. (*b*) If CTLp decay at a slow rate in the absence of antigen, we observe coexistence of multiple CTL clones directed against different epitopes.

shown with a mathematical model taking into account multiple CTL clones directed against different epitopes of the same virus population (Wodarz & Nowak 1999*a*). In such a scenario, the CTL clones are essentially in competition with each other (De Boer & Perelson 1994; Nowak *et al*. 1995). Competitive ability correlates with the CTL immunogenicity of the epitope, c_i. The CTL clone directed at the epitope with the largest c_i is the most superior competitor. The outcome of these competitive interactions depends on the life span of the CTL response in the absence of antigen.

According to equilibrium analysis, only the clone with the highest CTL immunogenicity, c_i, can survive at significant levels. Mathematically speaking, all other CD8 clones go extinct, although in practical terms, the spatial environment of the immune system could result in persistence of these clones at low levels. The reason is that the competitively superior CTL clone reduces virus load to levels too low to stimulate the weaker CTL clones. This result will be obtained if the life span of the CTLp response in the absence of antigen is short. Thus, lack of efficient CTL-mediated control of the infection correlates with the presence of an immunodominant CTL clone (figure 3).

On the other hand, if the life span of the CTLp response in the absence of antigen is long, the dynamics between virus replication and the CTL response converge to a quasi-equilibrium. Virus load at the quasi-equilibrium ($\hat{y}$) has similar properties as virus load at the true equilibrium (y^*). However, it is generally higher. Now, coexistence of multiple CTL clones directed against different epitopes is possible (figure 3). This is because the competitively superior CTL clone does not reduce virus load down to the true equilibrium, but only to the quasi-equilibrum, where virus load may still be sufficient to stimulate the weaker CTL clones. In summary, efficient long-term virus control does not lead to immunodominance, but to a broad CTL response directed against multiple epitopes.

These predictions are supported by data from an HIV-infected individual who has been classified as a long-term non-progressor (Harrer *et al*. 1996*a*). The patient had strong CTL responses, low viral load, and a stable CD4 T-cell count of > 500 cells μl^{-1} 15 years after infection (Harrer *et al*. 1996*a*). The study revealed the presence of a broad CTL response directed against multiple epitopes and maintained at high levels despite the low level of viraemia (Harrer *et al*. 1996*a*,*b*). This supports the prediction that broad CTL responses can result from a long life span of CTLp in the absence or at low levels of antigen. On the other hand, faster-progressing patients are characterized by narrow CTL responses (Borrow *et al*. 1997; Goulder *et al*. 1997). Although CTLs are present during the asymptomatic phase, these CTL seem short lived in the absence of antigen (Kalams *et al*. 1999*b*). Starting antiretroviral therapy in these patients results first in a transient increase of CTLp, followed by a rapid decline to low levels (Kalams *et al*. 1999*b*). The temporary increase in CTL numbers is likely to be caused by a trade-off between antigenic stimulation and virus-induced immune impairment. When viral replication is stopped, the amount of immune impairment is reduced, and before virus load has significantly declined, the number of CTLs can increase. Once virus load drops to low levels the antigenic stimulus is removed. The observation that the level of CTLp then also declines indicates that many of the CTLp seen in HIV-infected patients are short lived at low levels of antigen.

Previous theoretical work (Nowak *et al*. 1995) suggested that lack of immunodominance can be a consequence of an antigenically heterogeneous virus population. This hypothesis was supported by data from an HIV-infected patient with a relatively low CD4 T-cell count of around 200 cells μl^{-1} (Nowak *et al*. 1995). Hence, the presence of CTLs directed against multiple epitopes can indicate two alternative situations, depending on the level of virus load found in the patient: (i) if virus load is low, a broad CTL response results from the presence of efficient memory CTLs that are long lived in the absence of antigen, and have the potential to control virus replication in the long-term; and (ii) if virus load is high, the presence of CTLs directed against multiple epitopes indicates heterogeneity in the virus population caused by antigenic variation. The same study (Nowak *et al*. 1995) also suggested that a narrow CTL response indicates the presence of a homogeneous virus population and stable virus control. This hypothesis was supported by a patient with a higher CD4 T-cell count. However, although the CD4 T-cell count declined slowly in this patient, it reached about 300 cells μl^{-1} after about 50 months. Hence, this patient cannot be classified as a long-term non-progressor, and corresponds better to a typical progressor. We conclude that the narrow CTL response in this patient indicates that the CTLp are short lived in the absence of antigen, which in turn could be the reason for the steady loss of virus control and decline of the CD4 T-cell count. Indeed, there is evidence that in the presence of narrow CTL responses, antigenic escape mutants are likely to arise (Borrow *et al*. 1997; Goulder *et al*. 1997; Price *et al*. 1997). Emergence of escape mutants can result in increased viral loads, and evolution of antigenic escape has been associated with the development of AIDS (Goulder *et al*. 1997).

