Dynamics of recurrent viral infection

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In chronic viral infection, low levels of viral replication and infectious particle production are maintained over long periods, punctuated by brief bursts of high viral production and release. We apply well-established principles of modelling virus dynamics to the study of chronic viral infection, demonstrating that a model which incorporates the distinct contributions of cytotoxic T lymphocytes (CTLs) and antibodies exhibits long periods of quiescence followed by brief bursts of viral production. This suggests that for recurrent viral infections, no special mechanism or exogenous trigger is necessary to provoke an episode of reactivation; rather, the system may naturally cycle through recurrent episodes at intervals which can be many years long. We also find that exogenous factors which cause small fluctuations in the natural course of the infection can trigger a recurrent episode. Our model predicts that longer periods between recurrences are associated with more severe viral episodes. Four factors move the system towards less frequent, more severe episodes: decreased viral infectivity, decreased CTL efficacy, decreased memory T cell response and increased antibody efficacy.

Keywords: mathematical model; viral reactivation; memory T cell; CTL; antibody; recurrency

1. INTRODUCTION

Viruses are obligate intracellular parasites that rely on endless, sequential transmission from host to host for continued existence. Animal viruses have adopted two life styles to ensure this continuity: acute/highly contagious or persistent infections (Villarreal *et al.* 2000). The spread of acutely infectious viruses, such as measles, Ebola or influenza virus, depends on efficient and rapid transmission during the short window of time before the host immune responses clear the infection or before the virus kills its host. Other viruses, including herpesviruses, human and simian immunodeficiency viruses (HIV), hepatitis viruses and human papillomaviruses, establish persistent, long-lasting infections that allow viral spread during intermittent bouts of activity occurring throughout the host's life. For these viruses, primary infection is followed by the establishment of the virus in either a chronic or latent state.

In both chronic and latent infection, long periods of relative quiescence are typically punctuated by short bursts of high viral production and release. In latent infection, no viral replication can be observed during quiescent periods, but the viral genome is preserved in a completely or partially silent state within infected cells. This non-replicative state can periodically be reversed, with production of new virus occurring during reactivation episodes. In chronic viral infection, in contrast, viral replication and infectious particle production are continually maintained. For many such infections, recurrent episodes of high viral production and release interrupt relatively long periods of low-level viral replication. We note, however, that recurrent episodes are not common to all chronic infections (HIV, for example). Likewise, although most persistent viruses can easily be

classified as chronic or latent, some viruses simultaneously use both strategies. Hepatitis B virus, for instance, can maintain low levels of replication in hepatocytes, but be truly latent in peripheral blood lymphocytes or bone marrow cells.

Although tremendous progress has been made in understanding the molecular mechanisms underlying the maintenance of chronic and latent infections, intriguing questions remain. For instance, the basis for the failure of the innate and adaptive immune responses to eliminate chronic or latent viruses is still largely unknown, although it is clear that viral immune evasion mechanisms play a fundamental role in enabling escape from immune clearance (Redpath *et al.* 2001). Recently, the principles of homeostasis have been summoned to describe, qualitatively, the dynamic equilibrium between latent virus and immunological control (Ghazal *et al.* 2000).

In this work, we investigate a mathematical model of chronic viral infection. Clearly, a complete mathematical model of the immune system is not analytically tractable, due to the great complexity of the system. Recent theoretical work has shown the merit of simple immunological models (Antia *et al.* 1998; Wahl & Nowak 2000; Wodarz & Nowak 2000; De Boer *et al.* 2001; Luzyanina *et al.* 2001; Komarova *et al.* 2003) in explaining experimental results. Basic models usually include target cells (uninfected cells), infected cells and one or more other components, such as free virions, activated cytotoxic T lymphocytes (CTLs) or antibodies, depending on the focus of the work (Nowak & May 2000). The role of antibodies in infection control has also been recently examined from a theoretical perspective (Wodarz 2003). In this paper, we derive distinct expressions for the contributions of antibody and CTL, and include CTL differentiated from memory cells; these immune system components have typically been aggregated in previous approaches.

