Cytotoxic T-cell abundance and virus load in human immunodeficiency virus type 1 and human T-cell leukaemia virus type 1

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The correlation between virus load and specific cytotoxic T-lymphocyte (CTL) frequency during the chronic phase in human immunodeficiency virus type 1 (HIV-1) infection has been found to be negative in cross-sectional studies. We report here that, in infection with the related retrovirus human T-cell leukaemia virus type 1 (HTLV-1), the correlation is positive in asymptomatic carriers and zero in patients with the associated inflammatory disease HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP). We demonstrate that the direction of the correlation may depend on the efficacy of the CTL response using mathematical models. We conclude that the CTL response is effective in asymptomatic carriers of HTLV-1, but ineffective in patients with HAM/TSP. Virus-mediated impairment of specific CTL production in HIV-1 infection can account for the negative correlation observed.

Keywords: human immunodeficiency virus type 1; human T-cell leukaemia virus type 1; virus load; cytotoxic T lymphocyte; correlation; mathematical models

1. INTRODUCTION

Viral load is strongly correlated with the risk of disease in several persistent virus infections, such as human immunodeficiency virus type 1 (HIV-1) (Tersmette *et al.* 1988; Asjo *et al.* 1990; Munoz *et al.* 1992; Connor *et al.* 1993; Piatak *et al.* 1993; Mellors *et al.* 1995; Haynes *et al.* 1996) and human T-cell leukaemia virus type 1 (HTLV-1) (Bangham 1993). However, the factors that determine viral load are not well understood. In particular, the importance of the antiviral immune response is unknown. A normal efficient immune response to a virus comprises both innate components (natural killer cells, interferons and other cytokines) and acquired components (B and T cells and their products). However, cytotoxic T lymphocytes (CTLs) play a critical part in limiting viral replication (Lin & Askonas 1981; Koenig *et al.* 1993; Zinkernagel & Hengartner 1997; Schmitz *et al.* 1999). Therefore, individual variation in CTL responsiveness to a virus might be an important determinant of viral load and, thus, explain the different outcomes of infection in different individuals (Nowak & Bangham 1996). In this context, it is important to understand the correlation between CTLs and virus load and its implications for the role of CTLs in controlling the infection. This correlation may differ between acute infections and the (quasi-) equilibrium state observed in persistent infections. Furthermore, because these kinetic processes are multifactorial and nonlinear,

intuition is not always a reliable guide to a correct interpretation of the data.

A chronic inflammatory disease of the central nervous system, namely HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP), is strongly associated with a high HTLV-1 proviral load (Nagai *et al.* 1998). We have observed a significant positive correlation in healthy HTLV-1 carriers between the proviral load of HTLV-1 and the frequency of CTLs specific to an immunodominant A*02-restricted epitope in Tax, Tax11-19, as measured with fluorescent-labelled tetrameric complexes of HLA-A*02/β-2 microglobulin/Tax 11-19 peptide (figure 1*a*). However, there was no correlation in HAM/TSP patients (figure 1*b*). We have recently shown that the *HLA-A*02 and *Cw*08 genes are associated with a lower HTLV-1 proviral load and with protection from HAM/TSP (Jeffery *et al.* 1999, 2000). The likely explanation is that HLA-A*02- and HLA-Cw*08-restricted CTLs limit HTLV-1 replication and, thus, reduce the risk of HAM/TSP (Bangham *et al.* 1999; Jeffery *et al.* 1999, 2000).

Ogg *et al.* (1998) found a significant negative correlation between viral load and specific CTL frequency (again measured with HLA tetramers) among patients with HIV-1 infection (figure 1*c*). This is also in agreement with earlier studies on HIV-2 showing a negative correlation between CTL activity and viral load (Ariyoshi *et al.* 1995). At the same time, there is evidence that CTLs may be able to reduce HIV load significantly and delay the progression to acquired immune deficiency syndrome (AIDS) (Saah *et al.* 1998; Schmitz *et al.* 1999).

