Some basic properties of immune selection

Yoh Iwasa[a],*, Franziska Michor[b], Martin Nowak[b]

[a] *Department of Biology, Faculty of Sciences, Kyushu University, Hakozaki 6-10-1, Higashi-ku, Fukuoka 812-8581, Japan*
[b] *Evolutionary Dynamics Program, Harvard University, One Brattle Square, Cambridge, MA 02138, USA*

Abstract

We analyze models for the evolutionary dynamics of viral or other infectious agents within a host. We study how the invasion of a new strain affects the composition and diversity of the viral population. We show that—under strain-specific immunity—the equilibrium abundance of uninfected cells declines during viral evolution. In addition, for cytotoxic immunity the absolute force of infection, and for non-cytotoxic immunity the absolute cellular virulence increases during viral evolution. We prove global stability by means of Lyapunov functions. These unidirectional trends of virus evolution under immune selection do not hold for general cross-reactive immune responses, which introduce frequency-dependent selection among viral strains. Therefore, appropriate cross-reactive immunity can lead to a viral evolution within a host which limits the extent of the disease.
© 2004 Elsevier Ltd. All rights reserved.

Keywords: Virus dynamics; Evolution of virulence; Monotonic evolution; Immune selection; Theoretical immunology

1. Introduction

Many pathogenic microbes have high mutation rates and evolve rapidly within a single infected host individual. For example, the human immunodeficiency virus (HIV) can generate mutations, on the time-scale of weeks, which contribute to escape from immune responses and drug treatment (Hahn et al., 1986; Holmes et al., 1992; Fenyo, 1994; McMichael and Phillips, 1997; Borrow et al., 1997; Nowak and May, 2000; Wei et al., 2003). The continuous evolution of HIV within an infected individual over several years is likely to shift the balance of power between the immune system and the virus in favor of the virus. Hence, evolution of HIV within a host can drive disease progression (Nowak et al., 1991).

Here we analyse three models for the interaction between a virus population and immune responses (Perelson, 1989; McLean and Nowak, 1992; Perelson et al., 1993; De Boer and Boerlijst, 1994; Nowak et al., 1995a, b; Nowak and Bangham, 1996; De Boer and Perelson, 1998; Bittner et al., 1997; Perelson and Weisbuch, 1997; Wodarz et al., 1999; Regoes et al., 1998; Wahl et al., 2000; Nowak and May, 2000). The models describe deterministic evolutionary dynamics in terms of uninfected cells, infected cells, and strain-specific immune responses. This means, there are n virus strains (or mutants) which induce n immune responses. The immune responses are directed at the strains that induce them. Two of the three models describe cytotoxic immunity: the immune responses reduce the life-time of infected cells. The third model deals with non-cytotoxic immunity: the immune responses reduce the rate of transmission of virus from infected to uninfected cells. Virus mutants can differ in all virological and immunological parameters.

In the absence of immune responses only one virus strain can survive at equilibrium. This is the top competitor with maximum fitness. In the presence of immune responses there can be coexistence of several different strains. Consider a population of viral strains at a stable equilibrium. Imagine a new strain is generated by mutation. We calculate the conditions for the new strain to invade the existing population. There are three possibilities: (i) the new strain may simply be added to the existing population thereby increasing the number of strains by one; (ii) the new strain may invade the existing population and other strains may become extinct; (iii) the new strain may not be able to invade.

*Corresponding author. Tel.: +81-92-642-2639;
fax: +81-92-642-2645.
E-mail address: yiwasscb@mbox.nc.kyushu-u.ac.jp (Y. Iwasa).

The most fascinating question is whether we can find quantities that will consistently increase (or decrease) during such viral evolution. We can show that neither viral load nor diversity increases monotonically with virus evolution (although they are likely to increase in a probabilistic sense). Instead, we prove that any successful invasion of a new virus strain always decreases the total abundance of uninfected cells. This holds for all three models. For the models with cytotoxic immune responses, we find that any successful invasion increases the total force of infection, denoted by $\sum_{i=1}^{n} \beta_i y_i$. For the model with non-cytotoxic immune responses, we find that any successful invasion increases the total cellular virulence, denoted by $\sum_{i=1}^{n} a_i y_i$. Here y_i denotes the abundance of cells infected by strain i, while β_i and a_i denote, respectively, the infectivity and cellular virulence of strain i. Importantly, these results hold in the limit of strain-specific immunity, but not in the presence of general cross-reactive immunity. It remains an open problem to characterize the degree of cross-reactivity that is still compatible with our results.

Our analysis is part of a larger effort to understand the evolutionary dynamics of infectious agents (Sasaki, 1994; Levin and Bull, 1994; Gupta et al., 1998; Levin et al., 1997, 1999).

2. Cytotoxic immunity, model 1

Let us first consider a model, where cytotoxic immune responses reduce the life-time of infected cells. Denote by x the abundance of uninfected target cells and by y_i the abundance of cells infected with virus strain i. Let z_i denote the abundance of immune cells specific to strain i. Consider the following system of ordinary differential equations (cytotoxic immunity, or CTL model):

$$\frac{d}{dt}x = \lambda - dx - \sum_{i=1}^{n} \beta_i x y_i, \tag{1a}$$

$$\frac{d}{dt}y_i = (\beta_i x - a_i - p_i z_i) y_i, \quad i = 1, 2, 3, \ldots, n, \tag{1b}$$

$$\frac{d}{dt}z_i = c_i y_i - b_i z_i, \quad i = 1, 2, 3, \ldots, n. \tag{1c}$$

Target cells are supplied at a constant rate, λ, and die at a rate proportional to their abundance, dx. The infection rate is proportional to the abundance of uninfected and infected cells, $\beta_i x y_i$. Infected cells die at rate $a_i y_i$ because of viral cytopathicity. The immune response z_i is specific to virus strain i. The efficacy of the immune response in killing infected cells is given by p_i. Immune activity increases at a rate proportional to pathogen abundance, $c_i y_i$, and decreases at rate $b_i z_i$. In the present paper, we assume that virus load (the abundance of virions) is proportional to the amount of

(productively) infected cell. There is no need to model the dynamic of free virions explicitly, because it occurs (for HIV) on a much faster time scale than what we are considering here.

