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The dependence of viral parameter estimates on the assumed viral life cycle: limitations of studies of viral load data

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Estimation of viral parameters, such as the basic reproductive number (R_0) and infected cell life span, is central to the quantitative study of the within-host dynamics of viral diseases such as human immunodeficiency virus, hepatitis B or hepatitis C. As these parameters can rarely be determined directly, they are usually estimated indirectly by fitting mathematical models to viral load data. This paper investigates how parameter estimates obtained by such procedures depend on the assumptions made concerning the viral life cycle. It finds that estimates of the basic reproductive number obtained using viral load data collected during the initial stages of infection can depend quite sensitively on these assumptions. The use of models which neglect the intracellular delay before virion production can lead to severe underestimates of R_0 and, hence, to overly optimistic predictions of how efficacious treatment must be in order to prevent or eradicate the disease. These results are also of importance for attempts at estimating R_0 from similar epidemiological data as there is a correspondence between within-host and between-host models. Estimates of the life span of infected cells obtained from viral load data collected during drug treatment studies also depend on the assumptions made in modelling the virus life cycle. The use of more realistic descriptions of the life cycle is seen to increase estimates of infected cell life span, in addition to providing a new explanation for the shoulder phase seen during drug treatment. This study highlights the limitations of what can be learnt by fitting mathematical models to infectious disease data without detailed independent knowledge of the life cycle of the infectious agent.

Keywords: virus dynamics; parameter estimation; basic reproductive number; cell life span; non-exponential distribution

1. INTRODUCTION

Our understanding of the within-host dynamics of viral diseases such as human immunodeficiency virus (HIV), hepatitis B and hepatitis C has increased enormously with the development of more sensitive techniques allowing for the accurate measurement of viral load, even when the virus is present at low levels. These advances have been accompanied by a considerable theoretical effort aimed at extracting as much information as possible from longitudinal studies of virus load within an individual. Many of the viral parameters of interest cannot be obtained directly and, thus, must be estimated indirectly, usually by fitting mathematical models to viral load data.

Two particular kinds of study have yielded significant information about virus replication. Drug treatment studies, in which patients are treated with one or more anti-viral agents, have led to estimates for the life span of productively infected cells (Ho *et al*. 1995; Wei *et al*. 1995; Nowak *et al*. 1996; Perelson *et al*. 1996, 1997; Bonhoeffer *et al*. 1997; Grossman *et al*. 1998; Mittler *et al*. 1998, 1999; Neumann *et al*. 1998) and have unveiled an extremely dynamic picture of infection with a rapid turnover of infected cells and, hence, in order to maintain infection, a large number of infection events per unit time. The average life span of infected cells is central to the estimation of the viral generation time which, together with the mutation rate, determine the rate at which genetic diversity is generated by the virus—an important issue for diseases such as HIV as rapid viral evolution can lead to the emergence of viral strains resistant to the particular therapy being employed or to the generation of strains which escape recognition by the immune system (Coffin 1995).

Initial infection studies provided information about the maximum possible rate of viral replication (Lifson *et al*. 1997; Nowak *et al*. 1997; Little *et al*. 1999) as measured by the basic reproductive number R_0 (Anderson & May 1991), which is defined as the average number of secondary infections that would be caused by the introduction of a single infected cell into an entirely susceptible population of cells. This quantity, which is familiar from demographic and epidemiological theory, determines in simple situations whether a disease can first invade a population and then persist. In such situations, invasion is only possible if $R_0 > 1$ (see, for instance, figure 5 in Nowak *et al*. (1997)) and the same condition determines persistence as the system reaches an equilibrium state (the endemic equilibrium) at which the fraction of cells which remain susceptible equals $1/R_0$.

A successful vaccination or therapy regime corresponds to lowering R_0 to below 1 by reducing the number of secondary infections in some way. In an epidemiological setting, standard theory shows that this can be achieved if a fraction of $p_c = 1 - 1/R_0$ (or greater) of the susceptible population is removed by vaccination (Anderson & May 1991). In general, we call the proportion of potential secondary infections prevented by vaccination or therapy the vaccination proportion.

Since the viral parameters are crucial in the design of therapy and vaccination regimes, it is important to have confidence in their estimated values. Underestimation of

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R_0 is a serious problem as it leads to optimistic estimates for the critical vaccination proportion. Use of such an underestimate might lead us to believe that an imperfect treatment would be effective when the true value of the parameter would indicate otherwise.

Statistical techniques can be used to express our uncertainty in the estimates obtained using any particular model. Here we ask a different question, namely the sensitivity of parameter estimates to the assumptions underlying the model being used. Whenever we formulate a mathematical model, we simplify the underlying biology. Clearly, it is important to know how much difference these assumptions make to parameter estimates. Here we investigate the importance of the assumptions made concerning the life cycle of the virus.

