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Virus evolution within patients increases pathogenicity

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Abstract

Viruses like the human immunodeficiency virus (HIV), the hepatitis B virus (HBV), the hepatitis C virus (HCV) and many others undergo numerous rounds of inaccurate reproduction within an infected host. The resulting viral quasispecies is heterogeneous and sensitive to any selection pressure. Here we extend earlier work by showing that for a wide class of models describing the interaction between the virus population and the immune system, virus evolution has a well-defined direction toward increased pathogenicity. In particular, we study virus-induced impairment of the immune response and certain cross-reactive stimulation of specific immune responses. For eight different mathematical models, we show that virus evolution reduces the equilibrium abundance of uninfected cells and increases the rate at which uninfected cells are infected. Thus, in general, virus evolution makes things worse. An idea for combating HIV infection, however, is constructing a virus mutant that could outcompete the existing infection without being pathogenic itself.

Keywords: Immune selection; Directional evolution; HIV; Immune impairment; Cross-immunity

1. Introduction

Virus evolution as mechanism of disease progression in human immunodeficiency virus (HIV) infection has been a common theme for the last 15 years (Nowak et al., 1990, 1991, 1995; De Boer and Boerlijst, 1994; Sasaki, 1994; Regoes et al., 1998; Nowak and May, 2000; Iwasa et al., 2004). The basic theoretical idea is that a rapidly replicating HIV quasispecies establishes a permanent infection that goes through many viral generations within a short time. The immune system mounts responses to various viral epitopes, but the virus population escapes from many such responses by generating mutants that are not recognized in particular epitopes. During the cause of infection, virus evolution proceeds toward increasing pathogenicity by reducing immune control and increasing viral abundance. There is ample experimental evidence for this mode of disease progression: (i) the HIV population in any one infected host is fairly homogeneous during primary phase and heterogeneous afterwards (Nowak et al., 1991; Holmes et al., 1992; Bonhoeffer and Nowak, 1994; Bonhoeffer et al., 1995; Wolinsky et al., 1996); (ii) the average life cycle of HIV during the asymptomatic phase of infection is short, about 1–2 days (Wei et al., 1995; Ho et al., 1995; Perelson et al., 1996; Bonhoeffer et al., 1997); hence the HIV quasispecies can rapidly respond to selection pressure; (iii) HIV escapes from B-cell- and T-cell-mediated immune responses (Phillips et al., 1991; Nowak et al., 1995; Borrow et al., 1997; Goulder et al., 2001; Wei et al., 2003; Addo et al., 2003).

In a previous paper (Iwasa et al., 2004), we analysed models for the evolutionary dynamics of virus or other infectious agents within a host. We mathematically examined how the invasion of a new strain affects the composition and diversity of viral population in a host.

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We showed that—under strain specific immunity—the equilibrium abundance of uninfected cells always 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. However, we could also illustrate by two examples that 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.

In the present paper as a sequel to Iwasa et al. (2004), we show that, for some classes of models with virus-induced impairment of immune responses or cross-reactive immune stimulations, the same directional evolutionary trends hold as in the models without cross-immunity. These classes includes several different models studied in Regoes et al. (1998) with small modifications. We can also prove that these hold for models with cross-immunity, in which the abundance of a strain would enhance (rather than impair) the immune activities on other strains. We will discuss different functional forms of immune activation (De Boer and Perelson, 1995, 1998).

2. Model of immune impairment

We consider the following situation. Initially there are a certain number of strains coexisting in the system at equilibrium. Then a strain which is currently absent invades the system with a very small initial abundance. It may increase or decrease. If it increases, it may be included as an additional strain, increasing strain diversity. Alternatively, the invasion of a new strain may cause the extinction of one or more of the resident strains.