2.1. The equilibrium

The model given by Eq. (1) always has a single stable equilibrium, which can be calculated as follows. The equilibrium values of y_i and z_i can be written as functions of x, derived from Eq. (1b) and (1c). We denote these by $y_i(x)$ and $z_i(x)$ for $i = 1, 2, \ldots, n$. For given x, these values are either positive or zero, because some of these strains go extinct as an outcome of competition between strains, and have $y_i(x) = z_i(x) = 0$. The equilibrium abundance of infected cells is

$$y_i(x) = \begin{cases} \dfrac{b_i}{p_i c_i}(\beta_i x - a_i) & \text{for } x > \dfrac{a_i}{\beta_i}, \\ 0 & \text{for } x \leqslant \dfrac{a_i}{\beta_i}. \end{cases} \tag{2}$$

The immune response is proportional to the abundance of infected cells, $z_i(x) = c_i y_i(x)/b_i$. The equilibrium level of uninfected cells is determined by Eq. (1a), which can be rewritten as

$$\frac{\lambda}{x} = d + \sum_{i=1}^{n} \psi_i(x). \tag{3}$$

Here ψ_i is the force of infection by strain i. For Eq. (1), we have

$$\psi_i = \beta_i y_i. \tag{4}$$

From, Eqs. (2) and (4), $\psi_i(x)$ is zero for $x \leqslant a_i/\beta_i$, but is positive and an increasing function of x for $x > a_i/\beta_i$. Thus, the minimum level of uninfected cells that is needed to sustain virus strain i is given by a_i/β_i. The right-hand side of Eq. (3) is a sum of increasing functions, and hence it is also an increasing function of x, whilst the left-hand side of Eq. (3) is a decreasing function of x ranging from infinity to zero as x changes from zero to infinity. Hence there is always a single solution of Eq. (3), which is positive. Let x^* be the equilibrium number of uninfected cells. Let us renumber the strains so that a_i/β_i increases with i. Suppose that the equilibrium abundance of uninfected cells x^* satisfies

$$\frac{a_1}{\beta_1} < \cdots < \frac{a_k}{\beta_k} < x^* < \frac{a_{k+1}}{\beta_{k+1}} < \cdots < \frac{a_n}{\beta_n}. \tag{5}$$

From Eq. (2), the abundances of the first k strains are positive: $y_1^* > 0, \ldots, y_k^* > 0$, but the remaining strains are absent: $y_{k+1}^* = 0, \ldots, y_n^* = 0$. The immune responses are positive only for the first k types: $z_1^* > 0, \ldots, z_k^* > 0$, but $z_{k+1}^* = 0, \ldots, z_n^* = 0$.

This equilibrium is globally stable. The proof uses a Lyapunov function and is shown in Appendix A.
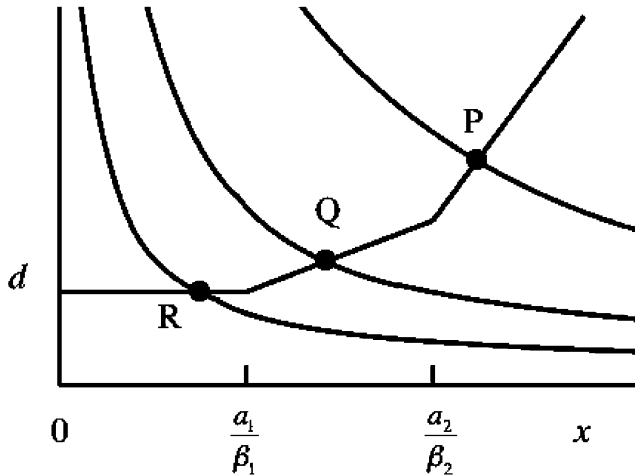
Fig. 1. Graphical representation of Eq. (3) for a population including two strains. Three arcs connected by kink is the right-hand side of Eq. (3), indicating per capita risk of uninfected cells. The curves with negative slopes are the left-hand side of Eq. (3), λ/x, with different value of λ. Horizontal axis is the abundance of uninfected cells. P, Q and R are for the equilibrium corresponding to three different values of λ. P indicates the equilibrium with two strains; Q, the one with strain 1 only; and R, the one without pathogen. The model is given by Eq. (1).

2.2. Graphical representation

To obtain the equilibrium solution x satisfying Eq. (3) and to know its parameter dependence and the change after invasion of a new strain, graphical representation of both sides of Eq. (3) is very useful. A typical case is illustrated in Fig. 1. Here there are two strains, differing in the minimum level of uninfected cells $0 < a_1/\beta_1 < a_2/\beta_2$. The solid curve indicates the right-hand side of Eq. (3). It is a constant d for $0 < x < a_1/\beta_1$, which corresponds to the absence of strains. It is positive for $x > a_1/\beta_1$, in which it is an increasing function of x. The arc for $a_1/\beta_1 < x < a_2/\beta_2$ corresponds to the state including strain 1 only, and the arc for $a_1/\beta_1 < x < a_2/\beta_2$, corresponds to the state including both strains 1 and 2. Three curves with negative slopes indicate λ/x with three different levels of λ. The x value of cross-points, indicated by solid circles, indicates the abundance of uninfected cells in the equilibrium. Corresponding to three different values of λ, Fig. 1 has three cross-points. The one denoted by P satisfies $x > a_2/\beta_2$, in which both strains have positive abundance at equilibrium. The cross-point denoted by Q satisfies $a_1/\beta_1 < x < a_2/\beta_2$, in which only strain 1 has positive abundance, and strain 2 is absent. The cross-point R with very small λ is for $0 < x < a_1/\beta_1$, in which neither strain exists in the system at equilibrium.

2.3. Invasion of a new strain

The possibility of invasion of a new strain into the population and its outcome can also be analysed from
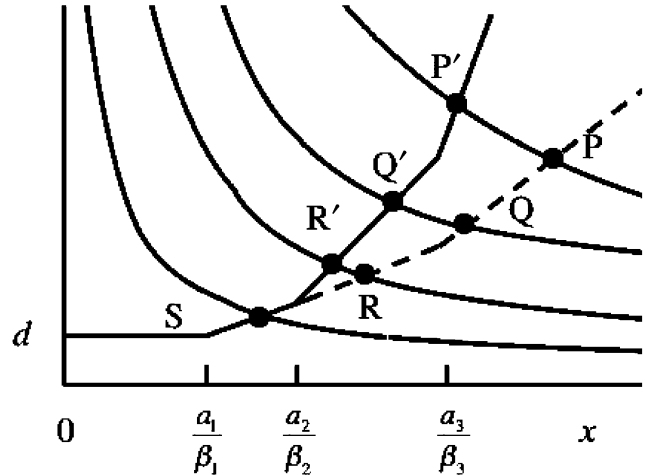

Fig. 2. Graphical representation of Eq. (3) for a population before and after the invasion of a new strain. Broken curve is for the population with strains 1 and 3. Solid curve is for the population with strain 2 is added. The explanation is the same as in Fig. 1.

the graphical representation of both sides of Eq. (3). The right-hand side of Eq (3) increases by $\psi_j(x)$. First, if the population before the invasion of a new strain j has a level of uninfected cells less than a_j/β_j, then the invasion is not successful. If instead the level of uninfected cells before the invasion is greater than a_j/β_j, then invasion by strain j is possible. As an outcome of invasion, the level of uninfected cells is always smaller than before the invasion, and the per capita loss of uninfected cells $d + \sum_{i=1}^{n} \psi_i(x)$ is always larger than before the invasion.