2. THE GENERAL MODEL OF VIRAL DYNAMICS

The basic model of viral dynamics (Ho *et al*. 1995; Wei *et al*. 1995; Nowak *et al*. 1996; Perelson *et al*. 1996) considers the interactions between target cells (x), productively infected cells (y) and infectious free virus particles (v). For the purposes of our study, it makes two important assumptions. First, the term describing the removal (i.e. death) of the infected cell population is given by their per capita death rate a multiplied by the number of infected cells. This is equivalent to assuming that the life span of infected cells can be described by an exponential distribution with mean $1/a$ or, equivalently, that the probability of an infected cell dying does not depend on the time since infection. It is further assumed that infected cells begin to produce free virions as soon as they are infected.

More realistic models consider a fuller description of the virus life cycle (Levy 1998), which consists of a number of different phases (cell entry, reverse transcription, integration in the host cell genome, production of early viral proteins, production of viral genomes and late viral proteins and assembly and release of virions). Initially, during the life cycle the death rate of cells is low but later increases as a consequence of viral cytopathogenicity or cytotoxic T lymphocyte (CTL)-mediated lysis (Klenerman *et al*. 1996). In reality, therefore, cell life spans are more likely to be described by less-dispersed distributions as cells are unlikely to die either long before or long after the mean life span. Virion release only begins part way through the life cycle as there is a delay or lag phase between the entry of a virion into a cell and the production of virions by the cell (Herz *et al*. 1996; Nowak *et al*. 1997; Grossman *et al*. 1998; Mittler *et al*. 1998) and virion release may even be concentrated in a short burst towards the end of the cell's life.

Accounting for details of the life cycle clearly leads to a more complex mathematical model as one has to keep track of the time since each cell became infected. We use a simple approach employing a mathematical device known as the method of stages (Jensen 1948; Cox & Miller 1965; Grossman *et al*. 1998; Mittler *et al*. 1998; Lloyd 2001). In its simplest form, the single compartment representing the infected cell population is replaced by a set of n subcompartments or stages with a newly infected cell entering the first, then passing through each of the n stages before dying (see fig. 1 in Grossman *et al*. (1998)). The number of cells in each of these stages is denoted y_j. The amount of time spent in each stage is exponentially distributed so the life span of the cells is described by the sum of n exponential distributions. Here we deploy the method of stages just as a mathematical device in order to include more realistic distributions of cell life spans; the stages do not correspond to biological phases of the cell's life cycle. In more complex models, stages—or collections of stages—could correspond to such phases.

The most general form of the model is given by

$$\mathrm{d}x/\mathrm{d}t = \lambda - \mathrm{d}x - \beta x v, \tag{1}$$
$$\mathrm{d}y_1/\mathrm{d}t = \beta x v - a_1 y_1, \tag{2}$$
$$\mathrm{d}y_2/\mathrm{d}t = p_1 a_1 y_1 - a_2 y_2, \tag{3}$$
$$\vdots$$
$$\mathrm{d}y_n/\mathrm{d}t = p_{n-1} a_{n-1} y_{j-1} - a_n y_n, \tag{4}$$

and

$$\mathrm{d}v/\mathrm{d}t = k_1 y_1 + k_2 y_2 + \ \ldots\ + k_n y_n - uv. \tag{5}$$

Here, the rate of virion production by infected cells in the jth stage is k_j, the average life span of a free virion is $1/u$, the average time that an infected cell spends in the jth stage is $1/a_j$ and p_j is the chance that a cell which leaves the jth stage passes into the $j+1$ stage. The coefficients p_j allow for the consideration of different assumptions concerning the chance of cell death during different stages of the viral life cycle. In all of what follows, we shall take $p_j = 1$ for all j, corresponding to the notion that infected cells only die as they leave the nth stage. We define y to be the total number of infected cells.

This general framework contains most of the models previously employed in the study of viral load data as special cases and, thus, provides a unifying framework for their analysis. For instance, if n is set equal to one, we recover the basic model of virus dynamics. A lag phase, modelling a delay between infection of cells and release of infectious virions, can be considered by setting $k_j = 0$ for the first m stages.

An assumption which we shall frequently make is that free virus dynamics occur on a much faster time-scale than infected cell dynamics. In the basic model, this is equivalent to assuming that k and u are large. In this case, a quasi-equilibrium is quickly established between the free virus and infected cell populations, with $v(t) \approx ky(t)/u$. The basic model reduces to the SIR (susceptible–infectious–recovered) model, which is familiar from epidemiology (Anderson & May 1991), allowing us to make use of the extensive theory derived for this model and its many variants.

