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The role of the cytotoxic T-lymphocyte response and virus cytopathogenicity in the virus decline during antiviral therapy

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Although it is clear that HIV can lyse HIV-infected CD4 T cells, it is still controversial whether the depletion of CD4 T cells seen in HIV-infected patients after years of asymptomatic disease is caused by the direct cytopathic effects of the virus or is mediated by the immune response. Assuming the initial decline in viraemia during highly active antiretroviral therapy (HAART) is caused by the death of cells productively infected with HIV, I investigate how the rate of the virus decline is affected by the efficiency of the cytotoxic T-lymphocyte (CTL) response. I find that whether the stronger immune response causes a more rapid virus decline depends critically on how the virus is controlled by the CTL response (lytic versus non-lytic mechanisms). Moreover, variation in the efficiency of the immune response does not always cause variation in the rate of the virus decline (and, therefore, in the death rate of infected cells), implying that the constancy of the virus decline rate measured in different patients does not necessarily indicate that the virus is cytopathic. The potential problems associated with the model and the approach undertaken are also discussed.

Keywords: HIV; productively infected cells; virus cytopathogenicity; cytotoxic T-lymphocyte response; mathematical model

1. INTRODUCTION

It is still unknown whether HIV is cytopathic *in vivo*, that is, whether the depletion of CD4 T cells serving as a primary target for HIV occurs mainly because of killing of virus-infected cells by the virus itself (Perelson 2002). An alternative hypothesis is that the virus is relatively non-cytopathic *in vivo* and the majority of cell death occurs because of immune-response-mediated destruction of virus-infected cells (Klenerman & Zinkernagel 1997). Both hypotheses are supported by indirect evidence at least *in vitro* but conclusive evidence of the relative role of these two processes *in vivo* is still lacking. An understanding of why virus-infected cells die may help to design better strategies for treatment for the disease.

HIV is clearly cytopathic *in vitro*: in culture, cells infected with the virus die more rapidly than uninfected controls (Levy 1998); it is likely that both the direct killing of infected cells by the virus and indirect killing of bystander uninfected cells contribute to this effect (McCune 2001). Similarly, cytotoxic T lymphocytes (CTLs) can lyse HIV-infected cells in a standard ^{51}Cr release assay (Klenerman *et al.* 1996; Yang *et al.* 1996). It has been difficult, however, to evaluate the relative role of these two processes in determining the lifespan of productively infected CD4 T cells. Using drugs preventing virus replication, it has been estimated that such cells live, on average, 1 day, with little variation between individuals with different CD4 T cell counts (Ho *et al.* 1995; Wei *et al.* 1995; Perelson *et al.* 1996; Perelson 2002). This observation has led to a suggestion that HIV must be highly cytopathic *in vivo*, killing CD4 T cells in *ca*. 1 day. Otherwise, if the lifespan of infected cells was determined by the immune response, one would expect much greater variation in the lifespan of infected cells between individuals with different immune responses (Nowak *et al.* 1996). This verbal argument is further supported by mathematical modelling suggesting that the lifespan of cells infected with non-cytopathic viruses should vary to a greater extent when measured in patients with different immune responses than should that of cells infected with highly cytopathic viruses (Klenerman *et al.* 1996).

The main problem with such verbal logic and supporting mathematical modelling, however, is that immune responses have not been incorporated explicitly into the models. Recently, Arnaout *et al.* (2000) analysing the dynamics of virus-infected cells and CTLs during highly active antiretroviral therapy (HAART) have found that the lifespan of virus-infected cells may be independent of the efficiency with which CTLs lyse virus-infected cells. Here, I extend their analysis and reanalyse factors that may lead to variation in the lifespan of productively infected T cells between patients.

I achieve this by assuming that the initial decline in viraemia during HAART is caused by the death of cells productively infected with the virus. (This follows from the fact that prior to HAART the majority of the virus is produced by productively infected cells; Perelson *et al.* (1996).) Therefore, the rate of virus decline is proportional to the death rate of virus-infected cells. Given that, I further focus on whether the efficiency of the CTL response may affect the decline rate of the virus during HAART (and, therefore, the lifespan of infected cells).