2.1. Model 1: cross-reactive immune impairment

Consider the following model of the virus–immunity dynamics:

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

$$\frac{dy_i}{dt} = (\beta_i x - a_i - p_i z_i) y_i, \tag{1b}$$

$$\frac{dz_i}{dt} = c_i y_i - b_i z_i \left(1 + u \sum_{j=1}^{n} \beta_j y_j\right), \tag{1c}$$

where x is the number of uninfected cells, y_i is the number of cells infected by viral strain i, and z_i is the intensity of immune reaction specific to viral strain i. Eq. (1a) indicates that the uninfected cells are supplied at a constant rate λ but will decay at rate d. The third term of Eq. (1a) is the rate at which uninfected cells become infected at a rate proportional to y_i. β_i is the rate of transmission of strain i. Here we do not model the dynamics of free viral particles explicitly, but we simply assume that the number of free viral particles would be proportional to the number of cells infected. This is valid as the number of free vial particles would change at a much shorter time-scales than those variables in Eq. (1) (Perelson et al., 1996; Ho et al., 1995; Regoes et al., 1998; Iwasa et al., 2004).

Eq. (1c) indicates that the immunity specific to viral strain i is activated at a rate proportional to the abundance of uninfected cells y_i and hence proportional to the number of free viral particles. The decay rate is not a constant but an increasing function of the total abundance of virus, $b_i(1 + u\sum_{j=1}^{n} \beta_j y_j)$. This assumption represents the effect that any viral strain impairs to some degree immune activity against other viral strains.

Now we consider the following situation. Initially there are one or a few strains in the host body, which may be maintained at equilibrium. Then a new strain is created by the mutation of an existing strain. The new invader is initially very rare, and it may just go extinct. But it may increase its abundance. If so, it may coexist with the resident strains, but it also may drive some of the resident strains to extinction, realizing a new equilibrium with a fewer coexisting strains. Over many years, a number of events of invasion of new strains occur possibly followed by change in the strain composition.

We ask whether or not there is any systematic difference between the equilibrium after the invasion of a new strain followed by the replacement and the one before the invasion. If so, we may be able to identify a unidirectional evolutionary trend of virus controlled by immune selection. Iwasa et al. (2004) examined the model Eq. (1) with $u = 0$, and proved mathematically that the successful invasion of a new strain always decreases the equilibrium abundance of uninfected cells, and always increases the total force of infection $\sum_{i=1}^{n} \beta_i y_i$. Based on a similar logic, we can prove the same evolutionary trend to hold for the model given by Eq. (1), which includes cross-reactive immune impairment ($u > 0$). To clarify, we state this as the following proposition:

Proposition 1. *In the model given by Eq.* (1)*, after a new strain succeeds in invasion, the equilibrium abundance of uninfected cells x always becomes less than the level before the invasion. The equilibrium total force of infection $\sum_{i=1}^{n} \beta_i y_i$ always increases after such an evolutionary change.*

In Iwasa et al. (2004), the global stability of the equilibrium is proved using a Lyapunov function for the system with $u = 0$ in Eq. (1). However, it is not possible

to construct a Lyapunov function for the general case of $u>0$. Here we simply assume that the equilibrium is stable, and if the new equilibrium is created after invasion, the system converges to it. This assumption is consistent with all the results of numerical analyses we have done. We examine the change in the equilibrium caused by the invasion of a novel strain, given the global stability.

Let $Y=\sum_{i=1}^{n}\beta_i y_i$. From Eqs. (1b) and (1c), we can express the equilibrium abundance of infected cells and its specific immune activity as functions of x and Y:

$$y_i=\frac{b_i}{c_i p_i}(1+uY)[\beta_i x-a_i]_+, \tag{2a}$$

$$z_i=\frac{1}{p_i}[\beta_i x-a_i]_+, \tag{2b}$$

where $[x]_+=x$, for $x>0$, and $[x]_+=0$, for $x\leqslant 0$. Hence the equilibrium abundance of infected cells is a function of uninfected cell abundance x, and the total intensity of immune reaction Y. Combining $Y=\sum_{j=1}^{n}\beta_i y_i$ with Eq. (2a), we have