How can these seemingly conflicting results be reconciled? In this paper we show that the direction of the

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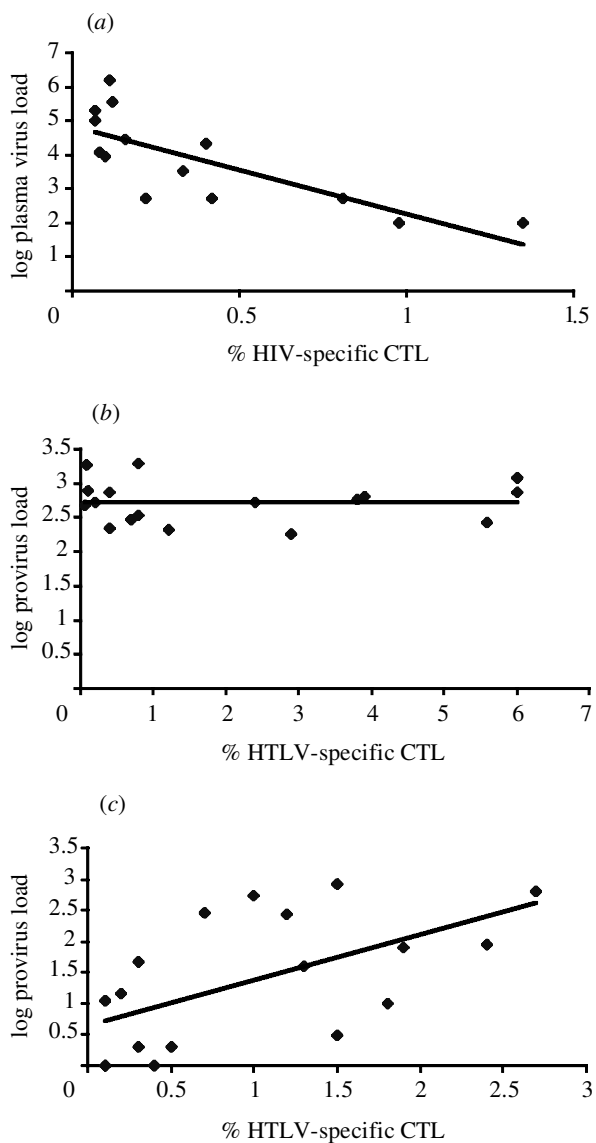†Both authors contributed in equal part to this work.

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Figure 1. Virus-specific CTL frequency is (*a*) negatively correlated with viral load in HIV-1 infection ($p = 0.01$), but (*c*) positively correlated in asymptomatic carriers of HTLV-1 ($p = 0.01$). (*b*) The correlation coefficient is zero in patients with HTLV-1-associated myelopathy ($p = 0.97$). The vertical axis in each panel shows (on a logarithmic scale) the percentage of CD8$^+$ peripheral blood mononuclear cells that bind the appropriate tetrameric complex(es) of class 1 HLA/β-2 microglobulin/retroviral peptide. The horizontal axis (again on a logarithmic scale) shows the plasma HIV load (*a*) or the proviral HTLV-1 load (*b,c*). Details of the HIV-specific tetrameric complexes are given in Ogg *et al.* (1998). The HTLV-1-specific tetrameric complex contains HLA-A*0201/peptide/β-2 microglobulin/Tax peptide (Jeffery *et al.* 1999). The Tax peptide corresponds to residues 11–19 (inclusive) of the HTLV-1 viral transcriptional transactivator, Tax, which is an immunodominant HLA-A*02-restricted CTL epitope (Kannagi *et al.* 1992). A note of caution: not all tetramer-positive cells captured in these studies might be functional. Hence, it would be interesting to also correlate virus load with CTL function, for example as measured by enzymed-linked immuno spot assays. Moreover, it is not known what fraction of the overall CTL response is captured by the tetramer measurements. (*a*) HIV, (*b*) HTLV: HAM/TSP patients, and (*c*) HTLV-1: asymptomatic carriers.