Fig. 2 illustrates the situation where two strains (strain 1 and strain 3) exist in the initial population, and then strain 2 invades it. The broken curve in Fig. 2 is for the population before the invasion including strains 1 and 3 only. It consists of three arcs connected by kinks. The four curves with negative slopes are λ/x for four different levels of λ. Both P and Q are the communities with two strains. Both R and S indicate a population with strain 1 only. In these equilibria, the abundance of uninfected cells satisfies $a_1/\beta_1 < x < a_3/\beta_3$, and strain 3 cannot be maintained.

Strain 2 with an intermediate value of a_2/β_2 is added to the population. Whether or not this invasion is successful, and if so what would be the outcome to the population composition can be known from the graph. A solid curve indicates the population with three strains. It consists of four arcs connected with kinks. These arcs correspond to: (1) the absence of strains, (2) strain 1 only, (3) strains 1 and 2, and (4) all three strains (strains 1–3), respectively. It has a cross-point with λ/x at P', Q', R' and S, for four different levels. At P', three strain coexist. At Q' and R' both strain 1 and species 2 coexist but strain 3 is absent. At S, only strain 1 exists.

Consider the case in which population indicated by P is realized before the invasion of strain 2. When the

strain 2 invades, the equilibrium would be shifted to P' in which all the three strains coexist. Hence, in this case the outcome of invasion is simply the addition of a new strain 2 without extinction of resident strain.

If the population before invasion is the one indicated by Q with strains 1 and 3. The outcome of the invasion of strain 2 is the one indicated by Q' in which strains 1 and 2 coexist, but strain 3 is not maintained. This implies that the invasion of strain 2 is successful, but it drives out one of the resident strain 3 to extinction—the replacement of strain 3 by strain 2 happens. The new level of uninfected cells x is too low for the strain 3 to be maintained.

If the original population is indicated by R that includes strain 1 only, the outcome of the invasion of strain 2 is given by R', including both strains 1 and 2. This is the addition of invading strain.

Finally when the original population is the one indicated by S. Strain 2 cannot invade this system, because the level of uninfected cells x is already too low for strain 2.

We can summarize these results as follows:

Proposition 1. *For model 1, any successful invasion reduces the number of uninfected cells, x, and increases the force of infection, $\sum_i \beta_i y_i$, at equilibrium.*

Note that the number of coexisting strains may not increase monotonically, because the invasion of a strain may cause the extinction of many existing residents. We also note that the total virus load $\sum_i y_i$ may decrease, but a properly weighted sum of viruses would increase all the time as stated in Proposition 1.

3. Non-cytotoxic immunity, model 2

Let us now consider the situation where the immune response decreases the rate of infection, but not the lifespan of infected cells. We have

$$\frac{\mathrm{d}}{\mathrm{d}t}x = \lambda - dx - \sum_{i=1}^{n}\frac{\beta_i x y_i}{1+\eta_i z_i}, \tag{6a}$$

$$\frac{\mathrm{d}}{\mathrm{d}t}y_i = \frac{\beta_i x y_i}{1+\eta_i z_i} - a_i y_i, \quad i=1,2,3,\ldots,n, \tag{6b}$$

$$\frac{\mathrm{d}}{\mathrm{d}t}z_i = c_i y_i - b_i z_i, \quad i=1,2,3,\ldots,n. \tag{6c}$$

This is the model studied by Regoes et al. (1998) (i.e. the case without immune impairment). The infection rate in the absence of immunity is given by β_i. Immune responses reduce the infection rate to $\beta_i/(1+\eta_i z_i)$.

Concerning the location of equilibrium, the invasion condition, and the outcome of a successful invasion, a similar argument as used for CTL model in the last

section applies to the non-CTL model Eq. (6). From Eqs. (6b) and (6c), the equilibrium abundance of virus strain i is

$$y_i(x) = \begin{cases} \dfrac{b_i}{\eta_i c_i}\left(\dfrac{\beta_i x}{a_i}-1\right) & \text{for } x > \dfrac{a_i}{\beta_i}, \\[2mm] 0 & \text{for } x \leqslant \dfrac{a_i}{\beta_i}. \end{cases} \tag{7}$$

As before, the immune activity is proportional to the pathogen abundance

$$z_i(x) = \frac{c_i y_i(x)}{b_i}.$$

At equilibrium we have

$$\lambda - dx = \sum_{i=1}^{n} a_i y_i(x). \tag{8}$$

Note that both sides of Eq. (8) are the total rate of production and death of uninfected cells, rather than per capita rates used in Eq. (3). The right-hand side of Eq. (8) is the total number of excess cell death per unit time due to the viral infection, and is called cellular virulence.