I find that whether the immune response affects the rate of virus decline depends, critically, on how the virus is controlled by CTLs. If the virus is controlled only either by killing virus-infected cells or by reducing the rate of

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virus replication (through a release of antiviral cytokines and chemokines), then variation in the efficiency of the immune response does not lead to variation in the lifespan of infected cells. This is simply because the efficiency of the immune response (that is the rate at which a given effector cell kills a virus-infected cell) is balanced by the number of HIV-specific CTLs. This, in turn, happens because, to balance the virus replication and virus death at the steady state, higher CTL efficiency requires fewer CTLs (and vice versa). The conclusion holds even if both mechanisms are acting together but only if they are interdependent (i.e. the efficiency of killing of infected cells is correlated with the efficiency of production of effector cytokines and chemokines). However, if the two mechanisms (killing and releasing cytokines) are independent, then variation in the efficiency of either effector mechanism will lead to variation in the measured lifespan of infected cells.

Thus, from the analysis it follows that variation in the lifespan of productively infected cells (or the absence thereof) is indicative of the cytopathogenicity of a virus only for a restricted set of assumptions and it is not yet clear whether these assumptions are fulfilled for HIV.

In § 2 I briefly describe the conventional results for the virus dynamics during HAART and their interpretation. In § 3 I analyse a simple mathematical model describing the dynamics of virus-infected cells and the immune response during HAART. In § 4 I discuss the implications of the main results of the analysis and the possible problems associated with the model and the approach undertaken.

2. VIRUS DYNAMICS DURING HAART

A striking property of HIV infection is that during the asymptomatic period of several years viraemia (viral load in blood) remains approximately constant (Levy 1998, pp. 317–321). The constant viraemia, however, results from a balanced production and clearance of the virus with more than 95% of the virus being turned over each day. This dynamic property of HIV infection has been discovered using drugs that suppress virus replication, namely reverse transcriptase and protease inhibitors (Ho *et al.* 1995; Wei *et al.* 1995; Perelson 2002).

When the drugs are administered, viral load declines in several phases (see figure 1). First, there is an initial delay in the virus decline owing to pharmacological and virus life-cycle delays (Herz *et al.* 1996; Perelson *et al.* 1996; Lloyd 2001; Nelson *et al.* 2001). Then, during the first phase, viral load declines rapidly with a half-life time constant of *ca.* 1 day. The overall drop in viraemia during this phase varies between patients, whereas the rate at which viral load declines is strikingly independent of a patient's CD4 T cell count (a measure of immune-system health). Approximately one to two weeks later, there is a second slower phase during which viral load decreases with an average half-life time of 15–30 days (Perelson *et al.* 1997). It has been proposed that the virus decline during the first phase represents the death of productively infected CD4 T cells whereas during the second phase cells of other types (such as macrophages or latently infected CD4 T cells) are being eliminated (Perelson *et al.* 1997). Although, as should be noted, other interpretations of the virus dynamics during HAART have also been suggested (Ferguson *et al.* 1999; Grossman *et al.* 1999; Arnaout *et al.* 2000; Hlavacek *et al.* 2000; Muller *et al.* 2001), in the following analysis I nevertheless assume that the virus decline in phase I (i.e. the first week of therapy) is caused by the death of productively infected cells. More specifically, I ask how the CTL response may affect the initial rate of virus decline and, therefore, the death rate of virus-infected cells.