One special case of particular interest occurs when the same exponential distribution is employed for each stage, in which case the overall life span is given by a gamma distribution. This distribution is specified by its mean and the parameter n. When n equals one, we recover the exponential distribution of the simplest model but, as n is increased, the distribution becomes more peaked with the limit $n \to \infty$ corresponding to all cells having exactly the same life span (i.e. a delta distribution). In order to compare models in the gamma-distributed case, we take the average life span of infected cells to equal $1/a$, which means that, for an n stage model, the average time spent in each stage is $1/(na)$. If we further assume that infected cells in each stage release virions at the same rate (so that

$k_j = k, p_j = 1$ and $a_j = na$), we have the simplest extension to the basic model and it includes only one extra parameter, i.e. n, compared to the basic model.

3. INITIAL BEHAVIOUR OF THE BASIC MODEL AND ESTIMATION OF R_0

In the absence of infection, target cells reach an equilibrium with $x = \lambda/d$. In order to study the initial behaviour after infection, we perturb this state by introducing a small amount of virus. Over the initial period, since there are few free virions and, hence, few infection events, the target cell population remains approximately constant at its pre-infection level. We assume that $x = \lambda/d$, a procedure equivalent to the standard mathematical technique of linearization. The model reduces to a set of linear equations, from which an expression for the initial rate of viral growth (r) can be derived.

An expression for R_0 in the basic model can be easily obtained since the number of new infections due to a single infected cell is given by multiplying the number of free virions released over the infected cell's life span by the number of cells infected by each free virion over its life span (Nowak *et al.* 1996; Bonhoeffer *et al.* 1997). This gives

$$R_0 = \frac{\beta\lambda k}{adu}. \tag{6}$$

Linearization of the model shows that the initial rate of viral growth r (Nowak *et al.* 1997) is given by $r^2 + (a + u)r - au(R_0 - 1) = 0$ and, assuming that free virus dynamics occurs on a faster time-scale than infected cell dynamics, this expression simplifies to one which is familiar from epidemiology (Anderson & May 1991), i.e.

$$r = a(R_0 - 1). \tag{7}$$

Thus, R_0 can be estimated from the initial rate of increase of viral load and the average life span of infected cells.

4. THE ESTIMATION OF R_0 IN MORE REALISTIC MODELS

The definition of R_0 is unaffected by issues of life span distribution and the timing of virion release since R_0 only depends on the total amount of infectious virions released over the whole life cycle and the number of newly infected cells which arise from each virion. Equation (6) for R_0 holds for all models parameterized so that the average number of virions released over the life span of an infected cell is k/a. However, the initial growth rate of a virus does depend on life cycle assumptions (Heesterbeek & Dietz 1996) and this has important implications in the estimation of R_0.

We assume that the virus life cycle can be modelled by a lag phase which is described by an m-stage gamma distribution with average duration $1/c$, followed by a virion production phase which is described by an n stage gamma distribution with average duration $1/a$. We further assume that virions are produced at a constant rate during this latter phase with an average of k/a virions released by each infected cell and that free virus dynamics occurs on a fast time-scale.

Anderson & Watson (1980) showed that the initial rate of viral increase r satisfies

$$aR_0\left\{1 - \left(\frac{r}{na} + 1\right)^{-n}\right\} = r\left(1 + \frac{r}{mc}\right)^{m}. \tag{8}$$

We now consider two special cases in order to illustrate the implications of life cycle assumptions on the estimation of R_0 from the initial behaviour of the infection.

(a) *A model with non-exponential infected cell life spans*

We first consider the simplest extension to the basic model, differing only in that the infected cell life span is gamma rather than exponentially distributed. Letting the duration of the intracellular delay $1/c$ tend to zero in equation (8) gives (Lloyd 1996)

$$r = aR_0\left\{1 - \left(\frac{r}{na} + 1\right)^{-n}\right\}. \tag{9}$$

Keeping R_0 fixed, less-dispersed distributions of infected cell life spans lead to a more rapid initial increase in viral load (figure 1) (Anderson & Watson 1980; Malice & Kryscio 1989; Lloyd 1996; Keeling & Grenfell 2000). In order to obtain the same growth rate in both the basic (exponential) and more realistic models, one needs a larger value of R_0 in the exponential case. This means that use of equation (7) for estimating R_0 from the initial growth rate overestimates the value of R_0 when the distribution is less dispersed than the exponential, as is the case in reality (Lloyd 1996; Keeling & Grenfell 2000). The overestimate is greatest for the least-dispersed distributions and it is possible to show numerically that the greatest overestimate of R_0 is 29.8%, which occurs with a life span of fixed length (i.e. $n \to \infty$) and when $R_0 \approx 2.1$. Such overestimates lead to conservative estimates for critical vaccination fractions; in other words, they lead us to believe that a given treatment might not work when in reality it would.