Fig. 3 illustrates the graphs of the both sides of Eq. (8). Again there is a single positive equilibrium x^* that satisfies Eq. (8). A similar result for the equilibrium, its parameter dependence, the invasibility of a new strain, and the outcome of the new strain can be discussed, in the same manner as in the last section (see caption to Fig. 3).
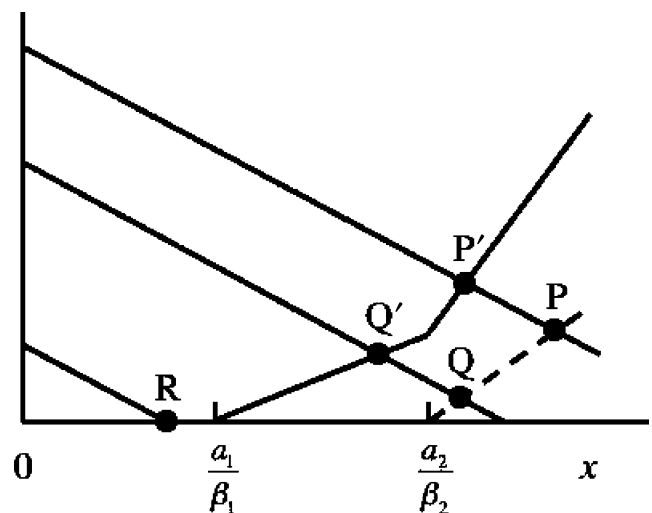

Fig. 3. Graphical representation of Eq. (8) for a population before and after the invasion of a new strain. The model is for non-CTL immunity (model 2). The lines with negative slope are for $\lambda - dx$ with different λ. Broken curve is for the population with strain 2 only; it changes to solid curves after invasion of strain 1. P and Q are the equilibria with strain 2, but R includes no strain. After invasion of strain 1, P shifts to P', in which strains 1 and 2 coexist (addition of species); Q shifts to Q', in which strain 2 is replaced by strain 1 (replacement). Strain 1 cannot invade R.

Concerning the location of the equilibrium, we can again conclude that (1) if the population before the invasion of a new strain j has the level of uninfected cells less than a_j/β_j, the invasion is not successful. (2) If instead the level of uninfected cells before the invasion is greater than a_j/β_j, invasion of strain j is successful. (3) As an outcome of invasion, the level of uninfected cells always decreases (x becomes smaller), and the total rate of new infection of cells, or the total rate of cellular virulence, $\sum_{i=1}^{n} a_i y_i(x)$, always increases. (4) The outcome of successful invasion can be accompanied by the extinction of some resident species, and this can be known from the new cross-point in the graph of both sides of Eq. (8). Hence, we have the following propositions.

Proposition 2. *For model 2, any successful invasion reduces the number of uninfected cells, x, and increases the total viral cytopathicity, $\sum_i a_i y_i$, at equilibrium.*

4. Cytotoxic immunity, model 3

Finally, we study model 3, given by

$$\frac{d}{dt}x = \lambda - dx - \sum_{i=1}^{n} \beta_i x y_i, \tag{9a}$$

$$\frac{d}{dt}y_i = (\beta_i x - a_i - p_i z_i)y_i, \quad i = 1, 2, 3, \ldots, n, \tag{9b}$$

$$\frac{d}{dt}z_i = (c_i y_i - b_i)z_i, \quad i = 1, 2, 3, \ldots, n. \tag{9c}$$

Here the immune response reduces the lifetime of infected cells, as in model 1, but the population growth rate of immune cells specific to strain i is proportional to their current number as well as the number of infected cells: the rate of immune cell production in Eq. (9c) is given by $c_i y_i z_i$ instead of $c_i y_i$ as in Eq. (1c). If viral abundance is kept constant, the immune activity shows an exponential increase in Eq. (9c), but a linear increase in Eq. (1c). Again, there is a single, globally stable equilibrium (see Appendix A).

In a similar vein as in the previous cases, we consider the equilibrium abundance of strain i and the number of specific immune cells. These values, when uninfected cell number x is given, are calculated as follows:

Case 1:

$$x < \frac{a_i}{\beta_i}, \quad y_i = 0, \quad z_i = 0. \tag{10a}$$

Case 2:

$$x = \frac{a_i}{\beta_i}, \quad 0 < y_i < \frac{b_i}{c_i}, \quad z_i = 0, \tag{10b}$$

y_i takes the value that realizes $x = a_i/\beta_i$.

Case 3:

$$x > \frac{a_i}{\beta_i}, \quad y_i = \frac{b_i}{c_i}, \quad z_i = \frac{1}{p_i}(\beta_i x - a_i). \tag{10c}$$

Again we have $\psi_i(y_i) = \beta_i y_i$ for the per capita risk of infection for uninfected cells. If we draw the relationship between $\psi_i(y_i) = \beta_i y_i$ and x, it is of a "step" shape. For x smaller than a_i/β_i, we have $\psi_i(y_i(x)) = 0$. For x greater than a_i/β_i, we have $\psi_i(y_i) = \beta_i b_i/c_i$. At the boundary between these two situations, $x = a_i/\beta_i$, the risk function can take any value between two values: $0 < \psi_i < \beta_i b_i/c_i$. Hence, ψ_i is not given as a function of x, but it should be adjusted to satisfy relationship $x = a_i/\beta_i$.

In models 1 and 2, immune activity is proportional to the pathogen abundance ($z_i = c_i y_i/b_i$). In contrast, in the current model given by Eq. (9), the immune activity level z_i is not proportional to the pathogen abundance. Even if there is some pathogen in the body, the immune activity stays zero if the pathogen abundance is lower than a threshold: $0 < y_i < b_i/c_i$ (case 2). When the pathogen abundance reaches the maximum value $y_i = b_i/c_i$, the immune activity becomes positive, and increases with the abundance of uninfected cells x, without changing the abundance of viral strain i (case 3). The number of cells infected by strain i cannot exceed the maximum value b_i/c_i, because it is suppressed to the level by the enhanced immune activity.

In the system of n viral strains and n specific immune reactions to them, the equilibrium must have either of the two types. The first is similar to the last two models. Namely the equilibrium abundance of the uninfected cells satisfies Eq. (5). It is greater than a_i/β_i of the first k strains, but is smaller than that of the remaining $n - k$ strains. From Eq. (10), number of cells infected by the first k strains are positive: $y_1^* > 0, \ldots, y_k^* > 0$, but the remaining strains are absent: $y_{k+1}^* = 0, \ldots, y_n^* = 0$. The immune reactions are positive for the first k types: $z_1^* > 0, \ldots, z_k^* > 0$, but $z_{k+1}^* = 0, \ldots, z_n^* = 0$. In addition to the equilibrium of this type, we have the second case in which the following relation holds:

$$\frac{a_1}{\beta_1} < \cdots < \frac{a_{k-1}}{\beta_{k-1}} < \frac{a_k}{\beta_k} = x^* < \frac{a_{k+1}}{\beta_{k+1}} < \cdots < \frac{a_n}{\beta_n}. \tag{11}$$

Here the equilibrium level of uninfected cells is adjusted to be exactly the same as a_k/β_k of strain k. For the first $k - 1$ strains, the number of cells infected by the strain and its immune reaction level are positive: $y_1^* > 0, \ldots, y_{k-1}^* > 0$, and $z_1^* > 0, \ldots, z_{k-1}^* > 0$. Both viral abundance and the specific immune activity are zero for the last $n-k$ strains: $y_{k+1}^* = 0, \ldots, y_n^* = 0$ and $z_{k+1}^* = 0, \ldots, z_n^* = 0$. What is quite notable is that there is one boundary strain, with positive abundance $y_k^* > 0$, but there is no immune activity specific to it: $z_k^* = 0$. For this strain, Case 2 holds at equilibrium.