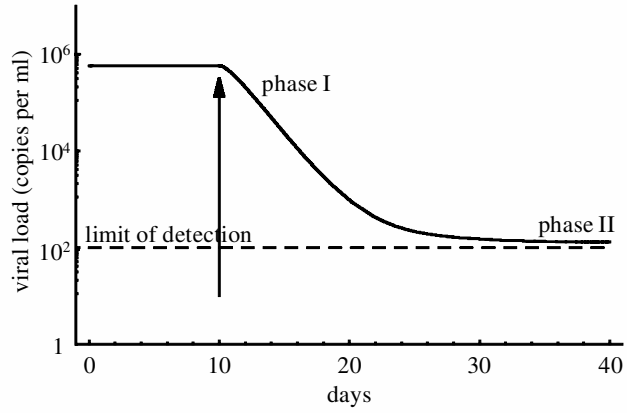

Figure 1. The dynamics of HIV during HAART. It is assumed that before day 10 the viral load is constant (asymptomatic phase) and then at day 10 the drugs suppressing virus replication are administered (i.e. the therapy starts at day 10). During phase I, viral load declines rapidly with a half-life time of $t_{1/2} = 0.8$ day (this phase corresponds to the death of productively infected cells). During phase II, viral load declines more slowly with a half-life time of $t_{1/2} = 30.7$ days (representing the half-life of latently infected cells).

3. THE MODEL

Although it is not known what controls HIV during the asymptomatic phase, CTLs are thought to play some part in restricting virus replication (Koenig *et al.* 1995; Evans *et al.* 1999; Jin *et al.* 1999; Metzner *et al.* 2000). There are a number of ways in which CTLs may play that part. First, HIV-specific CTLs can lyse virus-infected cells. Second, CTLs release chemokines such as β-chemokines (macrophage inflammatory protein (MIP)-1α, MIP-1β, regulated upon activation, normal T-cell expressed and secreted (RANTES)) that can prevent infection of new cells (Cocchi *et al.* 1995). Finally, CTLs release cytokines such as interferon-γ that can suppress the rate of virus production by virus-infected cells (Yang *et al.* 1997; Guidotti & Chisari 2001).

Since the last two mechanisms effectively reduce the rate of virus infection of uninfected cells, I assume that CTLs affect virus replication in two major ways: by killing virus-infected cells (lytic mechanisms) and by reducing the rate of virus replication (non-lytic mechanisms). Since free HIV particles are short lived (Perelson *et al.* 1996; Ramratnam *et al.* 1999; Zhang *et al.* 1999), the virus concentration is approximately proportional to the density of virus-infected cells. I also assume that changes in the total number of uninfected cells are small during the first week of HAART.

The remaining details of the model are as follows. Infected cells Y have a *per capita* growth rate r and a death rate owing to viral cytopathogenicity of α. The presence of the immune response reduces the replication rate of the virus to $r/(1 + aZ)$, where Z is the number of CTLs controlling the virus and a is the efficiency of CTLs in reducing the virus replication rate. Infected cells are also killed by CTLs at a *per capita* rate h. Stimulation of the immune response by the virus is described by a function $f(Y, Z)$. The mathematical model then becomes (see electronic Appendix A for a more general model; available on The Royal Society's Publications Web site):

$$\dot{Y} = \frac{rY}{1 + aZ} - \alpha Y - hYZ, \tag{3.1}$$

$$\dot{Z} = f(Y, Z). \tag{3.2}$$

During the chronic (asymptomatic) phase, viral load (and most probably the number of T cells productively infected with HIV) is approximately constant; therefore, the steady states of the model described by equations (3.1) and (3.2) should describe the asymptomatic phase of infection. These steady states are:

$$\frac{r}{1 + a\overline{Z}} = \alpha + h\overline{Z}, \tag{3.3a}$$

$$f(\overline{Y}, \overline{Z}) = 0. \tag{3.3b}$$

Administration of drugs preventing the production of infective virions (protease inhibitors) and/or *de novo* infection (reverse transcriptase inhibitors) reduces the rate of virus replication to the value $(1 - \rho)r$ with the drug efficiency ρ. Although the antiviral drugs may interfere with the proliferation of T cells, the mechanism of this phenomenon is not understood (Levy 1998, pp. 354–356); for simplicity, I assume that the drugs do not affect the immune response directly. However, the decline in viral load can indirectly affect the immune response. It has been shown that the number of HIV-specific CD8 T cells (measured by major histocompatibility complex class I + specific peptide tetramers) declines during HAART (Ogg *et al.* 1999; Casazza *et al.* 2001). It is not clear, however, whether the number of functional CD8 T cells changes correspondingly. In the model, I assume that the number of CTLs changes from the steady-state value given in equation (3.3*a*) with a *per capita* rate δ (which can be negative):

$$\dot{Y} = (1 - \rho)\frac{rY}{1 + aZ} - \alpha Y - hYZ, \tag{3.4}$$

$$\dot{Z} = f(Y, Z) \approx -\delta Z. \tag{3.5}$$

Since we do not know how CTLs control HIV, I consider three different scenarios: when CTLs are able only to lyse infected cells (i.e. $h > 0$ and $a = 0$), when CTLs can affect only virus replication (i.e. $h = 0$ and $a > 0$), and when both mechanisms are used by CTLs and are of the same order of magnitude.