(b) *A model with a latent period*

We now allow for a non-zero intracellular delay but, in order to simplify matters, we assume that the period of virion production is described by an exponential distribution of average duration $1/a$. This model is identical to those used by Grossman *et al.* (1998) and Mittler *et al.* (1998) in studying data from drug treatment studies. Setting $n = 1$ in equation (8) leads to the following expression for the initial growth rate of the virus:

$$r = a\left\{R_0\left(1 + \frac{r}{mc}\right)^{-m} - 1\right\}. \tag{10}$$

In the case of an exponential delay (i.e. $m = 1$) this implies that $R_0 = 1 + (r/a)[1 + (r + a)/c]$ (Nowak *et al.* 1997) and for a fixed delay ($m \to \infty$) that $R_0 = (1 + r/a)\exp(r/c)$ (Nowak *et al.* 1997). As the delay becomes shorter ($1/c \to 0$), the relationship (equation (7)) from the delay-free model is recovered.

Keeping R_0 fixed, the inclusion of a delay phase causes a reduction in the initial growth rate, with larger reductions obtained for less-dispersed delays (figure 1*b*). Reinterpreting this observation in terms of R_0, we see that, in order to maintain the same initial growth rate, one needs a larger R_0 if a delay is included and R_0 must be higher

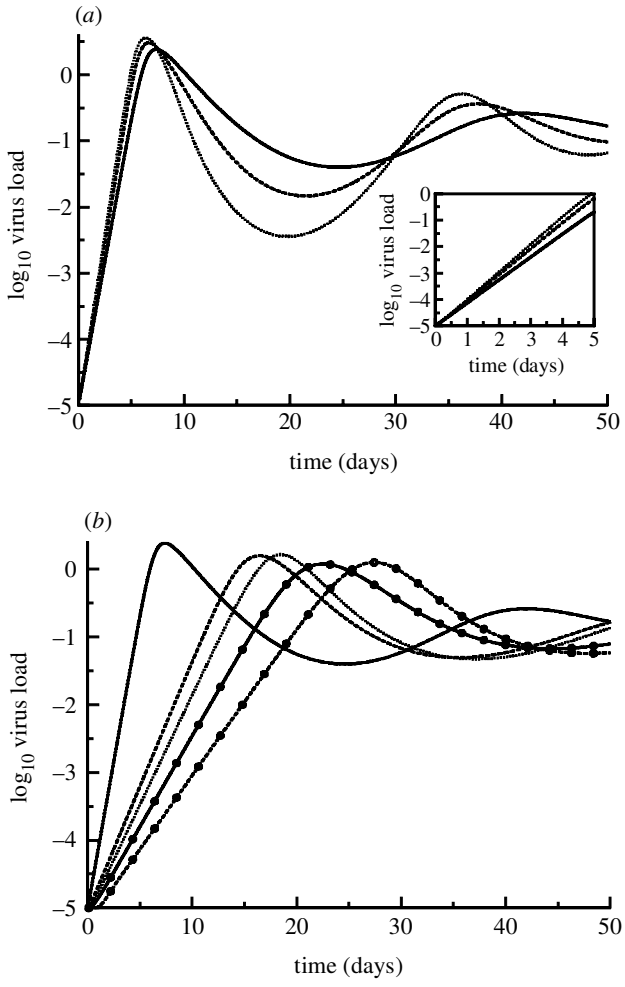

Figure 1. Initial behaviour and approach to the equilibrium in the model when different assumptions are made concerning the viral life cycle. (*a*) Model with gamma-distributed cell life spans. The solid line corresponds to $n = 1$ (i.e. exponential), the dashed line to $n = 2$ and the dotted line to $n = 5$. In each case, the average life span of the infected cell is taken to be two days and R_0 is taken to be 5. The inset highlights the initial behaviour and the increasing growth rate seen as n increases. Notice also the less rapid approach towards the equilibrium seen for larger values of n. (*b*) Model with various latent periods and with the duration of virion production being exponentially distributed with mean two days. In each case, R_0 is taken to be 5. The solid line corresponds to no latent period (i.e. the solid line in (*a*)), the dashed line to a one-day, exponentially distributed latent period and the dotted line to a one-day, five-stage, gamma-distributed latent period. The solid line with symbols corresponds to a two-day, exponentially distributed latent period and the dashed line with symbols to a two-day, five-stage gamma-distributed latent period. Notice that the changes in the initial growth rate are larger than seen in (*a*) and that the approach to the equilibrium again depends on the life cycle assumptions.

still with a less-dispersed delay. The simplest relationship between r and R_0, as provided by equation (7), significantly underestimates R_0 if a delay is not accounted for and this problem is worse for less-dispersed delays. As discussed earlier, the underestimate would lead to an overly optimistic estimate of p_c and, therefore, the critical vaccination coverage predicted by the basic model would not be enough to control infection.

As an example, Nowak *et al.* (1997) presented initial viral load data for simian immunodeficiency virus infection in macaques. An initial growth rate of 2.2 day^{-1} was observed for one individual. Using estimates for a obtained from drug treatment studies, the standard model of virus dynamics gives an estimate of 4.0 for R_0. Employing a delay model with a one-day lag between infection and production of virions increases the R_0 estimate to 13 (exponential delay) or 36 (fixed delay). Smaller differences were seen for either individuals with lower growth rates or if a shorter delay was assumed.