correlation between virus load and CTLs may depend on CTL efficacy *in vivo*. We made the following assumptions: (i) that activated CTLs are maintained by the continuous production of viral antigen, (ii) that infected subjects differ in the parameters that affect the efficiency of their CTL response to a given pathogen, and (iii) that an equilibrium (or quasi-equilibrium) is reached between virus replication and virus-specific CTL generation. Mathematical models that describe the dynamics of a lytic CTL response to persistent viruses predict that an efficient CTL response controlling the infection results in a positive correlation between CTLs and virus load. A weak CTL response, which is not efficiently controlling the infection, leads to the absence of a correlation. A negative correlation is observed if the virus actively inhibits the immune response even when the CTLs offer some control of the infection. We conclude that the negative correlation among HIV-1-infected patients is due to HIV-induced impairment of the immune response. On the other hand, the models predict that, for HTLV-1, the CTL response is effective in asymptomatic carriers and ineffective in HAM/TSP patients. This conclusion is consistent with a previous observation that the dominant target antigen of anti-HTLV-1 CTLs, the Tax protein, is subject to positive selection in healthy HTLV-1 carriers but not in those with HAM/TSP (Niewiesk *et al.* 1994; Bangham *et al.* 1999).

2. RESULTS AND DISCUSSION

Virus infection dynamics have recently been studied in the context of differential equations that take into account the number of uninfected host cells, infected cells, free virus particles and a lytic CTL response (Anderson & May 1979, 1991; Nowak & Bangham 1996; De Boer & Perelson 1998). However, for the purpose of the present paper, we can simplify this model in order to describe the interactions between a replicating virus population v and an immune response z. The simplified model is given by the following pair of differential equations:

$$\dot{v} = rv\left(1 - \frac{v}{k}\right) - pvz \tag{1}$$

and

$$\dot{z} = F(v, z) - bz. \tag{2}$$

The variable v denotes the virus population size (in terms of infected cells or free virus particles). The virus population grows at a density-dependent rate $rv(1 - v/k)$. The parameter r describes the overall replication rate of the virus, which includes the rate of infection and the number of virus particles produced by each infected cell, as well as the half-life of the free virus particles. The virus population may only grow up to a carrying capacity k, which is determined by the maximum possible number of infected host cells. The immune cell population grows in response to antigen at a rate $F(v, z)$ and decays at a rate bz. The immune system inhibits the virus population at a rate pvz. This is a general inhibition term and may include CTL-mediated lysis or an antibody response. Although this model is general and can be applied to any pathogen-specific and major histocompatibility complex (MHC)-restricted immune response, we will concentrate
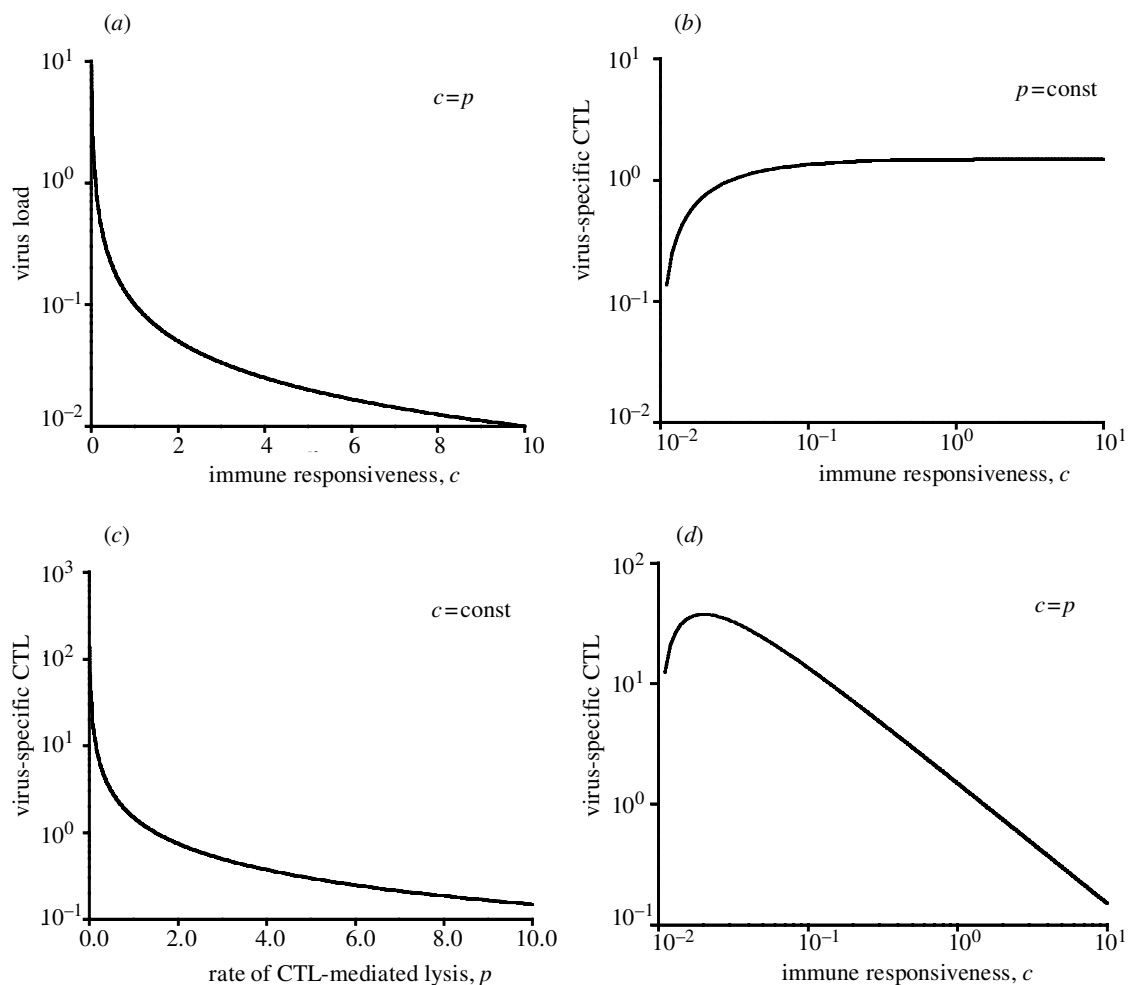
Figure 2. Effect of immunological parameters on the virus load and CTLs. Model 1 is used as an example. (*a*) An increase in the efficacy of the CTL response significantly reduces the virus load. We use the term 'efficacy of the CTL response' in order to include both the immune responsiveness c and the rate of CTL-mediated lysis p. The graph assumes that $c = p$. The three models examined differ in the exact way the immunological parameters influence the virus load. In model 1, the virus load is only reduced by a high rate of CTL proliferation c, while in model 3 it is only significantly reduced by a high rate of CTL-mediated virus inhibition p. In model 2, the virus load is influenced equally by both parameters. (*b*) An increase in the immune responsiveness c drives the equilibrium number of antiviral CTLs towards an asymptote. The parameter p is kept constant (const). (*c*) A high rate of CTL-mediated virus inhibition p significantly reduces the equilibrium number of CTLs. The parameter c is kept constant (const). (*d*) If the immune responsiveness c is linked to the rate of CTL-mediated virus inhibition p ($c = p$), the number of CTLs becomes a one-humped function of the overall efficacy of the CTL response. The baseline parameters were chosen as follows: $r = 1.5$, $k = 10$, $c = 1$, $p = 1$ and $b = 0.1$.

our discussion on the dynamics of a lytic CTL response to viral infections.

The virus will establish a persistent infection if $r > 0$. In the absence of an immune response, the virus replicates up to the carrying capacity k. The presence of an immune response reduces the viral load and the equilibrium expressions then depend on the exact model that describes the dynamics of CTLs. The exact form of the function $F(v, z)$ is unknown. In the following we analyse a variety of types of functional response and examine the effect of the efficacy of the CTL response on the correlation between the level of virus load and the frequency of CTLs at equilibrium.