(a) *CTLs only kill* ($a = 0$)

Initially, I assume that the rate of CTL decline during HAART is small, i.e. $\delta \ll r$ (Ogg *et al.* 1999; Casazza *et al.* 2001). Thus, the changes in the number of CTLs during initial days of HAART are also small; therefore, we can replace the number of CTLs $Z(t)$ by its stationary value $\overline{Z} = (r - \alpha)/h$ given in equation (3.3*a*) at $a = 0$. The dynamics of virus-infected cells then simply become:

$$\dot{Y} = (1 - \rho)rY - \alpha Y - hYZ \approx (1 - \rho)rY - \alpha Y - hY\overline{Z} = -\rho rY. \tag{3.6}$$

We find that the rate at which the number of infected cells declines is independent of the efficiency of the immune response h. This happens because lower h requires more CTLs to control the virus (see equation (3.3*a*)) and vice versa. A similar result was obtained by Arnaout *et al.* (2000). Moreover, the rate of virus decline is the product of the rate of virus replication r and the drug efficiency ρ. Thus, the variation in the rate of virus decline during the first phase can simply be because of the drug efficiency and growth-rate variation between different patients (Bonhoeffer *et al.* 1997).

If the rate of change of HIV-specific CTLs is not small ($\delta \sim r$), then the virus decline is not strictly exponential (see figure 2, dashed lines). Despite this fact, the initial decline rate is still independent of the strength of the immune response h.

(b) *CTLs only reduce the virus replication rate* ($h = 0$)

As in the previous case, by replacing $Z(t)$ with its stationary value $\overline{Z} = (r - \alpha)/(\alpha a)$ at $h = 0$ the dynamics of virus-infected cells become:

$$\dot{Y} = (1 - \rho)\frac{rY}{1 + aZ} - \alpha Y \approx (1 - \rho)\frac{rY}{1 + a\overline{Z}} - \alpha Y = -\rho\alpha Y. \tag{3.7}$$

Again, the rate of decline is independent of the strength of the immune response (now a), but now it is a product of the death rate of infected cells α and the drug efficiency ρ.

(c) *CTLs kill and affect the virus replication rate* ($h > 0$ *and* $a > 0$)

Applying the same technique as before at $\delta \ll r$, we find:

$$\begin{aligned}\dot{Y} &= (1 - \rho)\frac{rY}{1 + aZ} - \alpha Y - hYZ \\ &\approx (1 - \rho)\frac{rY}{1 + a\overline{Z}} - \alpha Y - hY\overline{Z} \\ &= -\rho\frac{rY}{1 + a\overline{Z}} + Y\left(\frac{r}{1 + a\overline{Z}} - \alpha - h\overline{Z}\right) \\ &= -\rho\frac{rY}{1 + a\overline{Z}},\end{aligned} \tag{3.8}$$

where $\overline{Z} = \left(\sqrt{(\alpha a - h)^2 + 4rha} - (\alpha a + h)\right)/(2ha)$, found by solving equation (3.3*a*).