Clearly, that estimates of R_0 can depend so sensitively on the model chosen is deeply troubling. In an instance such as this, it would be foolish to use this method unless we have a very good quantitative description of the infectious agent's life cycle. Since, despite many advances in recent years, we still do not have such a description of the HIV life cycle, estimates of R_0 from initial viral growth data must be treated with more than a little caution.

5. MODEL BEHAVIOUR NEAR EQUILIBRIUM AND DRUG TREATMENT

As the progess of the infection continues, the number of target cells drops and the rate of increase of virus decreases. Eventually an equilibrium is reached in which balances are reached between the production and removal of target cells and between the production and removal of infected cells. At this equilibrium, the number of target cells is given by $\lambda/(dR_0)$, i.e. the number of target cells at the disease-free equilibrium divided by R_0 (Nowak *et al.* 1997). This provides an alternative way of estimating R_0 if one can measure the size of the target cell population both before infection and once the post-infection equilibrium has been established.

Although, like R_0, this equilibrium level does not depend on the assumptions made concerning the viral life cycle, the dynamics of the model as it approaches the equilibrium (as measured, for instance, by the damping time of the oscillations about the equilibrium) are dependent on these assumptions (figure 1) (Grossman 1980; Culshaw & Ruan 2000; Lloyd 2001). Studies which use viral load data obtained as the system approaches equilibrium (e.g. Stafford *et al.* 2000) must take this into account if we are to have confidence in the parameter estimates they obtain.

Anti-retroviral drug therapy often consists of the administration of a combination of reverse transcriptase inhibitors and protease inhibitors. A reverse transcriptase inhibitor essentially prevents infection of new cells; thus, its effects can be modelled by setting β equal to zero. A protease inhibitor prevents already infected cells from producing infectious virions; instead the virions released are non-infectious. Models for the effects of protease inhibitors alone should consider both infectious and non-infectious virions (Perelson *et al.* 1996)—we do not discuss this simple modification in detail here.

We imagine that the populations have reached their equilibrium levels before the initiation of drug therapy at time $t = 0$ and that the effect of therapy is to prevent further infection events. It is clear from equations (1)–(5) that, in order to model viral load upon treatment, only the infected cell and free virus populations need be

followed and that these populations decline according to a linear model.

For the basic model, the free virus decline is given by (Wei *et al*. 1995; Bonhoeffer *et al*. 1997)

$$v(t) = v^*(u\mathrm{e}^{-at} - a\mathrm{e}^{-ut})/(u-a), \tag{11}$$

where v^* is the equilibrium viral load.

Assuming that the life span of free virions is much shorter than that of infected cells, one observes an initial shoulder phase, the duration of which is on the time-scale of $1/u$, followed by an exponential decline at rate a. The observed decay of free virus can therefore be used to estimate a and, hence, the life span of infected cells. Furthermore, it has been suggested that the shoulder phase can be used to estimate u, the free virion life span. Preempting the discussion which follows, however, Herz *et al*. (1996) noted that such estimates of u are sensitive to the inclusion of pharamacological and intracellular delays.

(a) *Estimation of infected cell life span using the more realistic model*

In this section, we use the simplest extension to the basic model, with a gamma distribution of infected cell life spans and with equal production of virions by each stage. Grossman *et al*. (1998) employed a similar model, but within the context of imperfect drug treatment and their discussion focuses on the effects of imperfect drug treatment rather than of non-exponential life spans (see the comments below). Notice that this extension differs from previous models, such as that of Mittler *et al*. (1998) which deployed gamma distributions for the intracellular delay, but assumed that the period of virion production was exponentially distributed. In the $n = 2$ case it is easily shown that

$$v(t) = \frac{v^*}{(u-2a)^2}\{u[(u-3a) + at(u-2a)]\mathrm{e}^{-2at} + a(4a-u)\mathrm{e}^{-ut}\}. \tag{12}$$

This is no longer simply the sum of exponentials (mathematically, this occurs because the linearization leads to repeated eigenvalues): the exponential $\exp(-2at)$ is multiplied by a first-order (i.e. linear) polynomial in t. For large enough t, the decay of $v(t)$ is dominated by the exponential term, i.e. the virus decays at rate $2a$. Comparing this with the decay at rate a seen in the basic model, the drug-induced decay of free virus can be seen to be more rapid in the more realistic model (figure 2).

It is possible to obtain analytical solutions for the more general model with larger values of n (see also Mittler *et al*. 1998), but these quickly become unwieldy and are not shown here. The important point is that the decay is dominated by a term proportional to $\exp(-nat)$. Thus, it is a general result that the use of more realistic models leads to more rapid decay of infected cells (figure 2). Moreover, the polynomial which multiplies this exponential term, which is of order $n-1$, leads to a lengthening of the shoulder phase as n increases (figure 2).