If we draw the graph of per capita loss of uninfected cells, $d + \sum_{i=1}^{n} \psi_i(x)$, it is a sum of n step functions.

Broken curve in Fig. 4 is for the initial population in which only strain 2 is available. If the cross-point with the curve of λ/x has x less than the critical point a_2/β_2, as indicated by point S, no viral strain is maintained at equilibrium. Both R and Q are the cases in which strain 2 exists with positive abundance, but no immune reaction is invoked ($y_2 > 0, z_2 = 0$). In contrast for the cross-point P, the equilibrium includes strain 2 and some immune reaction to it ($y_2 > 0, z_2 > 0$).

A step function drawn in a solid line in Fig. 4 is for the case in which both strains 1 and 2 with $0 < a_1/\beta_1 < a_2/\beta_2$. Now after the invasion of strain 1, P (with strain 2 only) shifted to P' in which both strains 1 and 2 exist. Hence this invasion ends up with the addition of strain 1 without losing resident strain 2. For the population indicated by R would be shifted to R', in which resident strain 2 goes extinct and it is replaced by strain 1. The cross-point labeled S has no strain, and the invader cannot be established there because the supply rate is too slow.

What is very interesting is the equilibrium indicated by cross-point Q. Here the invasion of strain 1 occurs, but the cross-point remains the same as before. To examine this case more closely, we choose parameters satisfying $a_2 b_1/\beta_2 c_1 < \lambda < a_2 b_2/\beta_2 c_2$. Then we consider the initial population composed only of strain 2. The equilibrium is

$$x = \frac{a_2}{\beta_2}, \quad y_2 = \frac{\lambda}{a_2} - \frac{d}{\beta_2}, \quad \text{and} \quad z_2 = 0. \quad (12a)$$

In this system, strain 1 can invade the population, and two strains coexist. The new equilibrium is

$$x = \frac{a_2}{\beta_2}, \quad y_1 = \frac{b_1}{c_1}, \quad z_1 = \frac{1}{p_1}\left(\beta_1 \frac{a_2}{\beta_2} - a_1\right),$$

$$y_2 = \frac{\lambda}{a_2} - \frac{1}{\beta_2}\left[d + \frac{\beta_1 b_1}{c_1}\right], \quad \text{and} \quad z_2 = 0, \quad (12b)$$
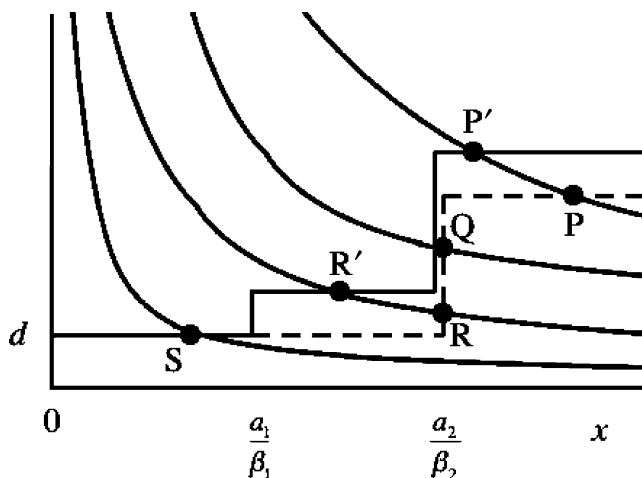

Fig. 4. Graphical representation of Eq. (3) for a population before and after the invasion of a new strain. The model is given by Eq. (9).

which has exactly the same number of uninfected cells x as before invasion of strain 1. And yet the strain composition is changed greatly. Equilibrium x does not decrease after invasion, but may remain the same as before, even if there is a considerable change in strain composition.

In this argument, we used the equilibrium values of y_i and z_i for a given x. If x were in fact a constant, the dynamics of y_i and z_i would be the same as the Lotka–Volterra predator–prey system with neutral stability—a boundary case between stability and instability, in which the system would fluctuate forever. However in the present model, x is not fixed but changes responding to y, and hence the dynamics of x, y_i and z_i make the equilibrium globally stable. In Appendix A, using a Lyapunov function, we can prove that the equilibrium obtained as above is globally stable, and that the system starting from any initial point would converge to it.

We have the following propositions:

Proposition 3. *For model* 3, *any successful invasion of a new mutant leads to a reduced or an unchanged equilibrium abundance of uninfected cells,* x. *In these two situations, the force of infection,* $\sum_{i=1}^{n} \beta_i y_i$, *increases or remains the same, respectively.*

The analysis can be applicable not only to these three models, but also a wider class of models of immune system in which the force of infection can be written as the sum of contributions of different strains: $\sum_{i=1}^{n} \psi_i(x)$, which always increases by the addition of a new strain. Addition of a mutant strain always increase this function and makes the equilibrium level of uninfected cells smaller.

5. Cross-immunity violates the trends

In the argument leading to the general decrease principle, we assumed that the equilibrium risk of infection $\psi_i(x)$ is given as a function of uninfected cell abundance x, only, irrespective of the presence of other strains. This assumption is valid for the cases of Eqs. (1), (6) and (9) in which the dynamics of the ith pathogen y_i and the corresponding immune activity z_i include only themselves and x, but are independent of the abundance of other strains nor their immune reactions (i.e. y_j, z_j, $j \neq i$). The immune reactions of different specificity interact only through the abundance of uninfected cells x, which is their common resources.

However, in general cases the immune reaction to a specific pathogen can be affected by the presence of other pathogens, named cross-immunity. In the presence of cross-immunity, the decrease in the equilibrium abundance of uninfected cells no longer holds, as illustrated by the following two examples.