In this case, the rate of virus decline depends upon both a and h, characterizing the strength of the immune response. However, equation (3.8) can be rewritten assuming $h = ca$, where c is a constant:

$$\dot{Y} \approx -\frac{r\rho}{1 + (\sqrt{(\alpha - c)^2 + 4rc} - \alpha - c)/(2c)}Y, \tag{3.9}$$

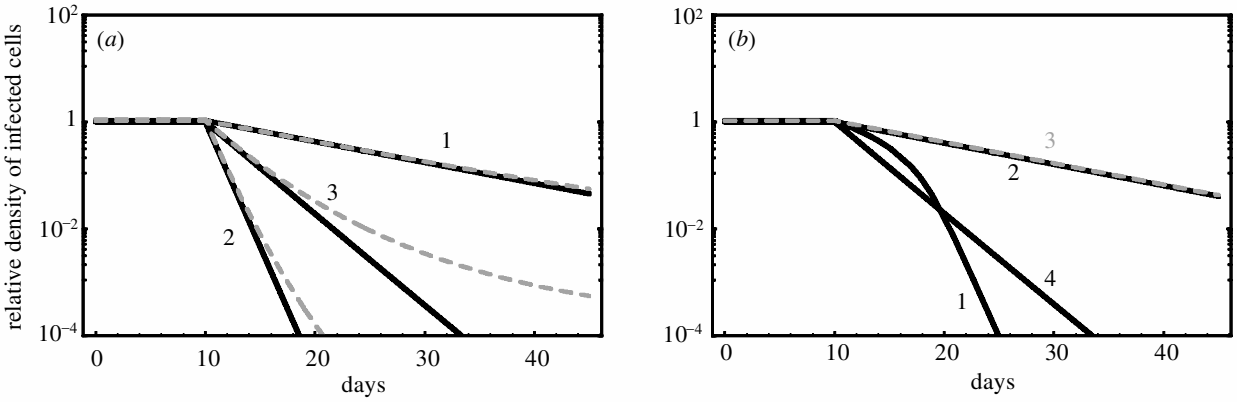

Figure 2. The influence of the immune response on the virus dynamics during HAART. (*a*) The virus decline when the efficiency of the immune response varies. Three parameter combinations are shown: (1) $a = 0.1$, $h = 0$ (only non-lytic mechanisms; slope $= -\rho\alpha$); (2) $a = 0$, $h = 0.02$ (only lytic mechanisms; slope $= -\rho r$); (3) $a = 0.1$, $h = 0.02$ (both lytic and non-lytic mechanisms). Two rates of CTL decline are considered: $\delta = 10^{-3}$ (solid lines) and $\delta = 5 \times 10^{-2}$ (dashed lines). (*b*) The virus decline when saturation in the killing rate is considered (i.e. in equation (3.1) the term hYZ is replaced with $hYZ/(1 + Y/K_1 + Z/K_2)$). (1) Saturation in the killing rate at high densities of infected cells leads to non-exponential virus decline ($K_1 = 10^{-1}$, $K_2 = 10^3$). Saturation in the killing rate at high densities of CTLs reduces the decline rate: (2) $K_1 = 10^3$, $K_2 = 10^{-1}$; (3) $K_1 = 10^{-1}$, $K_2 = 10^{-1}$ (dashed line). (4) Virus decline in the absence of saturation ($K_1 = 10^3$, $K_2 = 10^3$, $a = 0.1$, $h = 0.02$). Other parameters: $r = 1.2$, $\alpha = 0.1$, $\rho = 0.9$.

from which it follows that the rate of virus decline depends only on the ratio of effector-cell efficiencies and not on their absolute values. Importantly, if a and h vary independently between different individuals, leading to $c = h/a$ varying from 0 to ∞, then the rate of virus decline will also vary from $\rho\alpha$ to ρr (figure 2*a*). A similar conclusion holds if the two effector mechanisms are correlated (i.e. $h = ca$) but with a constant c differing between patients. If either of these assumptions were true for HIV, the observed small variation in the virus decline rate would imply that $r \approx \alpha$, i.e. that almost all deaths of virus-infected cells occur because of virus cytopathogenicity. Unfortunately, as far as I am aware, it is not known whether there is a correlation between the two effector mechanisms for HIV-specific CTLs.

Thus, I find that whether the strength of the immune response controlling the virus affects the measured life-span of virus-infected cells depends critically on how the virus is controlled and whether the effector mechanisms by which the virus is controlled are correlated.