Apart from the above considerations, a narrow CTL response could also arise in specialized circumstances that have not been taken into account in our models. In HTLV-I infection, a single dominant CTL response directed against the Tax protein is observed (Daenke *et al*. 1996). While this could result from a CTLp response that is short-lived in the absence of antigen, another explanation is that Tax is the only antigen exposed to CTLs, and that infected cells are killed before any other viral proteins can be presented on the cell surface (C. R. M. Bangham, personal communication). In addition, the presence of specific viral antigens that are highly immunogenic compared with alternative viral antigens could result in a narrow CTL response, even if it is efficient and can control viral replication in the long term.

4. VIRUS-INDUCED IMMUNE IMPAIRMENT

Experiments with LCMV-infected mice have shown that the development of a long-lived memory CTL response requires CD4 T-cell help (Borrow *et al*. 1996, 1998; Thomsen *et al*. 1996, 1998). In helper-deficient mice, antiviral memory CTLs go extinct following the primary response. This eventually leads to a resurgence of virus load and uncontrolled viral growth. In HIV infection, the high virus load attained during the acute phase has been shown to be associated with the absence of significant CD4 T-cell proliferative responses (Kalams *et al*. 1999*a*; Kalams & Walker 1998; Rosenberg *et al*. 1997, 1999; Rosenberg & Walker 1998). This absence of CD4 T-cell help could result in the failure to generate memory CTLp that are long lived in the absence of antigen. According to theory, this early impairment could be the reason for persistent HIV replication and eventual loss of virus control (Wodarz & Nowak 1999*b*). Our argument is supported by the observation that the HIV-specific CTL response decays at a relatively fast rate following significant drug-induced reduction of virus load (Kalams *et al*. 1999*b*). This indicates that many of the CTLs seen in HIV-infected patients are short lived and thus do not have the capacity to control viral replication in the long term. However, it is possible that limited CD4 cell help is available, resulting in the generation of suboptimal memory CTLp, which can control viral replication for a certain period of time. Once these memory CTLp have fallen below a critical threshold, virus grows uncontrolled resulting in the development of AIDS. Variation in the duration of the asymptomatic phase of the infection might be due to differences in the initial level of memory CTLp generated in the primary phase of HIV infection.

The degree of virus-induced immune impairment depends on the rate of viral replication relative to the CTL responsiveness of the host (Wodarz *et al*. 1998; Wodarz & Nowak 1999*b*) (figure 4). If the viral replication rate is low relative to the CTL responsiveness, the amount of immune impairment is small. The virus is cleared, or contained at low levels in the long term. Within this parameter region, variation in the rate of viral replication results in a positive correlation between virus load and CTLs (figure 4). If the rate of viral replication is fast compared with the CTL responsiveness of the host, the degree of immune impairment is strong. In this parameter region, variation in the rate of viral

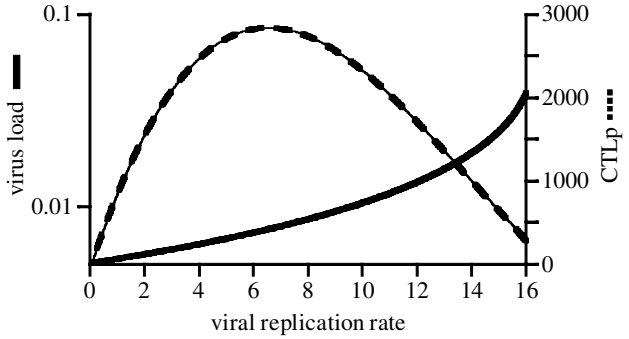

Figure 4. The relationship between viral replication, virus load and CTLp in a mathematical model taking into account immune impairment. The faster the rate of viral replication, the higher the degree of immune impairment. If the degree of immune impairment is relatively low, there is a positive correlation between CTL and virus load. If the degree of immune impairment is relatively high, there is a negative correlation between virus load and CTL.

replication results in a negative correlation between CTLs and virus load (figure 4). If the amount of immune impairment is still stronger, virus replication can drive the memory CTL response extinct. This can also depend on the initial conditions. Extinction of memory CTLp is promoted by low initial numbers of CD8 and CD4 T cells, as well as by a high initial virus load. Note that extinction of memory CTLp does not correspond to the complete absence of CTL in the patient, since we define memory CTLp as responses that are long lived in the absence or at low levels of antigen and depend on CD4 cell help. As discussed in the previous paragraph, many of the CTLs seen in HIV-infected patients could be short lived, unable to achieve efficient long-term control.