Three of the most interesting predictions which emerge from this basic model are: (i) the system persists for long

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intervals during which the populations of free virus and infected cells are extremely small; these intervals are followed by brief bursts of high virus release which are then efficiently contained by the immune response; (ii) the interval between recurrent episodes can be extremely long when antibody binding is efficient, and is greatly reduced when antibody levels are deficient; and (iii) in contrast, more efficient cytotoxic action by CTLs reduces the interval between recurrent episodes. To elucidate the dependence of these predictions on the specific assumptions of our basic model, we investigated a series of alternative models, described in detail in the electronic supplementary material. All three of the predictions described above were common to the seven models we explored.

2. A MODEL WITH EXPLICIT EXPRESSIONS FOR ANTIBODY AND CYTOTOXIC T LYMPHOCYTE

We extend an established immune system model (Nowak & May 2000; Wodarz & Lloyd 2004) to include five populations, the major players in recurrent infection: uninfected cells (x), infected cells (y), free virions (v), activated CTLs specific to the virus (z) and antibodies specific to the virus (u). After primary infection, we also include a population of long-lived memory T cells (z_M). The remaining characters are positive parameters,

$$\dot{x} = \lambda - dx - \beta xv, \quad (2.1)$$

$$\dot{y} = \beta xv - ay - pyz, \quad (2.2)$$

$$\dot{z} = cyz - bz + hyz_M, \quad (2.3)$$

$$\dot{u} = \xi z - \eta u - kuv, \quad (2.4)$$

$$\dot{v} = ey - kuv - \gamma xv - qv. \quad (2.5)$$

In equation (2.1), we assume that uninfected cells are produced from a pool of precursor cells at a constant rate, λ , and die with rate d . We use mass-action kinetics to model the interactions of healthy cells with free virus; β gives the rate at which free virus infects cells. In Model 4 of the electronic supplementary material, we also include density-dependent proliferation of target cells.

In equation (2.2), we assume that infected cells are created through the infection of an uninfected cell; we thus ignore vertical transmission through the proliferation of infected cells. Infected cells die with rate a , or may be killed by activated, virus-specific CTLs at rate p .

In equation (2.3), we assume that activated CTLs, specific to the virus, proliferate at rate cy . In reality, CTL proliferation is induced and stimulated by antigen-presenting cells in the lymph nodes; in the basic model, we make the simplest assumption (Nowak & May 2000) that this proliferation rate is roughly proportional to the number of infected cells. The influence of the helper T cell population on the activation and proliferation of CTLs is incorporated in the parameter c ; therefore, a limitation of this model is that we neglect dynamic changes in the CD4 cell population. In Models 2 and 3 of the electronic supplementary material, we explore two alternative density-dependent models of CTL proliferation. Activated CTLs die with natural death rate b .

Since the population of memory T cells will also be important in recurrent infections, we also include the term hyz_M in equation (2.3) to model CTL differentiated from memory T cells. Here, z_M is the population of virus-specific memory T cells, and hy is the rate at which newly

activated CTLs are produced from memory T cells. Although in reality CTL differentiation from memory T cells occurs in response to infected cells through intermediaries, we assume that the number differentiated at any time is roughly proportional to the total population of infected cells at that time. In the basic model, we assume that any decay in the population of memory T cells is negligible over the time course of interest, and thus z_M is constant. We relax this assumption in Model 6 of the electronic supplementary material. We also assume that CTLs differentiated from memory T cells are indistinguishable from those deriving from the activation of naïve T cells (Wodarz *et al.* 2000); this assumption is relaxed in Model 7 of the electronic supplementary material. During the primary effector stage, virus-specific memory T cells are absent, giving $z_M = 0$.