4. DISCUSSION

I analysed how the immune response, or, more precisely, the variation in the efficiency of the immune response, affects the rate of virus decline during the first week of HAART. I found that the result critically depends on how the immune response (CTLs in particular) controls the virus. For instance, if CTLs only kill virus-infected cells, or only reduce the rate of virus replication (by releasing antiviral cytokines and chemokines), then the variation in killing/suppressing efficiency does not affect the rate of virus decline. This is simply because, to control the virus at the steady state, the efficiency of the immune response (per effector cell) must be balanced by the number of virus-specific CTLs (see also Bucy 1999). A similar conclusion holds even when both mechanisms are acting and are correlated (i.e. $h = ca$), with a coefficient of proportionality that is constant between different individuals. However, if the killing efficiency is independent of the suppressing efficiency (i.e. $h \neq ca$), then independent variation in h and a leads to a variation in the rate of virus decline that is bounded by ρr and $\rho\alpha$. Unfortunately, it is not clear whether the release of cytokines and chemokines is correlated with the release of perforin and granzymes for antigen-specific CD8 T cells. Although both processes are triggered when there is an appropriate ligand for the T-cell receptor, the conclusive evidence on the linkage between the two effector mechanisms as far as I am aware is still lacking.

It is also important to note that although the model described by equations (3.1) and (3.2) does not include the dynamics of the free virus and uninfected cells, the major conclusion holds in a more general model (see electronic Appendix A). However, functional changes in the killing term, such as to include saturation in the killing rate as the number of CTLs or infected cells increases, may affect the rate of virus decline (figure 2*b*). It is not clear, however, whether saturation in the killing rate occurs *in vivo*. By contrast, the functional form of how CTLs reduce the replication rate of the virus does not affect the major result (not shown).

Fortunately, it is possible to test the presented mathematical model. The model predicts that the rate of virus decline during antiviral therapy should be independent of the number of functional CTLs, specific to the virus, that existed prior to treatment (see, for example, equations (3.6) and (3.7)). For HIV infection, both viral load and the CTL number may be accurately estimated (Piatak *et al.* 1993; Sun *et al.* 2003). If, however, the opposite is found (i.e. the rate of decline *is* correlated with the number of functional CTLs specific to the virus), this would imply that: (i) both lytic and non-lytic mechanisms are involved in controlling the virus; and (ii) either the effector mechanisms are uncorrelated or the correlation is different in different patients (i.e. c varies between patients).

It is also necessary to emphasize that, despite the rela-

tive robustness of the model predictions, there are several potential problems associated with the model and with the approach undertaken.

(i) The model assumes that the initial virus decline is the result of the death of cells productively infected with the virus. In HIV infection, however, the virus decline may be affected by the release of the virus from lymphoid tissues into the blood and in some circumstances may not represent the death of infected cells (Hlavacek *et al.* 2000; Muller *et al.* 2001).

(ii) The key assumption of the model is that the virus and the immune response are at a steady state prior to drug administration. Although this is most probably correct for the total population sizes of the virus and the HIV-specific CTLs, there might be a very dynamic change in clone composition in both populations with new viral variants arising and new CD8 T-cell responses generated, i.e. the population structures of the virus and the CTLs may not be at equilibrium.

(iii) The model, in its simplest form, predicts that the number of virus-specific CTLs is determined by the parameters r, α, h and a and as a consequence is independent of viral load (see equation (3.3a)). This seems to conflict with experimental observation where negative, positive or no correlation between the number of CTLs specific to a given epitope of the virus and viral load has been reported (Novitsky *et al.* 2003, and references therein). Since the underlying mechanisms of such a relationship have not been elucidated, I did not investigate how the model can be modified to include these observations even though such attempts have been made (Wodarz *et al.* 2001).

The analysis conducted in this paper should in no way be considered to provide an answer to whether or not HIV is cytopathic. Rather, I tried to demonstrate that in attempting to understand why a given virus is cytopathic a simple analysis or common logic might simply be misleading. The dynamics of HIV and other infections such as hepatitis B virus and hepatitis C virus during antiviral therapy are complex with the immune response most probably playing an important role. Hopefully, future research will shed some light on whether the immune-response efficiency affects the rate of virus decline during HAART as well as on whether HIV is controlled by the immune response during the asymptomatic phase and how such control, if it exists, is managed.

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