The strength of the CTL response is determined by two parameters. These are the rate of CTL proliferation (or immune responsiveness, which is denoted by c) and the rate of CTL-mediated lysis of infected cells (which is denoted by p). In our analysis we assume that the rate of

CTL proliferation c is proportional to the rate of CTL-mediated lysis p. This is justified because both processes depend on T-cell receptor (TCR) recognition of viral antigen on the surface of infected cells in conjunction with MHC molecules. We introduce three different functional responses for modelling CTL dynamics *in vivo* (see Appendix A): model 1 is a nonlinear Lotka–Volterra response, model 2 a linear response and model 3 is a density-dependent response. We examined the effect of immunological parameters on the level of virus load and CTLs at equilibrium and determined how these parameters influence the correlation between CTL abundance and virus load.

The immunological parameters had a qualitatively identical effect on virus load and CTL frequency/abundance at equilibrium in all models examined. These relationships are shown in figure 2 using model 1 as an example. An increase in the overall efficacy of the
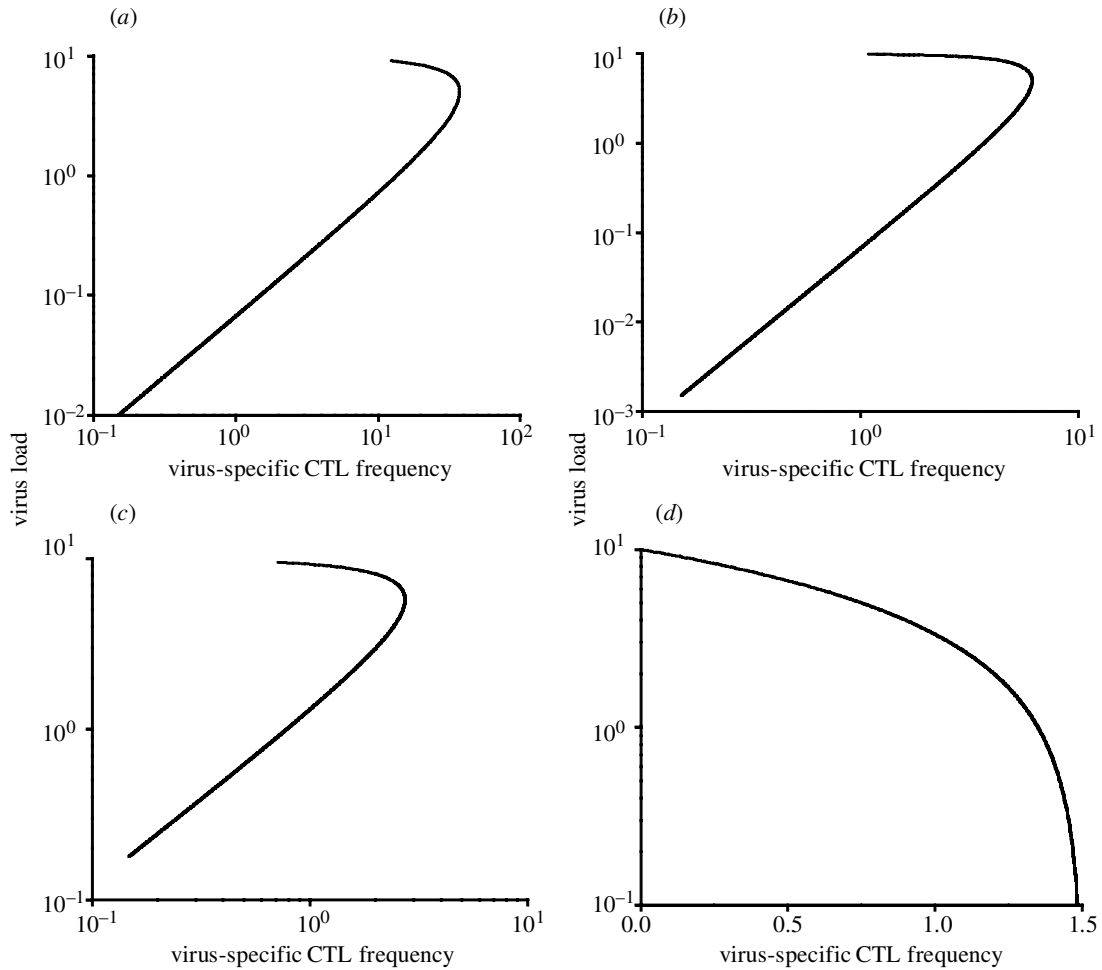
Figure 3. The correlation between virus load and CTL frequency. $(a-c)$ Correlations obtained from models 1–3, respectively. The predictions are qualitatively identical. If the CTL response is effective, thereby keeping the virus load at low levels, the correlation is positive. This correlation is abolished if the CTL response is weak and the virus load is high. The immune responsiveness thresholds below which the correlation turns (c_T) are respectively given for models 1–3 as follows:

$$c_T < 2b/k \tag{3}$$

$$c_T < \sqrt{\frac{br}{k}}, \tag{4}$$

and

$$c_T < b + \sqrt{\frac{b\varepsilon r}{k}}. \tag{5}$$

(d) Virus-induced impairment of the immune response results in a negative correlation between the virus load and CTL frequency. We used a general model of immune impairment, assuming that increased levels of virus load reduce the amount of CTL generation. The functional response is given by $F(v, z) = cvz/(qv + 1)$. The CTL response will persist if $q < (ck/b - 1)/k$. That is, if the degree of immune impairment lies above a threshold, the CTL response collapses and the virus can grow up to its carrying capacity. Virus persistence controlled by the CTL response is described by the equilibrium

$$v^{(5)} = b/(c - bq) \tag{6}$$

and

$$z^{(5)} = \frac{r[k(c - bq) - b]}{pk(c - bq)}. \tag{7}$$

The baseline parameters were chosen as follows: $r = 1.5$, $k = 10$, $c = 1$, $p = 1$, $b = 0.1$, $\omega = 0.0001$ and $\varepsilon = 1.2$. (a) Model 1, (b) model 2, (c) model 3 and (d) immune impairment.

immune response (either in c or p or both parameters) results in a significant reduction in virus load (figure 2a).

In contrast, the immune responsiveness of the host c and the rate of lysis p may have an opposing influence on the equilibrium number of CTLs (figure 2b,c). An increase in the immune responsiveness c only significantly increases the number of CTLs for low values of c. Higher values of c drive the number of CTLs towards an asymptote. Thus, in strong immune responders an increase in immune responsiveness does not significantly influence the abundance of CTLs. The reason is as follows. Low immune responders (low c) develop a high virus load because they cannot control viral replication efficiently. The resulting large antigenic load provides a strong stimulus to the weak CTL response. On the other hand, in high immune responders the smaller antigenic stimulus more efficiently elicits CTLs. Therefore, at equilibrium there is little difference between the two groups in the abundance of virus-specific CTLs. This has been observed in HTLV-1 infection (Jeffery *et al.* 1999).

On the other hand, an increase in the rate of lysis p always leads to a significant reduction in CTLs. The more efficient the CTLs are at eliminating infected cells, the lower the equilibrium abundance of CTLs. This is equivalent to predator–prey dynamics: if each predator is very efficient at killing the prey, a given prey population will not be able to sustain a large number of predators. In contrast, if each predator is inefficient and consumes only a small fraction of the prey population, a large number of predators may coexist at equilibrium.

Because of these patterns, the equilibrium number of CTLs becomes a one-humped function of the efficacy of the immune response if the immune responsiveness c and the rate of CTL-mediated lysis p are linked (figure 2d). This translates into the following correlations between virus load and CTLs (figure 3a–c). The direction of the correlation depends on the efficacy of the immune response (c and p). If the immune response is strong (high values of c and p), thereby suppressing the virus to low levels, we observe a positive correlation between the virus load and CTLs. On the other hand, if the efficacy of the immune response lies below a threshold and the virus load is relatively high (low values of c and p), the positive correlation is abolished and turns negative. However, according to the model, this correlation is very weak, since at high loads a decrease in the efficacy of the immune response pushes the number of CTLs towards extinction while hardly influencing the virus load, which remains near carrying capacity. The threshold of CTL efficacy was calculated and is given in figure 3a–c.