Turning to the inverse problem of the estimation of infected cell life spans from an observed decay in free virus following drug therapy, since the rate of viral decay in the more realistic model gives an estimate for na (as opposed to a in the basic model), the estimated value of a will be smaller in more realistic models, leading to longer estimates

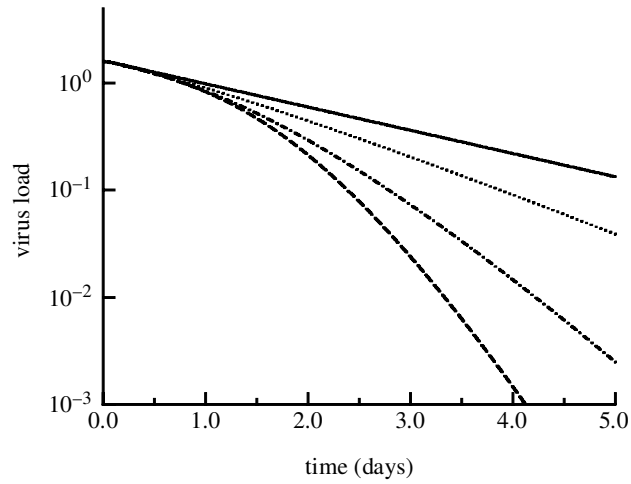

Figure 2. Viral load decay curves obtained from the basic (solid line) and more realistic models using gamma distributions with $n = 2$ stages (dotted line), $n = 5$ stages (dotted-dashed line) and $n = 10$ stages (dashed line). The other model parameters are taken to be $a = 0.5\ \mathrm{day}^{-1}$, $u = 30\ \mathrm{day}^{-1}$ and $k = 30\ \mathrm{day}^{-1}$. The system is in equilibrium at time $t = 0$ in each simulation and, in order to have the same equilibrium virus load in all three simulations, the basic reproductive ratio R_0 is taken to be the same. Notice that the parameters chosen imply that the dynamics of free virus occur on a fast time-scale: the shoulder phase mainly arises for these parameter values because of the non-exponentially distributed cell life spans.

for infected cell life spans. Furthermore, the new model offers an alternative explanation for the shoulder phase: in addition to reflecting free virus dynamics, it also in part arises from non-exponential infected cell life spans. (Notice that increased estimates of infected cell life span are due to the more complex relationship between the half-life of viral decay and the life span of infected cells within the more realistic framework; they do not lead to a lengthening of the predicted time taken to clear the virus, as obtained by extrapolating the curve of viral load versus time.)

Whilst the decay is no longer strictly exponential, it may be impossible to distinguish it from an exponential, particularly for experimental data which are subject to noise. This is illustrated in figure 3 in which various models are fitted to a viral load decay curve obtained upon treatment with a protease inhibitor (Perelson *et al*. 1996). (As the treatment study from which these data were obtained used a protease inhibitor alone, we used the more complex model formulation involving non-infectious and infectious free virions, as mentioned above.)

As our purpose here is to point out the ways in which life cycle assumptions can affect viral parameter estimates rather than making detailed parameter estimates, our illustration only involves data from a single patient. The models with n equal to 2 or 3 actually fit the data better for this patient than the basic model, whereas the fit with $n = 5$ is worse. Whilst there are systematic differences between the curves obtained from the different models, the quality of the available data makes it difficult to differentiate statistically between the different model fits. The important point is that the use of more realistic life cycles tends to increase the estimated life span, although it is interesting to note that these increases lie