Example 1. Symmetric cross-immunity in cytotoxic immunity model

Consider the following mode 1 of cytotoxic (CTL) immune activity:

$$\frac{\mathrm{d}}{\mathrm{d}t}x = \lambda - dx - \sum_{i=1}^{n} \beta_i x y_i, \tag{13a}$$

$$\frac{\mathrm{d}}{\mathrm{d}t}y_i = \left(\beta_i x - a_i - p_i \sum_{j=1}^{n} c_{ij} z_j\right) y_i,$$
$$i = 1, 2, 3, \ldots, n, \tag{13b}$$

$$\frac{\mathrm{d}}{\mathrm{d}t}z_j = \sum_{j=1}^{m} y_i c_{ij} - b_j z_j, \quad j = 1, 2, 3, \ldots, m. \tag{13c}$$

Here i distinguishes viral strains, and j indicates epitopes. z_j is the number of immune cells specific to epitope j. The number of epitopes is m, which can be different from the number of strains n. If two strains share a common epitope, the abundance of one strain stimulates the immune reaction to the epitope and affects the other strain, which causes cross-immunity. In Eq. (13), c_{ij} is the rate of stimulation of strain i to activate the immune reaction to the jth epitope. The same matrix is used in Eq. (13b), which indicates that a strain stimulating an epitope is more likely to be suppressed by the corresponding immunity.

Here we consider a case of two strains and 1 epitope ($n = 2$, $m = 1$). We choose the following parameters: $\beta_1 = \beta_2 = a_1 = a_2 = 1$, $d = 0$, $c_1 = 1$, $c_2 = 5$, $p_1 = 10$, $p_2 = 1$. We have

$$\frac{\mathrm{d}}{\mathrm{d}t}x = \lambda - (y_1 + y_2)x, \tag{14a}$$

$$\frac{\mathrm{d}}{\mathrm{d}t}y_1 = (x - 1 - 10z)y_1, \tag{14b}$$

$$\frac{\mathrm{d}}{\mathrm{d}t}y_2 = (x - 1 - 5z)y_2, \tag{14c}$$

$$\frac{\mathrm{d}}{\mathrm{d}t}z = (y_1 + 5y_2 - 1)z. \tag{14d}$$

There are equilibrium with only strain 1

$$x = \lambda, \quad y_1 = 1, \quad y_2 = 0, \quad z = \frac{\lambda - 1}{10} \tag{15a}$$

and another equilibrium with only strain 2

$$x = 5\lambda, \quad y_1 = 0, \quad y_2 = \frac{1}{5}, \quad z = \frac{5\lambda - 1}{5}. \tag{15b}$$

We assume that $\lambda > 1$. There is no equilibrium in which both strains 1 and 2 coexist. In fact comparing per capita rate of increase for strains 1 and 2, it is always the case that strain 2 has a higher rate of increase if z is positive. The equilibrium Eq. (15a) is unstable against the invasion of strain 2, and Eq. (15b) cannot be invaded by strain 1. Hence we can conclude that Eq. (15a) is invaded by mutant strain 2 which replaces strain 1. The

system converges to Eq. (15b). The evolutionary change makes the number of uninfected cells at equilibrium five times greater than before. Hence, the conjectured statement of monotonic decrease in uninfected cell number does not hold.

Example 2. Asymmetric cross-immunity in non-cytotoxic immunity.

The second examine is the model for non-cytotoxic immunity (model 2). Suppose that strain 1 does not stimulate the immunity, but has a high infection rate β, reducing the equilibrium abundance of uninfected cells effectively. Strain 2 can invade the population dominated by strain 1, but it strongly activates the immunity, which suppresses not only strain 2 but also strain 1. Due to the presence of this activated immunity, strain 1 cannot invade the population dominated by strain 2. Strain 2 has a smaller effectiveness of infecting cells than strain 1, resulting in more uninfected cells x at equilibrium. To represent the idea, we consider the following example:

$$\frac{\mathrm{d}x}{\mathrm{d}t} = \lambda - x\left(1 + \frac{y_1}{1 + z_2} + \frac{2y_2}{1 + z_2}\right), \tag{16a}$$

$$\frac{\mathrm{d}y_1}{\mathrm{d}t} = \left(\frac{x}{1 + z_2} - 1\right)y_1, \tag{16b}$$

$$\frac{\mathrm{d}y_2}{\mathrm{d}t} = \left(\frac{2x}{1 + z_2} - 1\right)y_2, \tag{16c}$$

$$\frac{\mathrm{d}z_2}{\mathrm{d}t} = y_2 - z_2. \tag{16d}$$

First the equilibrium in with strain 1 only is: $x = 1$, $y_1 = \lambda - 1$, $y_2 = 0$, $z_2 = 0$. The strain 2 can invade this equilibrium, and it replaces the resident strain 1. This is because strain 2 has always greater per capita rate of population increase than strain 1 (compare Eqs. (16b) and (16c)). The final equilibrium with strain 2 only is

$$x = \frac{\lambda + 1}{3}, \quad y_1 = 0, \quad y_2 = \frac{2\lambda - 1}{3}, \quad z_2 = \frac{2\lambda - 1}{3}.$$

The abundance of uninfected cells increases if $\lambda > 2$.

6. Discussion

Here, we show that for a wide range of models describing the dynamical interaction of viruses and the immune system, virus evolution reduces the equilibrium abundance of uninfected cells. Consider a population of different virus strains that coexist in a particular host in the presence of strain specific immune responses. If a new strain invades this population then complicated changes can occur in the composition of the virus population. A number of strains could die out as a

result, and then the diversity of viral strains in the host may decrease. The total viral load may also decrease. However, a successful invasion will reduce the equilibrium abundance of uninfected cells. This result holds in great generality. Furthermore, in specific models we show that any successful invasion will either increase the total force of infection or the total viral cytopathicity. Note that viral evolution in such systems need not increase viral load or diversity, although both of these quantities are likely to increase.

Our results do not hold for general cross-reactive immunity. In this case, it is possible that viral evolution increases the equilibrium abundance of uninfected cells, reduces viral cytopathicity and reduces the force of infection. This has important implications for a completely new approach to anti-viral therapy: persistent infections in a host individual could be combated by introducing specific strains that reduce the extent of disease and/or eliminate infection (see also, Bonhoeffer and Nowak, 1994). An ordinary form of cross-immunity is the one in which the presence of a particular antigen enhances the immune activity to other antigens, but it may impair the immune reaction, as studied by Regoes et al. (1998).