If an effective CTL response which controls the virus infection results in a positive correlation between the virus load and CTLs, how can we explain the negative correlation found among HIV-1-infected patients? We suggest that the most likely reason for this negative correlation is HIV-induced impairment of the CTL response (Kalams & Walker 1998). This may be due to high levels of viral cytopathicity, replication and diversity (Nowak *et al.* 1991, 1995; Van't Wout *et al.* 1994; Fouchier *et al.* 1996; Wodarz *et al.* 1998). If we assume that patients differ mainly in their degree of virus-induced immune impairment, the models predict a negative correlation between virus load and CTLs in cross-sectional studies (figure 3d).

If the virus load is high, there is strong impairment of the immune system, which in turn results in low numbers of CTLs. On the other hand, patients with a low virus load experience less immune suppression, resulting in higher levels of CTLs at equilibrium.

Up to now we have analysed the correlation between CTLs and virus load assuming that the CTL response mainly lyses infected cells and that patients differ mainly in their immunological parameters. CD8^{+} T cells can also act by a non-lytic mechanism, i.e. via the secretion of cytokines. In this case, the outcome depends on the exact assumptions we make about the mechanisms underlying non-lytic effector activity. If the secretion of cytokines with antiviral activity is completely independent of TCR triggering, the parameters c and p are unlinked and patients differ in c alone. The models then predict a zero correlation between CTLs and the virus load, regardless of the *in vivo* efficacy of the antiviral CD8^{+} T cells. The reason is that the immune responsiveness (c) does not have a significant influence on the equilibrium number of CD8^{+} T cells. However, if (as is more likely) the release of cytokines by CD8^{+} T cells also depends on TCR triggering, then the parameters c and p are linked. In this case, the results for non-lytic effector activity will be the same as for a lytic response.

Apart from these considerations, differences in the virus load among patients may be mainly due to non-immunological parameters. In this case, the correlation between the virus load and CTL abundance is positive. For models 2 and 3 this can be expressed as $v^{(2)} = bz^{(2)}/c$ and $v^{(4)} = \varepsilon z/(1-b/c) - \omega$ (see Appendix A). However, in this case the correlation has again no implications for the *in vivo* efficacy of the antiviral CTLs.

HTLV-1 is a non-cytopathic virus that persistently replicates without seriously harming the immune system of the host (except in the 1% who develop leukaemia). The positive correlation observed in this study between the virus load and CTL frequency in asymptomatic carriers (figure 1) indicates that the CTL response is efficient (high values of c and p) and plays an important role in controlling the virus and preventing the development of disease. In contrast, the lack of a correlation in HAM/TSP patients (figure 1) indicates that disease is associated with a weak CTL response (low values of c and p), which fails to keep the virus under control (Bangham *et al.* 1999). Note that one central result from the models is that, even if the CTL response is inefficient, the CTLs may still be present at relatively high levels. These conclusions are consistent with the previous observation of positive selection on the Tax protein of HTLV-1 in asymptomatic carriers and with the recent finding of a class I MHC-associated reduction in HTLV-1 load and protection against HAM/TSP (Jeffery *et al.* 1999, 2000).

On the other hand, in HIV-1 infection the negative correlation between virus load and CTL frequency (figure 1) indicates that the virus actively impairs the generation of virus-specific CTLs. Although CTLs may limit HIV-1 replication early in the course of infection (Rowland-Jones *et al.* 1997), HIV-induced immune impairment results in low numbers of CTLs at high viral loads, probably because T-cell help is required for CTL generation (Rosenberg *et al.* 1997; Kalams & Walker 1998; Rosenberg & Walker 1998; Kalams *et al.* 1999).