In equation (2.4), we assume that the antibody production rate is proportional to the number of CTLs. This production rate should, of course, be proportional to the B cell population (Bocharov & Romanyukha 1991); however, since antigen-specific B and T cells are activated and proliferate in the lymph nodes via similar mechanisms (Seiden & Celada 1991), we invoke the additional assumption that the B and T cell populations specific to the virus are roughly proportional to each other. Although this assumption is important to the tractability of our model, it does not affect our main conclusions; in Model 5 of the electronic supplementary material, we explicitly include the population of B cells and obtain the same results. The natural decay rate of the antibody population is given by η . The parameter k reflects the efficacy of the antibody in neutralizing free virus. We do not consider antibody binding to infected cells, since the role of antibody-dependent cell-mediated cytotoxicity in host defence is still controversial.

In equation (2.5), the number of infectious virions released from one infected cell per unit time is given by e , the number of virions absorbed by one uninfected cell per unit time is given by γv , and q is the natural clearance rate of the virus. The term γxv is typically neglected but is included here, since the free virus population may be very small during the latent stage of the infection.

3. RESULTS

(a) *The model exhibits recurrent infection*

Figure 1 demonstrates the interesting dynamical behaviour of this system. We see that the virus and infected cell populations persist at extremely low levels for long intervals, and that brief bursts of viral production are quickly controlled by the antibody response and by newly activated CTLs. We note that no exogenous 'trigger' is required to initiate reactivation of the virus; instead, the system naturally cycles through periods of relative quiescence and periods of viral release.

Mathematically, although we omit the proofs here, we find that for a wide range of parameter values, the system moves through a Hopf bifurcation and exhibits a periodic state; the trajectory of the periodic state has a very slow velocity 'near' the (unstable) virus-free equilibrium, and then quickly cycles through state-space close to an (also unstable) virus-persistent equilibrium. In generating figure 1, we examined the analytically simpler case when the parameter $z_M = 0$, since the inclusion of the memory

Figure 1. The model predicts long periods of quiescence followed by brief bursts of recurrent viral production. Parameters are: $k=0.08 \text{ particle}^{-1} \mu\text{l}^{-1} \text{d}^{-1}$, $p=0.015 \text{ cell}^{-1} \mu\text{l}^{-1} \text{d}^{-1}$, $d=0.1 \text{ d}^{-1}$, $a=0.5 \text{ d}^{-1}$, $b=0.2 \text{ d}^{-1}$, $\xi=100 \text{ molecules cell}^{-1} \text{d}^{-1}$, $\eta=0.05 \text{ d}^{-1}$, $e=50 \text{ virions cell}^{-1} \text{d}^{-1}$, $\lambda=100 \text{ cells } \mu\text{l}^{-1} \text{d}^{-1}$, $\beta=1.5 \times 10^{-4} \text{ virion}^{-1} \mu\text{l}^{-1} \text{d}^{-1}$, $c=0.015 \text{ cell}^{-1} \mu\text{l}^{-1} \text{d}^{-1}$, $\gamma=5 \times 10^{-3} \text{ cell}^{-1} \mu\text{l}^{-1} \text{d}^{-1}$, $q=3 \text{ d}^{-1}$, $z_M=0$. Populations on the y-axis are shown per μl .

Figure 2. The time evolution of the system variables, rescaled to illustrate comparative timing and correlations between populations. Parameters are as given in figure 1, except $z_M h = 10^{-4} \text{ d}^{-1}$ and $k = 0.01 \text{ particle}^{-1} \mu\text{l}^{-1} \text{d}^{-1}$.

T cell population ($z_M \neq 0$) substantially complicates the analysis. We consider $z_M \neq 0$ in figures 2 and 5.

Figure 1 illustrates that the period between episodes of viral production and release can be of the order of years. The length of this period, of course, depends on the parameter values. It is well accepted that, even for the most intensively studied viral infections, a large number of the parameters in a model, such as equations (2.1)–(2.5), have not been experimentally determined. This does not, however, diminish the value of the insights gained through careful analysis of such a model (Wodarz & Lloyd 2004). The results we report here are robust for a wide range of parameter values, and are likewise applicable to a range of

viral infections. For the sake of numerical simulation, we note that the values of d , a , b , η , q and λ used in equations (2.1)–(2.5) can be relatively well determined from experiments. We then use rough estimates of the size of the quasi-equilibrium populations of x , y , z , u and v to guide our choice of parameter values, along with the condition that the basic reproductive ratio is greater than one but not too large: $1 < R_0 = \beta\lambda/ad < 3$.