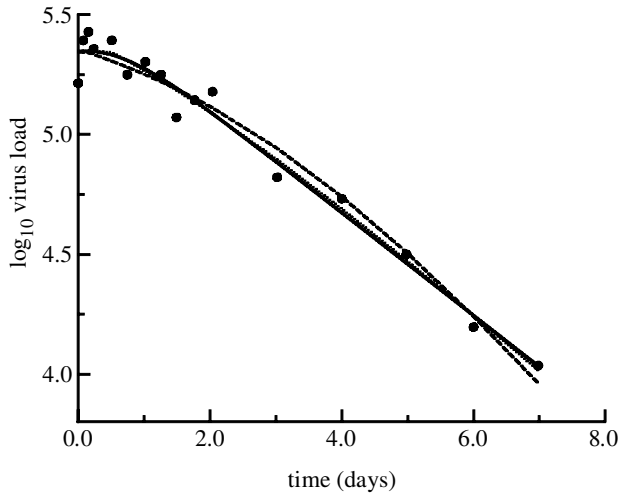

Figure 3. Example of viral load decay seen upon treatment with a protease inhibitor (Perelson *et al.* 1996), together with model fits obtained using the basic model (solid line) and the more realistic model with $n = 2$ (dotted line) and $n = 5$ (dashed line). In each case, a modified form of the model appropriate for the action of protease inhibitors alone (Perelson *et al.* 1996) was fitted by nonlinear least-squares regression (Press *et al.* 1986). In addition, in each case, a pure delay time τ, which was intended to represent the pharmacological delay, but which could also model a pure intracellular delay (Herz *et al.* 1996), was also allowed for when fitting the data. The fitted parameters for the basic model were $a = 0.494\ \text{day}^{-1}$, $u = 2.6\ \text{day}^{-1}$ and $\tau = 0$ days, those for the realistic model with $n = 2$ were $a = 0.322\ \text{day}^{-1}$, $u = 50\ \text{day}^{-1}$ and $\tau = 0.419$ days, those for the realistic model with $n = 3$ (curve not shown) were $a = 0.266\ \text{day}^{-1}$, $u = 50\ \text{day}^{-1}$ and $\tau = 0.227$ days and those for the realistic model with $n = 5$ were $a = 0.221\ \text{day}^{-1}$, $u = 50\ \text{day}^{-1}$ and $\tau = 0.101$ days. These parameters correspond to estimated average infected cell life spans of 2.02, 3.10, 3.76 and 4.52 days (basic model and realistic model with $n = 2$, 3 and 5, respectively). The parameter u was constrained to be no greater than 50 in the model fitting process.

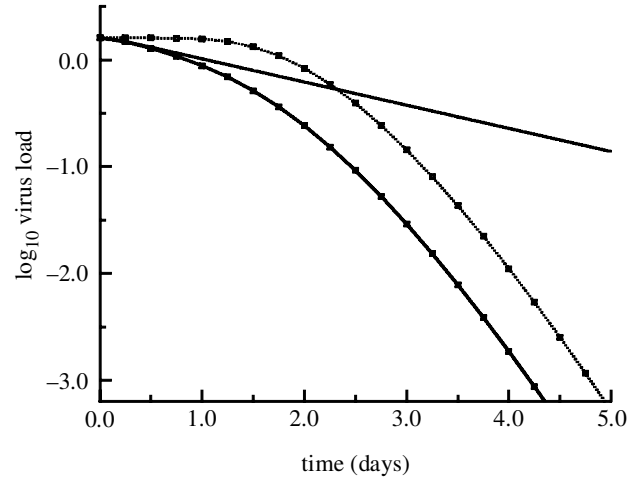

Figure 4. Viral load decay curves for models in which virions are released at a constant rate over an infected cell's life span (solid curves) and in which virions are released in a single burst at the end of an infected cell's life (dotted curve). This makes no difference to the behaviour of the basic model (solid curve without symbols), but increases the length of the shoulder phase, without changing the final decay rate, in the more realistic model (curves with squares, obtained from a ten-stage model). The parameter values are $a = 0.5\ \text{day}^{-1}$ and $k = u = 10\ \text{day}^{-1}$.

below the theoretical maximum increase, presumably because the increased length of the shoulder phase in more realistic models increases the time taken for the decay rate to reach its asymptotic value. We also note that estimates of u (the free virion clearance rate) are larger when the new model is employed since the shoulder phase is now attributed to the non-exponential distribution of cell life spans, as well as free virion dynamics. A similar effect was noted by Mittler *et al.* (1998) as the less-dispersed intracellular delays they employed also led to a lengthening of the shoulder phase.

A counter-intuitive consequence of the consideration of non-exponential waiting times in different phases of the viral life cycle is that the virus decay need no longer reflect the slowest phase of the life cycle. If n is large enough it can be the case that $na > u$, in which case the free virus dynamics can dominate the overall virus decay, even though $u \gg a$. We notice that a similar argument explains why the fixed-length intracellular delay (i.e. corresponding to n tending to infinity) employed by Herz *et al.* (1996) did not affect the rate of virus decay observed in their model, even in the case when the intracellular delay was longer than the time-scale over which cell death occurs. We remark that this behaviour would not be observed if an exponential delay phase (or any other distribution with moderate dispersion) were employed.

In order to investigate the effect of the timing of free virion production on viral decay rates, we relax the assumption that free virions are produced continuously and instead assume that virions are only released during the final stage. (Of course, this life cycle could alternatively be described as a gamma-distributed latent phase, followed by an exponentially distributed phase of virion release (Grossman *et al.* 1998; Mittler *et al.* 1998).) In order to make a fair comparison, we keep the total virion production per infected cell constant, which means that k_n is taken to equal nk whilst all other k_j are zero. This modification is seen to have no effect on the asymptotic decay of free virus, but it further lengthens the shoulder phase (figure 4). (This result is predicted by analysis of the model as the k_js do not affect the decay rates obtained upon linearization of the model, whereas the factors which multiply the exponential terms are dependent on k_j.) Thus, in general, the shoulder seen in virus decline reflects more than just free virion dynamics and the intracellular delay, as it also reflects the convolution of the infected cell life span distribution with the distribution describing timing of virion release (only part of which reflects the intracellular delay). We suggest that, without much more detailed independent biological information concerning these distributions, it is unlikely that viral parameters can be estimated with a high degree of confidence from the shoulder phase.