5. THERAPY AND LONG-TERM IMMUNOLOGICAL CONTROL

In § 4 we have shown that lack of memory CTLp, and thus lack of efficient immunological control, could be the result of a fast initial rate of viral replication. This prediction is supported by experimental data from macaques infected with different strains of SIV (Lifson *et al*. 1997). Peak viraemia as well as post-acute viral set point were correlated with the initial rate of growth of the virus population. The level of set-point virus load in turn has been shown to correlate with the rate of disease progression (Mellors *et al*. 1995). With these results in mind, therapy regimens limiting the initial growth rate of HIV could allow for the development of efficient CD4 T-cell responses, and consequently for the development of memory CTLp that are long lived in the absence of antigen. Such a scenario has been explored by mathematical models (Wodarz & Nowak 1999*b*) (figure 5). The therapy regimen provides the immune system with an antigenic stimulus, while reducing the degree of immune impairment. Consequently, sufficient levels of memory CTLp are generated, and the virus can be controlled in the absence of long-term continuous treatment (figure 5). Such therapy can therefore turn a potential fast progressor into a state of long-term non-progression. Success of treatment depends on the exact

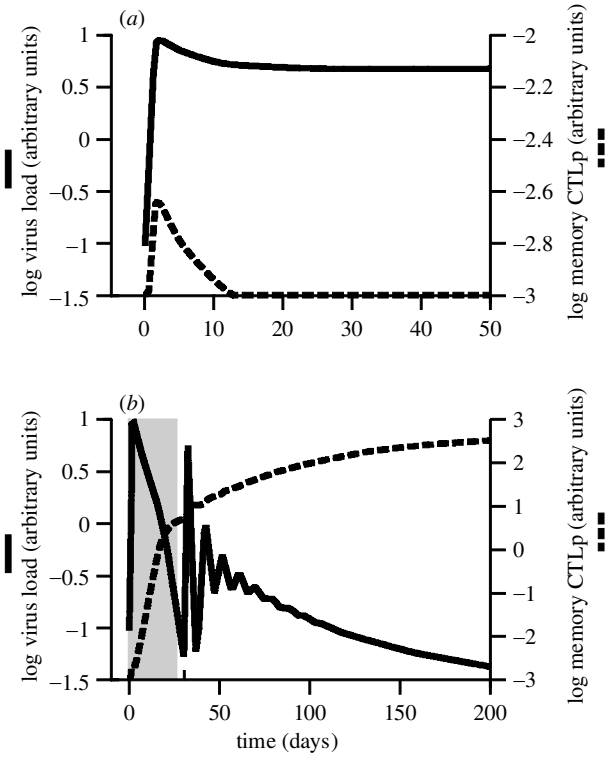

Figure 5. Primary HIV infection. Shading indicates drug therapy. (*a*) Basic dynamics. The virus population replicates up to a peak and subsequently settles to a stable equilibrium. The memory CTLp initially expand, but are subsequently exhausted due to HIV-induced impairment of the T-helper-cell response. Note that we only take into account an efficient memory CTL response, dependent on CD4 cell help. The model considers a simplified scenario, excluding less efficient CTL responses that may be independent of CD4 cell help and that may not control the virus in the long term. Hence, virus load does not fall by a very large amount after the peak, and the memory CTLp response declines to low levels. The depicted scenario may correspond to fast progressing disease. (*b*) Effect of drug therapy. Administration of antiretroviral drugs during the primary phase of the infection minimizes the degree of HIV-induced immune impairment. Consequently, CTL memory becomes established in response to the increased viral load. Once CTL memory has been established, it may control HIV in the long term in the absence of continued therapy.

timing and duration of therapy (Wodarz & Nowak 1999*b*).

This treatment schedule can be modified to achieve long-term immunological control in patients who are chronically infected with HIV and are in the asymptomatic phase of the infection (Wodarz & Nowak 1999*b*) (figure 6). In traditional treatment regimens, virus load falls to very low levels, but when drugs are withdrawn, virus load re-emerges to pre-treatment levels. In dynamical terms, withdrawal of drugs in a chronically infected patient is similar to events occurring during acute infection: the HIV population grows from low levels. According to mathematical models, a temporary drug window followed by a second phase of drug treatment can result in the development of an efficient memory CTL response that is long lived in the absence