It will be interesting to analyse the correlation between HIV load and CTL frequency separately in long-term non-progressors and faster progressors. Our models suggest that, among non-progressors, the number of CTLs will be highest and the correlation could be positive since the CTLs successfully control the infection and the amount of immune impairment is minimal. In fact, this has been observed with CD4$^+$ cell proliferative responses in simian immunodeficiency virus (SIV)-infected macaques (Lifson *et al.* 2000). Animals efficiently controlling the virus following drug treatment during primary infection showed a positive correlation between proliferative responses and virus load, while the correlation was negative among animals characterized by less efficient immunological control of SIV replication.

We conclude that the direction of the correlation between CTLs and virus load in cross-sectional studies may depend on the efficacy of the CTL response (as defined by the values of c and p) if patients differ mainly in ther immunological parameters and if the rate of CTL proliferation is proportional to the rate of CTL-mediated lysis. A positive correlation is consistent with a strong CTL response controlling the infection; the absence of a correlation may result from a weak CTL response unlikely to prevent disease. Finally, a negative correlation between virus load and CTLs suggests that the virus has the upper hand and actively inhibits the immune response.

APPENDIX A

(a) *Model 1*

Nowak & Bangham (1996) suggested a CTL response where the CTL proliferation is proportional to both the number of infected cells (v) and CTLs (z), i.e. $F(v, z) = cvz$. The CTL population becomes established if, in the absence of an immune response, the virus load lies above a threshold required for activation, i.e. if $ck > b$. In this case, the equilibrium expressions for the virus load and CTLs are given by

$$v^{(1)} = b/c \tag{A1}$$

and

$$z^{(1)} = \frac{r(kc - b)}{pkc}. \tag{A2}$$

(b) *Model 2*

We assume that the growth of the CTL response is simply proportional to the virus load, i.e. $F(v, z) = cv$. Biologically, this may correspond to the production of effector cells in response to antigen. In this case, a CTL response becomes established if $r > 0$. The equilibrium expressions for the virus load and CTLs are given by

$$v^{(2)} = \frac{brk}{rb + pkc} \tag{A3}$$

and

$$z^{(2)} = \frac{rkc}{rb + pkc}. \tag{A4}$$

(c) *Model 3*

We analyse a density-dependent model where the growth of the immune response declines with an increasing ratio of CTL to virus load and stops if that ratio crosses a threshold. In biological terms, such dynamics may be due to the dilution of cytokines or spatial interference if the CTL population becomes much larger than the number of infected cells. This can be expressed as $F(v, z) = cz[1 - \varepsilon z/(v + \omega)]$. A similar model has been used for describing predator interference in ecology (Hassell 1981; May 1981). The CTL population will become activated if the rate of proliferation is greater than the rate of death, i.e. if $c > b$. In the absence of an infection, the CTLs may persist at a background level, corresponding to the population of recirculating CTLs. This is described by $v^{(3)} = 0$ and $z^{(3)} = \omega(c - b)/(c\varepsilon)$. The parameter ω determines the level of CTLs in the absence of an infection and may be connected to immunological memory. That is, if the host is naive, the value of ω is low and there are only few virus-specific CTLs recirulating through the body in the absence of antigen. On the other hand, after exposure the value of ω is higher, resulting in immunological memory, i.e. an elevated number of CTL precursors after the clearance of the infection. Since CTLs may persist independent of virus replication, the condition for the establishment of an infection now depends on immunological parameters and is given by

$$r > \frac{p\omega(c - b)}{c\varepsilon}. \tag{A5}$$

High levels of CTL persistence in the absence of an infection (ω) and high rates of CTL-mediated lysis (p) may lead to the extinction of the virus population. Virus replication controlled by a CTL response is described by

$$v^{(4)} = \frac{k[c\varepsilon r - p\omega(c - b)]}{c\varepsilon r + kp(c - b)} \tag{A6}$$

and

$$z^{(4)} = \frac{r(c - b)(\omega + k)}{c\varepsilon r + kp(c - b)}. \tag{A7}$$

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