The monotonicity of evolution might be understood as analogous to the rule of resource competition in ecology. If there are n strain sustained by the same resources R, the strain with the strongest competitive advantage is the one with the smallest (Tilman, 1982). In this context, there is no possibility of stable coexistence, but the outcome of the new strain invasion always ends up with either the failure of invasion or the replacement. The successful invasion and replacement would causes the equilibrium resource level lower than the before.

In the present context, multiple strains can coexist because of the specific immune reactions. Here the strains are competitors that consume the common resources, uninfected target cells, and the presence of specific immune activities corresponds to the specific parasites or predators. Whilst these allow the stable coexistence of multiple strains, it also affect the rule of resource depletion. For this to hold, immune activity must be quite specific.

Acknowledgements

Appendix A

Here we consider the global stability of the equilibrium. The argument in the text is on the location of the equilibrium. For these to hold, we need to make sure that the equilibrium is stable. We can use Lyapunov functions to do this.

A.1. Stability of Model 1, given by Eq. (1)

Here we prove the global stability of the positive equilibrium of Eq. (1). Let x^*, y_1^*, z_i^* (for $i = 1, 2, ..., n$) are the values at the positive equilibrium. We need to distinguish the strains with positive abundance at equilibrium, and those with zero abundance. We assume $y_1^* > 0, ..., y_k^* > 0$, and $y_{k+1}^* = 0, ..., y_n^* = 0$, and also $z_1^* > 0, ..., z_k^* > 0$, and $z_{k+1}^* = 0, ..., z_n^* = 0$. Eq. (2) is rewritten as

$$\frac{d}{dt}x = \left[\lambda\left(\frac{1}{x} - \frac{1}{x^*}\right) - \sum_{i=1}^{n}\beta_i(y_i - y_i^*)\right]x, \qquad (A.1a)$$

$$\frac{d}{dt}y_i = [\beta_i(x - x^*) - p_i(z_i - z_i^*)]y_i,$$
$$i = 1, 2, 3, ..., k, \qquad (A.1b)$$

$$\frac{d}{dt}y_i = [\beta_i(x - x^*) - p_i(z_i - z_i^*)]y_i + (\beta_i x^* - a_i)y_i,$$
$$i = k+1, ..., n, \qquad (A.1c)$$

$$\frac{d}{dt}z_i = c_i(y_i - y_i^*) - b_i(z_i - z_i^*), \quad i = 1, 2, 3, ..., n. \qquad (A.1d)$$

We consider the following function:

$$V = (-x^* \ln x + x)$$
$$+ \sum_{i=1}^{n}(-y_i^* \ln y_i + y_i)$$
$$+ \sum_{i=1}^{n}\frac{p_i}{2c_i}(z_i - z_i^*)^2. \qquad (A.2)$$

Then after standard calculation, we can show that

$$\frac{dV}{dt} = -(x - x^*)^2\frac{\lambda}{xx^*}$$
$$- \sum_{i=1}^{n}\frac{p_i b_i}{c_i}(z_i - z_i^*)^2$$
$$- \sum_{i=k+1}^{n}(a_i - \beta_i x^*)y_i$$
$$\leqslant 0. \qquad (A.3)$$

Hence Eq. (A.2) monotonically decreases with time. This suggests that the equilibrium is in fact globally stable. We also can show that $V(x, y_1, ..., y_n, z_1, ..., z_n)$ has a Hessian negative definite, and has the minimum only at the equilibrium. By examining the dynamics in detail, we can show that trajectories will not stay in the set in which the equality of Eq. (A.3) holds except for the equilibrium, and $V(x, y_1, ..., y_n, z_1, ..., z_n)$ is a Lyapunov function. We can conclude that all the

trajectories converge to the equilibrium (La Salle and Lefschetz, 1961).

A.2. Stability of Model 3, given by Eq. (9)

Here we prove the global stability of the positive equilibrium of Eq. (9). Let x^*, y_i^*, z_i^* (for $i = 1, 2, \ldots, n$) are the values in the equilibrium obtained by the method given in the main text. We need to distinguish the pathogens with positive abundance at the equilibrium, and those with zero abundance. We assume $y_1^* > 0, \ldots, y_k^* > 0$, and $y_{k+1}^* = 0, \ldots, y_n^* = 0$, and also $z_1^* > 0, \ldots, z_{k-1}^* > 0$, and $z_{k+1}^* = 0, \ldots, z_n^* = 0$. However, unlike model 1 or 2, both $z_k^* > 0$ and z_k^* are possible. Eq. (9) is rewritten as

$$\frac{d}{dt} x = \left[\lambda \left(\frac{1}{x} - \frac{1}{x^*} \right) - \sum_{i=1}^{n} \beta_i (y_i - y_i^*) \right] x, \qquad (A.4a)$$

$$\frac{d}{dt} y_i = [\beta_i(x - x^*) - p_i(z_i - z_i^*)] y_i,$$
$$\text{for } i \text{ s.t. } y_i^* > 0, \qquad (A.4b)$$

$$\frac{d}{dt} y_i = [\beta_i(x - x^*) - p_i(z_i - z_i^*)] y_i$$
$$- (a_i - \beta_i x^*) y_i, \quad \text{for } i \text{ s.t. } y_i^* = 0, \qquad (A.4c)$$

$$\frac{d}{dt} z_i = c_i(y_i - y_i^*) z_i, \quad \text{for } i \text{ s.t. } z_i^* > 0, \qquad (A.4d)$$

$$\frac{d}{dt} z_i = c_i(y_i - y_i^*) z_i - (b_i - c_i y_i^*) z_i,$$
$$\text{for } i \text{ s.t. } z_i^* = 0. \qquad (A.4e)$$

The suffixes of i's for $y_i^* = 0$ and those for $z_i^* = 0$ may differ in this model, and hence the classification of strains are more complicated than Eq. (A.1). However, from the way to construct the equilibrium, we know that $a_i - \beta_i x^* > 0$ for all i s.t. $y_i^* = 0$; and $b_i - c_i y_i^* > 0$ for all i s.t. $z_i^* = 0$. Now we consider the following function:

$$V = (-x^* \ln x + x)$$

$$+ \sum_{i=1}^{n} (-y_i^* \ln y_i + y_i)$$

$$+ \sum_{i=1}^{n} \frac{p_i}{c_i} (-z_i^* \ln z_i + z_i), \qquad (A.5)$$

then we can show that using dynamics Eq. (9) the following holds:

$$\frac{dV}{dt} = -(x - x^*)^2 \frac{\lambda}{xx^*}$$

$$- \sum_{i:y_i^*=0} (a_i - \beta_i x^*) y_i$$

$$- \sum_{i:z_i^*=0} (b_i - c_i y_i^*) z_i$$

$$\leqslant 0. \qquad (A.6)$$

Hence Eq. (A.5) monotonically decreasing with time. Note that the Hessian matrix of (A.5) is negative definite everywhere, implying that the equilibrium is the global minimum. By examining the set in which the equality of (A.6) holds, we can conclude that Eq. (A.5) is the Lyapunov function of the dynamics Eq. (9), and that the equilibrium is globally stable (La Salle and Lefschetz, 1961).