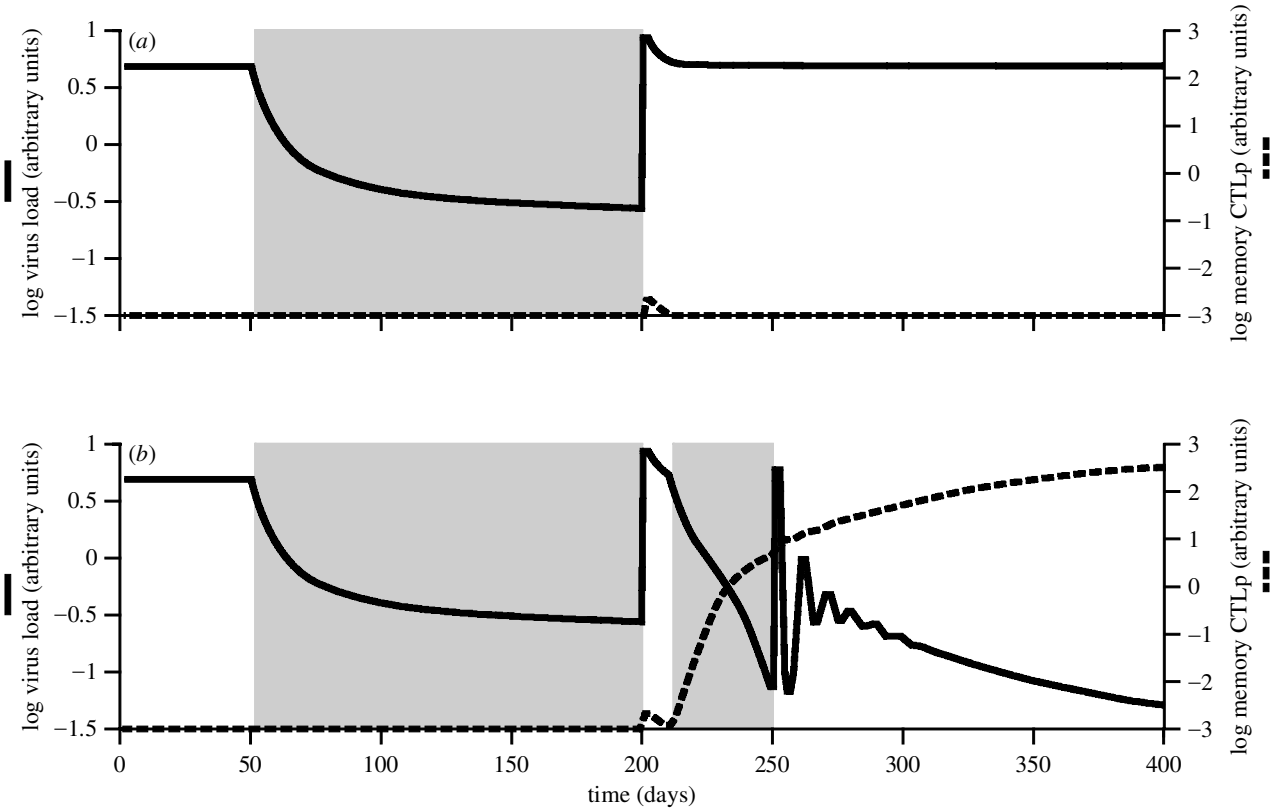

Figure 6. Asymptomatic period of the infection. Shading indicates drug therapy. (*a*) Efficient drug therapy reduces virus load to low levels. However, if the drugs are withdrawn, virus load re-emerges to pre-treatment levels. Although the rise in virus load boosts the immune system, virus-induced immune impairment prevents the development of CTL memory. Note again, that we only consider memory CTLp as defined in the text (see Appendix A(a) and Wodarz *et al*. 1999*b*), dependent on the presence of CD4 cell help. Hence, before therapy, the figure does not show persistence of less efficient CTLs at higher levels, which may not control the infection in the long term and which may be maintained by continuous viral replication in HIV-infected patients. (*b*) Treatment regimen required to re-establish CTL memory. It consists of four phases. The first phase of treatment reduces virus load to low levels. This is followed by a drug holiday allowing the virus to replicate, thereby boosting the immune system. While virus load increases, the secondary phase of therapy is initiated. This suppresses the amount of virus-induced immune impairment and allows the establishment of CTL memory in response to the increased virus load. Finally, drug therapy can be stopped for good once CTL memory has been generated. The virus is now controlled in the long term by the immune system. Note that after the second phase of therapy virus load transiently rises and oscillates before being controlled by CTL memory. This is because during the second phase of therapy the CTL effector response will have declined to low levels, allowing the virus to initially attain a positive growth rate. However, this does not indicate failure of the treatment regimen. Furthermore, it is important to point out that the secondary phase of treatment reduces virus load to lower levels in a shorter period of time than the primary phase of treatment. The reason for this is that the secondary phase of treatment is associated with a rising CTL memory response, which accelerates the death rate and consequently the decay rate of infected cells during therapy. This underlines the notion that the effect of drug treatment on virus load is enhanced by the presence of an efficient CTL response.