Figure 2 shows the time evolution of system (2.1)–(2.5) for the case $z_M \neq 0$; populations have also been rescaled for visual comparison. This rescaling allows us to better understand the sequence of events which occur during recurrence. During the latent period (roughly days 0–25

Figure 3. (a) The period between recurrent episodes, T , and (b) the peak number of infected cells, $y_{\max}$, increase with the efficacy of the antibody response; recurrent infections are less frequent, but more severe if the antibody response is strong. Parameter values are as given in figure 1, except that $\beta = 10^{-5} \text{virion}^{-1} \mu\text{l}^{-1} \text{d}^{-1}$. Insets show time courses of infected cells for two values of k .

Figure 4. (a) The period between recurrent episodes, T , and (b) the total number of infected cells, $y_{\max}$, decrease with a stronger CTL response; recurrent infections are more frequent, but less severe if the CTL response is strong. Parameter values are as given in figure 1, except that $\beta = 10^{-5} \text{virion}^{-1} \mu\text{l}^{-1} \text{d}^{-1}$, and k is fixed at $0.02 \text{particle}^{-1} \mu\text{l}^{-1} \text{d}^{-1}$.

and again towards the right-hand side of the figure), the populations of virus and infected cells are slowly growing from levels several orders of magnitude below their peak values. Free virus may be well below detectable levels for much of this period. Eventually, the infected cell population grows sufficiently large and stimulates a CTL response, which increases initially due to memory T cell differentiation, and later due to proliferation. This is followed by a strong antibody response. Each of these arms of the immune system contributes to the decline of both the free virus and infected cell populations, and subsequently maintains them at extremely low levels, allowing the uninfected cell population to recover. Finally, the immune response decays—note that the CTL response decays more quickly than the antibody response, due to the longer half-life of antibodies. Eventually, both the CTL and antibody populations are reduced substantially; the virus is then able to escape immune pressure and the cycle begins again.

(b) Factors that affect recurrence

In figure 1, the time between recurrent episodes, T , is about a year. This period, however, can be many years long, and is sensitive to a number of parameters in the model. We explored in detail the effects of varying several parameters, both numerically and analytically. The most

interesting results of these investigations are summarized in figures 3–5.

(i) Antibody response

In figure 3a, we see that T sensitively depends on the efficacy of the antibody response, k . As the antibody strength increases, so does the time between recurrences. In figure 3b, we find that the maximum number of infected cells during the recurrent episode, $y_{\max}$, also increases with antibody efficacy when k is small. For larger values of k , the peak number of infected cells cannot increase further, but this peak broadens, as illustrated by the insets. Since the lifetime of infected cells has not increased, a broader peak implies that more cells in total are infected during each recurrent episode. Thus, if the antibody response is strong, recurrent infections are less frequent but more severe.

We also explored this effect for viruses with different infectivities, β , by numerically determining the length of the period, T , for a range of values of β and k . These results are shown in detail in the electronic supplementary material. We find that T increases as the infectivity decreases; the period is longer for viruses that infect new cells less efficiently. We also see that if the infectivity is relatively low and the antibody efficacy is high, the period

Figure 5. The period between recurrences, T , and peak infected cell population, $y_{\max}$, decrease when the memory T cell population, $z_M h$, increases. Other parameters are as provided in figure 1.

between recurrences can be long, longer than the lifespan of the host. See the electronic supplementary material for more detail.