6. DISCUSSION

In this study, we have only considered the changes which result simply from relaxing the assumptions made by the basic model concerning the life cycle of the virus.

The inclusion of other biological details leads to yet more complex models and may have an even more important impact on parameter estimates obtained from viral load data. For instance, within the context of drug treatment studies, the inclusion of an explicit immune response or the trapping of virus particles by cells, such as follicular dendritic cells, have both been shown to have important effects on estimates of cell life spans (Arnaout *et al*. 2000; Hlavacek *et al*. 2000).

In the preceding discussion of the effects of anti-viral therapy, it was assumed that drug treatment was perfect, totally preventing ongoing viral replication. In the case of HIV, where there is considerable evidence for ongoing viral replication, even when drug therapy has reduced the viral load to below the detection limit of current assays (see Ramratnam *et al*. (2000) and references therein), this assumption is clearly incorrect. From a modelling standpoint, this complicates the picture considerably, as the equations for infected cells and free virus no longer decouple from the target cell equation and, thus, the dynamics of the target cell population must be considered. Furthermore, ongoing replication reduces the rate of virus decline below that which would be observed if drug treatment were completely effective. Consequently, life span estimates obtained using models which assume complete blocking of infection will overestimate the true life span (Perelson *et al*. 1996; Grossman *et al*. 1998; Ding & Wu 1999; Nelson *et al*. 2000). Notice, however, that our observation that non-exponential life spans can lead to decay curves consistent with an exponential decay argues against the reasoning of Grossman *et al*. (1998), who suggested that the knowledge that life spans are not exponentially distributed meant that another explanation was needed for the exponential decay kinetics of free virus, namely that infection is ongoing in the face of drug treatment.

An important issue that is often inadequately addressed is whether the basic model can adequately describe both the initial behaviour of the infection and the equilibrium behaviour using the same set of parameters (see, however, Stafford *et al*. 2000). This in turn has important implications for estimation of viral parameters and their use in the design of control and prevention strategies. For instance, estimation of R_0 from initial viral load data makes use of the infected cell life span estimate obtained from drug treatment studies. This assumes that the average life span of infected cells is the same during the initial stages of infection as it is when the equilibrium state has been reached. If cell death is largely due to virus cytopathicity this should be the case, assuming that there are no major changes in the virulence of the virus. If cell death is largely due to specific immune responses, such as killing by CTL, this will be a poor approximation as specific immune responses are likely to be weak during initial infection. Furthermore, the basic model assumes that target cell dynamics are essentially the same during all stages of infection. In the case of HIV, several types of immune cells can be infected, including macrophages and activated CD4 T cells. Different virus strains can preferentially infect different target cell pools and, with the evolution of the virus, the importance of different cell pools could change over time. In order to allow productive infection, CD4 T cells must be activated, raising the possibility that this pool of target cells could increase in size upon initial infection. Models which account for these and other phenomena which the basic model does not capture have been developed (see, for instance, McLean & Kirkwood 1990; McLean & Nowak 1992; De Boer & Perelson 1998; Murray *et al*. 1998; Callaway *et al*. 1999; Wodarz *et al*. 1999), but their behaviour is often much more complex and they do not inherit many of the simple properties of the basic model. Crucially, the condition $R_0 > 1$ may not determine invasion and persistence (see also Doebeli 1998; Dushoff 1996). In some situations, the invasion process cannot be captured by a linear model. Whether an infectious dose leads to the establishment of disease can depend on the size of the dose (Wodarz *et al*. 1999). Alternatively, if the death rate of infected cells depends on an immune response which can develop over time, a virus can initially invade before later being eradicated by the immune response.

Assumptions made concerning the viral life cycle can clearly have an important effect on estimates of viral parameters obtained by fitting mathematical models to viral load data. We have seen here that model misspecification can have an effect which is considerably larger than the uncertainty in parameter estimates which arises from noise in the data. Whilst this latter source of error can be addressed by statistical techniques, this study suggests that our lack of detailed understanding of the biological details of the virus life cycle poses a much greater constraint on parameter estimation. One issue of particular importance is the question of parameter identifiability; the above discussion of the shoulder phase during drug treatment shows that many factors influence this phase of the decay. Without additional independent information, we suggest that, in practice, it is very difficult to disentangle these effects in order to describe free virus dynamics, the distribution of life spans of infected cells and the timing of virion production separately. To conclude, whilst the study of viral load data has provided much important information on the dynamics of viral diseases, care must be taken to avoid its over-interpretation when detailed information concerning the viral life cycle is not available.

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