of antigen (figure 6). The drug window allows the generation of an antigenic stimulus, while reapplication of therapy prevents increased amounts of immune impairment. This results in sufficient CD4 T-cell help to ensure the development of long-lived memory CTLp that have the capacity to control HIV in the absence of long-term continuous treatment. If the immune system is already relatively weak, repeated phases of intermittent therapy are required to progressively achieve virus control (Wodarz & Nowak 1999*b*) (figure 7). According to mathematical models, this can be achieved even more efficiently by combining drug therapy with vaccination (Wodarz & Nowak 1999*b*) (figure 7), although this approach has practical limitations at the moment (Letvin 1998).

6. PRE-EXPOSURE VACCINATION

These dynamics also have implications for vaccination strategies aimed at people who are uninfected but at risk of virus exposure. The effectiveness of an HIV vaccine usually tends to be evaluated according to whether it can prevent infection of a significant fraction of people compared with control groups. However, the findings discussed in this paper suggest that the aim of an HIV vaccine might not so much be prevention of infection, but modulation of the dynamics so that an efficient, sustained CTL memory response is maintained, resulting in long-term immunological control of the virus. This notion is explained graphically in figure 8. Experimental data indicate that prevention of infection might be very difficult to

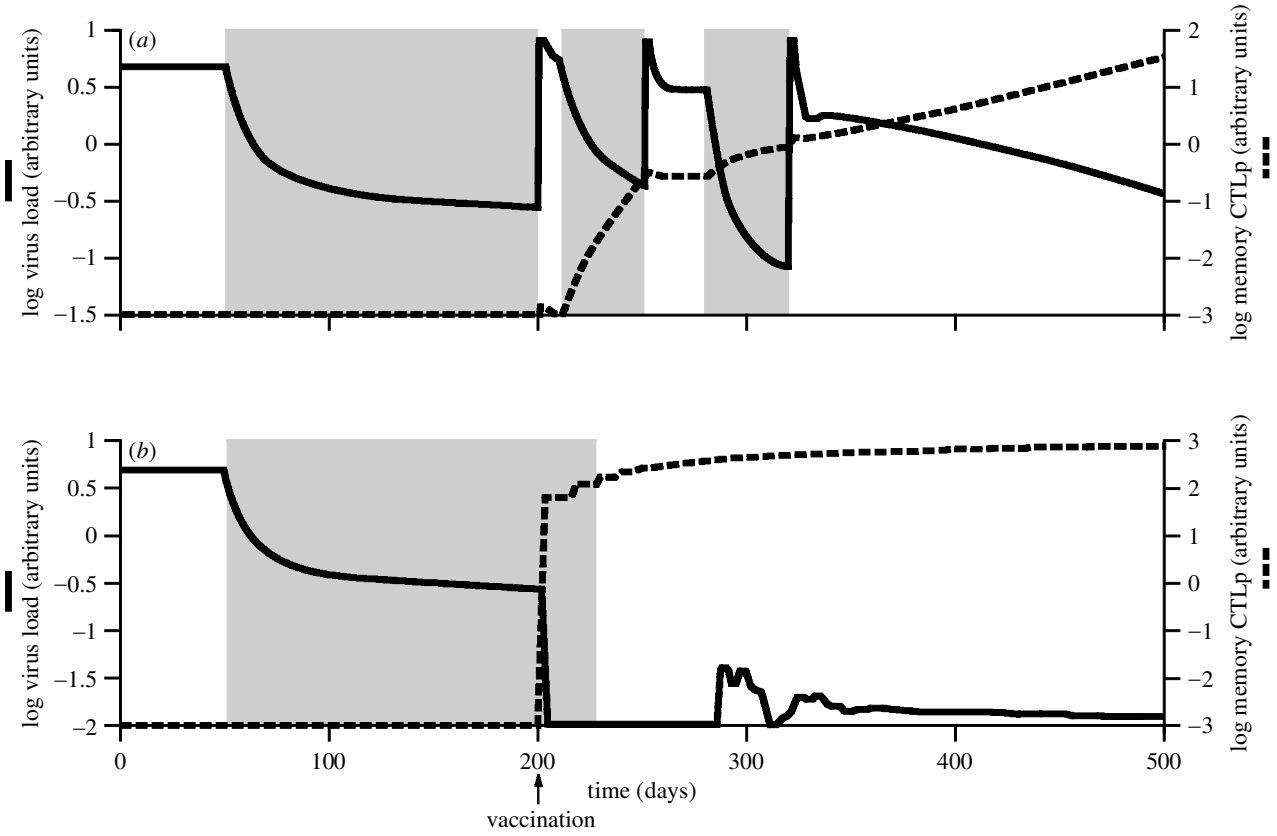

Figure 7. Modifications of the basic treatment window regimen resulting in the re-establishment of CTL memory during the asymptomatic period of the infection. Again, only memory CTL responses generated in the presence of CD4 cell help are considered. Since we assume that CD4 cell help is significantly impaired, these CTL are at low levels before start of therapy. Shading indicates drug therapy. (*a*) Multiple drug windows. In low immune responders and in patients with advanced HIV disease, the basic drug window treatment regimen may only result in partial establishment of CTL memory and failure to control the virus. Further phases of drug therapy separated by treatment windows may successively boost the CTL response, resulting in the eventual generation of efficient CTL memory and long-term control of HIV. (*b*) Drug therapy in conjunction with vaccination with persisting antigen. While virus load is kept at low levels due to antiviral therapy, the patient is vaccinated with a cocktail of immunogenic HIV peptides. This boost induces the establishment of CTL memory while drug treatment keeps HIV-induced immune impairment to a minimum. During the generation of CTL memory, HIV load sharply drops to very low levels. This is because the CTL response is boosted in the absence of HIV replication, allowing the rising CTL to reduce virus load to ever-decreasing values. This could theoretically clear the infection. However, the presence of latently infected cells and reservoirs inaccessible to CTLs renders this goal difficult to achieve. Thus, when drug treatment is stopped, virus load is likely to transiently increase before being controlled in the long term by CTL memory.