(ii) Cytotoxic T lymphocyte efficacy

The parameter p in our model reflects the efficacy of the CTL response, that is, the rate at which CTLs find and kill infected cells. This efficacy thus reflects the overall efficiency of the process of epitope presentation, CTL recognition and killing of target cells. Thus, viral strategies of immune evasion which interfere with this process, for example, would reduce the value of p . In figure 4, we examine the effect of CTL efficacy on the recurrent period T and on the severity of the recurrent infection. In contrast with the effect of antibody efficacy, increasing the efficacy of the CTL response decreases both the period and the number of infected cells, i.e. recurrence is more frequent but less severe. We also examine the interaction between this effect and viral infectivity, β , as illustrated in the electronic supplementary material. We find that T gradually decreases with increasing CTL response, and decreases markedly with increasing infectivity. If both infectivity and CTL efficacy are low, the period between recurrences can be long.

(iii) Memory T cell response

The inclusion of memory T cells in our system ($z_M \neq 0$) changes the dynamical structure of the model. Figure 5 demonstrates that when the memory response increases, the period between recurrences decreases, as does the maximal number of cells infected during the recurrent episode. In the electronic supplementary material we also demonstrate that this effect of the memory response on T is robust across a range of viral infectivities, β . Thus, our model predicts, perhaps counter-intuitively, that a larger population of memory T cells results in more frequent, less severe recurrent infections. When z_M is increased further, recurrent episodes are no longer observed, and the system remains in a virus-persistent equilibrium state. In exploring the effects of antibody and CTL efficacy when $z_M \neq 0$, we obtain similar results as those illustrated in figures 3 and 4 (not shown), but as expected T is reduced in each case.

Figure 6. Four main factors move the system toward less frequent, more severe recurrent episodes. Ab, antibody; memT, memory T cell.

(iv) Fluctuations in host health

Although the factors affecting clinical recurrence are not well elucidated, a number of known ‘stressors’ are thought to act as triggers for recurrent episodes. These include overall determinants of health, such as sleep and diet, as well as diverse factors, such as exposure to sunlight or emotional stress. We model the overall effects of these factors by adding a stochastic component to one or more of the populations in system (2.1)–(2.5) during numerical integration, as described in greater detail in the electronic supplementary material. We find that small random fluctuations in the infected cell or CTL populations can ‘trigger’ a recurrent episode, i.e. recurrent episodes happen more frequently, and with less regularity. It is also interesting to note that these episodes, when triggered, are less severe. This is presumably related to the fact that more frequent episodes are generally less severe, as seen in figures 3–5.

4. DISCUSSION

We demonstrate that a model of viral infection which separately incorporates the contributions of CTLs and antibodies exhibits long periods of quiescence followed by brief bursts of viral production. This suggests that for recurrent viral infections, no special mechanism or exogenous trigger is necessary to provoke an episode of reactivation; rather, the system may naturally cycle through recurrent episodes at intervals which can be many years long. This behaviour was also observed in six alternative forms of the model explored in the electronic supplementary material. Similar oscillatory behaviour was also predicted, at least for some parameter ranges, in a model of persistent infection with lymphocytic choriomeningitis virus (LCMV; *Luzyanina et al. 2001*). We also find that exogenous factors which cause small fluctuations in the natural time course of the infection can shorten this interval, triggering a recurrent episode.

Our model also predicts that longer periods between recurrences are associated with more severe recurrent episodes. This agrees well with recent clinical work, in which recurrence after a long period is found to be associated with higher mortality (*Luo et al. 2001*). Four main factors can move the system towards less frequent, more severe episodes: increased antibody efficacy, decreased CTL efficacy, decreased memory T cell responses and decreased viral infectivity (figure 6). Some of these results are, on the surface, quite surprising; we discuss each of these factors below.

- (i) Our model predicts that recurrence is more frequent when antibody responses are weak. These findings are consistent with the clinical observation that recurrent infection by viruses which are typically cleared in a healthy host