References

Bittner, B., Bonhoeffer, S., Nowak, M.A., 1997. Virus load and antigenic diversity. Bull. Math. Biol. 59, 881–896.

Bonhoeffer, S., Nowak, M.A., 1994. Can live attenuated virus work as post-exposure treatment? Immunol. Tod. 16, 131–135.

Borrow, P., Lewicki, H., Wei, X., Horwitz, M.S., Peffer, N., Meyers, H., Nelson, J.A., Gairin, J.E., Hahn, B.H., Oldstone, M.B., Shaw, G.M., 1997. Antiviral pressure exerted by HIV-specific cytotoxic T lymphocytes (CTLs) during primary infection demonstrated by rapid selection of CTL escape virus. Nat. Med. 3, 205–211.

De Boer, R.J., Boerlijst, M.C., 1994. Diversity and virulence thresholds in AIDS. Proc. Natl Acad. Sci. USA 91, 544–548.

De Boer, R.J., Perelson, A.S., 1998. Target cell limited and immune control models of HIV infection: a comparison. J. Theor. Biol. 190, 201–214.

Fenyo, E.M., 1994. Antigenic variation of primate lentiviruses in humans and experimentally infected macaques. Immunol. Rev. 140, 131–146.

Gupta, S., Ferguson, N., Anderson, R., 1998. Chaos, persistence, and evolution of strain structure in antigenically diverse infectious agents. Science 280, 912–915.

Hahn, B.H., Shaw, G.M., Taylor, M.E., Redfield, R.R., Markham, P.D., Salahuddin, S.Z., Wong-Staal, F., Gallo, R.C., Parks, E.S., Parks, W.P., 1986. Genetic variation in HTLV-III/LAV over time in patients with AIDS or at risk for AIDS. Science 232, 1548–1553.

Holmes, E.C., Zhang, L.Q., Simmonds, P., Ludlam, C.A., Leigh Brown, A.J., 1992. Convergent and divergent sequence evolution in the surface envelope glycoprotein of HIV-1 within a single infected patient. Proc. Natl Acad. Sci. USA 89, 4835–4839.

La Salle, J., Lefschetz, S., 1961. Stability by Liapunov's Method, with Applications. Academic Press, New York.

Levin, B.R., Bull, J.J., 1994. Short-sighted evolution and the virulence of pathogenic microorganisms. Trends Microbiol. 2, 76–81.

Levin, S.A., Grenfell, B., Hastings, A., Perelson, A.S., 1997. Mathematical and computational challenges in population biology and ecosystem science. Science 275, 334–343.

Levin, B.R., Lipsitch, M., Bonhoeffer, S., 1999. Population biology, evolution, and infectious disease: convergence and synthesis. Science 283, 806–809.

McLean, A.R., Nowak, M.A., 1992. Interactions between HIV and other pathogens. J. Theor. Biol. 155, 69–86.

McMichael, A.J., Phillips, R.E., 1997. Escape of human immunodeficiency virus from immune control. Ann. Rev. Immunol. 15, 271–296.

Nowak, M.A., Bangham, C.R.M., 1996. Population dynamics of immune responses to persistent viruses. Science 272, 74–79.

Nowak, M.A., May, R., 2000. Virus Dynamics. Oxford University Press, Oxford.

Nowak, M.A., Anderson, R.M., McLean, A.R., Wolfs, T.F.W., Goudsmit, J., May, R.M., 1991. Antigenic diversity thresholds and the development of AIDS. Science 254, 963–969.

Nowak, M.A., May, R.M., Sigmund, K., 1995a. Immune-responses against multiple epitopes. J. Theor. Biol. 175, 325–353.

Nowak, M.A., May, R.M., Phillips, R.E., Rowland-Jones, S., Lalloo, D.G., McAdams, S., Klenerman, P., Kope, B., Sigmund, K., Bangham, C.R.M., McMichael, A.J., 1995b. Antigenic oscillations and shifting immunodominance in HIV-1 infections. Nature 375, 606–611.

Perelson, A.S., 1989. Modeling the interaction of HIV with the immune system. In: Castillo–Chavez, C. (Ed.), Mathematical and Statistical Approaches to AIDS Epidemiology. Lecture Notes in Biomathematics, Vol. 83. Springer, New York, pp. 350–370.

Perelson, A.S., Weisbuch, G., 1997. Immunology for physicists. Rev. Mod. Phys. 69, 1219–1267.

Perelson, A.S., Kirschner, D.E., De Boer, R., 1993. Dynamics of HIV infection of CD4+ T cells. Math. Biosci. 114, 81–125.

Regoes, R.R., Wodarz, D., Nowak, M.A., 1998. Virus dynamics: the effect of target cell limitation and immune responses on virus evolution. J. Theor. Biol. 191, 451–462.

Sasaki, A., 1994. Evolution of antigen drift/switching: continuously evading pathogens. J. Theor. Biol. 168, 291–308.

Tilman, D., 1982. Resource Competition and Community Structure. Monographs in Population Biology. Princeton University Press, Princeton, NJ 296pp.

Wahl, L.M., Bittner, B., Nowak, M.A., 2000. Immunological transitions in response to antigenic mutation during viral infection. Int. Immunol. 12, 1371–1380.

Wei, X., Decker, J.M., Wang, S., Hui, H., Kappes, J.C., Xiaoyun, W., Salazar, J.F., Salazar, M.G., Kilby, J.M., Saag, M.S., Komarova, N.L., Nowak, M.A., Hahn, B.H., Kwong, P.D., Shaw, G.M., 2003. Antibody neutralization and escape by HIV-1. Nature 422, 309–312.

Wodarz, D., Lloyd, A.L., Jansen, V.A.A., Nowak, M.A., 1999. Dynamics of macrophage and T-cell infection by HIV. J. Theor. Biol. 196, 101–113.