achieve, since latently infected cells are generated very early in the infectious process. Prevention of infection would require that the virus does not grow at all on infection of the host. This can only be achieved if immune effectors are immediately present at the time and location of infection. Typically, there is a lag phase until immune effectors become activated and migrate to the site of infection, especially if infection occurs in the periphery. Hence, it might be unlikely that initial virus growth is prevented. However, vaccination could result in reduced initial virus growth, small degrees of immune impairment, maintenance of CTL memory and long-term immunological control.

7. CONCLUSION

The insights summarized in this review provide a theoretical framework in which to analyse the mechanisms underlying HIV-induced immune impairment, and to evaluate the potential of therapy to modulate HIV dynamics in order to achieve long-term immunological control. Improvement of our understanding requires detailed clinical investigations under carefully controlled conditions.

An important experimental model is SIV infection in macaques. In this setting, treatment during acute infection has been explored systematically (Lifson *et al*. 1999). The study has shown that depending on the initiation and duration of treatment during acute infection, various degrees of immunological control can be achieved in the absence of continuous therapy. Control is associated with the development of significant CD4 T-cell proliferative responses. In agreement with theory, the correlation between CD4 T-cell proliferative responses and virus load at equilibrium depended on the degree of immunological control achieved (Wodarz *et al.*, this issue). Animals showing efficient control of virus replication showed a positive correlation. Treatment in these animals

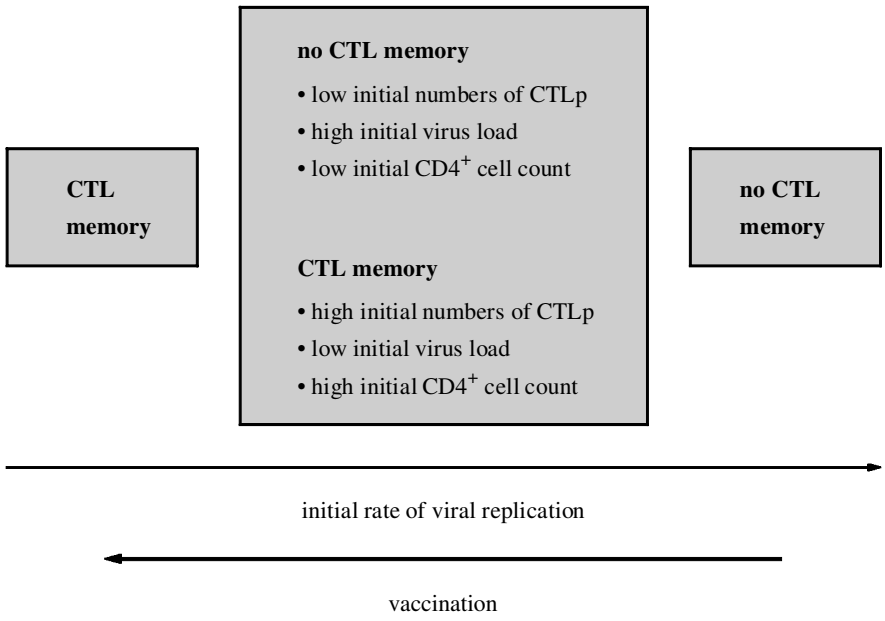

Figure 8. The effect of CTL pre-exposure vaccination. Pre-exposure vaccination could modulate the race between initial virus spread and expansion of specific immunity. This could shift the dynamics towards maintenance of a sustained memory CTL response and long-term immunological control, rather than to impaired immunity and disease progression.

might have achieved a level of control not previously observed in HIV-infected patients. On the other hand, animals showing less efficient virus control were characterized by a negative correlation, indicating higher degrees of immune impairment.