(pathological recurrence) is strongly associated with antibody (B cell) immunodeficiency (Roberts & Stiehm 2001; Ballow 2002). In addition, B cell-deficient mice have recently been shown to be unable to control recurrence of LCMV (Thomsen *et al.* 1996; Planz *et al.* 1997; Bachmann *et al.* 2004). In LCMV-infected mice, the absence of antibody responses results not only in increased viral loads, but also in increased CTL exhaustion, indicating that antibodies play a role both in virus clearance and in protecting T cells from over-exposure. Failure to mount an effective antibody response has also been suspected in hepatitis C virus-infected patients, who initially control the virus through strong T cell responses, but subsequently lose these virus-specific T cells and progress to chronic hepatitis (Gerlach 1999). Thus, experimental data and our findings concur in suggesting that the use of neutralizing antibodies or therapeutic vaccination might effectively improve the treatment of chronic viral infections. Our model predicts that antibody therapy would be particularly helpful in substantially delaying the re-emergence of viruses with low efficiencies of infection, while it may be less effective with highly infectious viruses. In addition, although strengthening the antibody response could potentially extend the period between recurrences beyond the lifespan of the host, if re-emergence should occur as a consequence of system perturbations, the severity of the episode would be increased.

- (ii) In contrast, our model predicts that recurrence is less frequent when CTL responses are weak. The lack of symmetry between the strength of the antibody and CTL responses can in a sense be attributed to 'competition' between CTL and antibody in controlling the virus. If the CTL response is weak, the infection is cleared more slowly, and ultimately a more effective antibody response is stimulated. The longer half-life of antibodies as opposed to activated CTLs postpones recurrence. We note that CTL efficacy, as stated previously, reflects the overall efficiency of the process of epitope presentation, CTL recognition and killing of target cells. Thus, a 'weak' CTL response in this sense does not imply a smaller population of CTLs, but less effective clearing of infected cells per CTL. Changes to this parameter could be mimicked in experimental settings by, for example, mutating specific viral epitopes to alter their immunogenicity and, consequently, the efficiency by which infected cells displaying those epitopes in the context of MHC class I are recognized and killed by activated CTLs.
- (iii) Our model predicts that when the memory T cell population is sufficiently large, recurrence does not occur. While this concurs with intuition, it is perhaps more difficult to understand the additional observation that when recurrence does occur, episodes are more frequent when the memory T cell population is larger, or is activated more rapidly. Some evidence that large memory responses do not necessarily correlate with rare reactivation episodes comes from cytomegalovirus

(CMV) immunobiology. Despite the very large CMV-specific memory T cell populations present in latently infected healthy individuals (Gillespie 2000), CMV reactivation is assumed to occur quite frequently and to be rapidly controlled. Thus, although CMV infection is followed by true viral latency with no production of viral particles, as opposed to the chronic infection modelled here, similar results are observed. Finally, our results suggest that recurrent episodes will be more severe when memory T cells are deficient. The importance of memory T cells in controlling virus recurrence is clearly exemplified by the high amounts of virus recovered in LCMV-infected mice that have been artificially impaired in their ability to generate memory T cells (Borrow *et al.* 1996; Thomsen *et al.* 1998).

- (iv) Finally, we predict that viruses which infect cells less efficiently will also recur less frequently. Although this again concurs with intuition, we find that during recurrent episodes, the number of infected cells is greater for viruses with lower infectivities. This is largely due to the gradual decline in immunological pressure between recurrences.

Taken together, these predictions suggest that exposure of T cells to continuous but low amounts of antigen, such as in the case of viruses with low infectivity, may be optimal to achieve continual protection and to delay virus outbreaks. Chronic viruses with high infectivity but with effective CTL evasion abilities might also recur less frequently and generate higher numbers of infected cells during each re-emergence. Viral escape mechanisms are key in establishing successful virus–host relationships that last for prolonged periods of time. True viral latency accompanied by no viral gene expression can be viewed as an extreme means of avoiding CTL attack, but other strategies include production of specific CTL interference molecules, inhibition of antigen presentation and viral epitope mutation (Phillips 1991; Alcamì & Koszinowski 2000; Timm 2004). These tactics allow infected cells to persist, but at the same time may continually stimulate T and B cells at a very low level and ultimately generate longer-lasting immune responses.

In future work, we hope to expand our model to incorporate other components of the immune system which are known to play a role in persistent infection, including CD4⁺ helper T cells, innate immunity cells and immunomodulatory cytokines.

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