Determinants of immunological control, as well as intermittent therapy regimens, have also been explored in HIV-infected patients. In agreement with theory, long-term non-progressors are characterized by persistent levels of CTLs despite very low levels of antigen, and by broad CTL responses directed against multiple epitopes (Harrer *et al*. 1996*a*). Treatment during primary infection, in conjunction with intermittent therapy, has significantly modulated viral dynamics, achieving a certain degree of immunological control in a number of patients who would otherwise be faster progressors (Lisziewicz *et al*. 1999; Ortiz *et al*. 1999). Virus control in these patients was associated with a high level and breadth of the CTL response (Ortiz *et al*. 1999). This indicates the presence of significant levels of memory CTLp that are long lived in the absence of antigen (Wodarz & Nowak 1999*a*). Further experimental and clinical data, in conjunction with mathematical analysis, are required to improve our understanding of these therapy regimens, in particular the relationship between timing of therapy and successful virus control.

APPENDIX A. MATHEMATICAL MODELS DISCUSSED IN THIS PAPER

(a) *Basic dynamics between CD8 cells and virus replication*

To analyse the dynamics of antiviral CTL responses, we use the basic virus infection model taking into account uninfected (x) and infected (y) host cells. We assume that the CTL pool consists of two populations: the precursors (w) and the effectors (z). The model is given by the following set of differential equations:

$$\left.\begin{aligned} \dot{x} &= \lambda - dx - \beta xy \\ \dot{y} &= \beta xy - ay - pyz \\ \dot{w} &= cwy(1-q) - bw \\ \dot{z} &= cqyw - hz \end{aligned}\right\}. \qquad \text{(A1)}$$

Target cells are produced at a rate λ, die at a rate dx and become infected by virus at a rate βxy. Infected cells die at a rate ay and are killed by CTL effector cells at a rate pyz. On contact with antigen, CTLp proliferate at a rate cyw and differentiate into effector cells at a rate $cqyw$. CTL precursors die at a rate bw, and effectors die at a rate hz. Note that we have chosen a simplified and phenomenological model of CTL memory generation, since the exact mechanisms underlying the establishment of memory are still unknown. Our conclusions are therefore independent of the exact differentiation pathway of antiviral CTLs. In addition, our results are independent from the mode of CTL action. Although our models describe a lytic CTL response, mathematical models describing non-lytic antiviral effector mechanisms mediated by $CD8^+$ T cells have shown that these two pathways of CTL-mediated antiviral activity are qualitatively similar in the aspects relevant to the current analysis.

Before the host has been infected with the virus in question, the system is at the equilibrium, E0, given by $x^{(0)} = \lambda/d$, $y^{(0)} = 0$, $w^{(0)} = 0$, $z^{(0)} = 0$. In practice, the number of virus-specific CTLp in the absence of the infection, $w^{(0)}$, will be greater than zero. Thus, more precisely we should write and $w^{(0)} = \eta/b$. However, as long as η is small this represents only a small perturbation to the dynamics given by system (A1).

Persistent infection of a naive host requires the basic reproductive ratio of the virus, R_0, to be greater than unity. The basic reproductive ratio of the virus denotes the average number of infected cells produced by one infected cell at the beginning of the infection. For model (A1) it is given by $R_0 = \beta\lambda/da$. Subsequent virus replication may either be limited by target cell availability, or by the CTL response. Target-cell-limited virus growth is described by equilibrium (E1), given by

$$x^{(1)} = a/\beta,\ y^{(1)} = \lambda/a - d/\beta,\ w^{(1)} = 0,\ z^{(1)} = 0.$$

If the immune system of the host is strong enough, the CTL population may expand in response to the virus infection. The condition for CTL expansion is given by $c(1-q)y^{(1)} > b$. If this condition is fulfilled, the system moves to equilibrium E2, given by

$$x^{(2)} = \frac{\lambda c(1-q)}{dc(1-q)+b\beta},\ y^{(2)} = \frac{b}{c(1-q)},$$
$$w^{(2)} = \frac{z^{(2)}h(1-q)}{bq},\qquad z^{(2)} = \frac{\beta x^{(2)} - a}{p}.$$

For low values of b, the system takes a long time to equilibrate. After an initial transient phase, the dynamics lead to a quasi-equilibrium at ($\hat{y}$), at which virus load decays only at a very small rate. Virus load at the quasi-equilibrium is higher than at the true equilibrium, but has similar properties. Hence, virus load at the quasi-equilibrium can be approximated by $\hat{y} = \alpha y^{(2)}$, where $\alpha > 1$. After a time period of $1/b$, the system approaches the true equilibrium, $y^{(2)}$.

(b) *Multiple epitopes*

The basic model can be extended to include multiple CTL clones ($I = 1 \ldots n$) specific for different viral epitopes of the same virus population. The model is given by the following set of differential equations:

$$\left.\begin{aligned} \dot{x} &= \lambda - dx - \beta xy \\ \dot{y} &= \beta xy - ay - y\textstyle\sum_{1=1n} p_i z_i \\ \dot{w}_i &= c_i yq(1-q) - bw_i \\ \dot{z}_i &= c_i qyw_i - hz_i \end{aligned}\right\}. \qquad \text{(A2)}$$

Assuming the existence of n CTL clones specific for different epitopes, we can rank them according to their competitive abilities, expressed by the value of c_i: $c_1 > c_2 > c_3 > \ldots > c_n$. If y has equilibrated, w_i declines with an exponential rate, described by $w_i(t) = \exp(-b(1 - c_i/c_1))t$. Only the most superior competitor survives. However, if b is small, the system takes a very long time to equilibrate. The system will remain at a quasi-equilibrium for a time-span of approximately $1/b$. Virus load at the quasi-equilibrium ($\hat{y}$) has the same properties as virus load at equilibrium, but is characterized by a higher value. That is, $\hat{y} = \alpha(b/c(1-q))$, where $\alpha > 1$. In this case, w_i declines at a rate $w_i(t) = \exp(-b(1 - (c_i/c_1)a)t)$. Hence, the CTL clone w_i persists if $c_i > c_1$.

(c) *CTL responses and immune impairment in HIV infection*

Our basic model describing the dynamics of CTL responses can be adapted to HIV infection by assuming that generation of an effective and sustained CTL response depends on $CD4^+$ T-cell help, and that infection of $CD4^+$ T cells results in impaired help. This is expressed in the following set of differential equations (Wodarz *et al.* 1998):

$$\left.\begin{aligned} \dot{x} &= \lambda - dx = \beta xy \\ \dot{y} &= \beta xy - ay - pyz \\ \dot{w} &= cxyw = cqyw - bw \\ \dot{z} &= cqyw - hz \end{aligned}\right\}. \qquad \text{(A3)}$$

The model is derived from (A1) and assumes that the target cells for the virus are $CD4^+$ T cells. It includes the additional feature that expansion of the CTLp population is proportional to both antigen (y) and the number of uninfected $CD4^+$ T cells (x) capable of delivering T-cell help.

If $R_0 > 1$, the system may converge to one of two equilibria. The pathogen may replicate in the absence of an efficient, sustained CTL response. This is described by equilibrium (E1):

$$x^{(1)} = a/\beta,\qquad y^{(1)} = \lambda/a - d/\beta,\qquad w^{(1)} = 0,\qquad z^{(1)} = 0.$$

On the other hand, an efficient, sustained CTL response may be established and this is described by equilibrium (E2):

$$y^{(2)} = \frac{b}{c(x-q)},\ w^{(2)} = \frac{hz^{(2)}}{cqy^{(2)}},\ z^{(2)} = \frac{\beta x^{(2)} - a}{p},$$

where $x^{(2)}$ is given by a solution of a quadratic equation:

$$x^{(2)} = \frac{c(\lambda + dq) - b\beta + \sqrt{[c(\lambda + dq) - b\beta]^2 - 4c^2\lambda qd}}{2cd}.$$

If $cy^{(1)}(x^{(1)} - q) > b$ equilibrium (E1) loses stability and the system converges to equilibrium (E2), i.e. a lasting CTL response invades. If this condition is not fulfilled equilibrium (E1) is stable. However, equilibrium (E2) may or may not be stable depending on host and viral parameters. If equilibrium (E2) is complex ($[c(\lambda + d1) - b\beta]^2 < 4c^2\lambda qd$) or negative ($x^{(2)} < q$ or $\beta x^{(2)} < a$), a persisting CTL response can never be established. On the other hand, if equilibrium (E2) is positive and real, both equilibria (E1) and (E2) are stable and the outcome depends on the initial conditions. A low initial number of CTLp, a high initial virus load, as well as a low initial $CD4^+$ T-cell count promote the absence of an efficient, sustained CTL response. Overall, a high replication rate of the virus as well as a low immune responsiveness of the host shift the dynamics between HIV and the immune system in favour of the virus.

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