$$\frac{Y}{1+uY}=\sum_{i=1}^{n}\frac{\beta_i b_i}{c_i p_i}[\beta_i x-a_i]_+ \tag{3}$$

at equilibrium. From, Eq. (2a), y_i is zero for$x\leqslant a_i/\beta_i$, but is positive and an increasing function of x for $x>a_i/\beta_i$. The minimum level of uninfected cells that is needed to sustain virus strain i is by a_i/β_i. On the other hand, Eq. (1a) indicates that $Y=(\lambda/x)-d$ holds at equilibrium. Hence, we have

$$\frac{Y}{1+uY}=\frac{\lambda-dx}{u\lambda+(1-ud)x}. \tag{4}$$

The right-hand side of Eq. (3) is a sum of increasing functions, and hence it is also an increasing function of x. In contrast Eq. (4) is a decreasing function of x. It is equal to $1/u$ at $x=0$, and to 0 at $x=\lambda/d$. Hence, there is always a single positive solution x^* at which Eq. (3) is equal to Eq. (4). x^* is the equilibrium number of uninfected cells.

Fig. 1 plotted Eq. (3) and Eq. (4), in which the horizontal axis is x, and the vertical axis is $Y/(1+uY)$. Eq. (3) is a piecewise straight line with a positive slope. Eq. (4) appears as a curve with a negative slope. Using graphical representation of Eqs. (3) and (4), we can show the equilibrium solution x^*, its parameter dependence, the condition for invasibility of a new strain, and the outcome of a successful invasion.

The possibility of invasion of a new strain into the population and its outcome can be known from figures such as Fig. 1. After invasion, Eq. (3) increases by $\beta_j y_j(x)$. If the population before the invasion of strain j has a level of uninfected cells less than a_j/β_j, the invasion is not successful. If instead the level of uninfected cells before the invasion is greater than

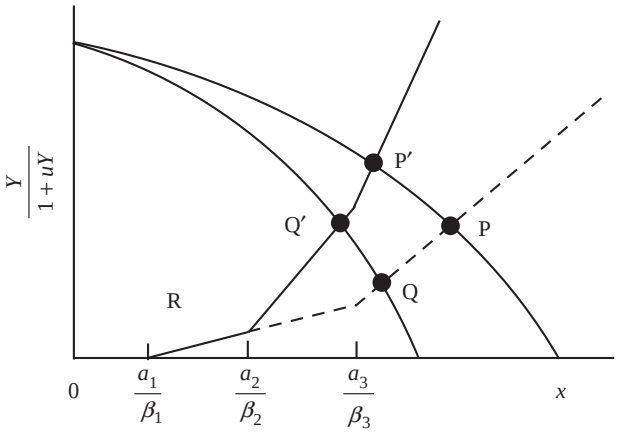

Fig. 1. Graphical representation of Eqs. (3) and (4) for a population before and after the invasion of a new strain. The model is given by Eq. (1). Broken curve is for the population with strains 1 and 3. Solid curve for the population with strain 2 is added. Three arcs connected by kink is Eq. (3), indicating per capita risk of uninfected cells. The curves with negative slopes are Eq. (4), with different λ. Horizontal axis is the abundance of uninfected cells x. P and Q are for the equilibrium corresponding to different values of λ, both including two strains. After invasion of strain 2, Eq. (3) would change to a solid curve and the equilibrium would shift to P′ and Q′. All three strains coexist in P′. But strain 3 is replaced by strain 2 in Q′.

a_j/β_j, then strain j can increase. As an outcome of invasion, the cross-point would shift to above, and hence toward left. Hence the level of uninfected cells x becomes smaller than before the invasion, and $Y/(1+uY)$ is larger than before the invasion, and hence $Y=\sum_{i=1}^{n}\beta_i y_i$ should increase.

Fig. 1 illustrates the situation where two strains (strains 1 and 3) exist in the initial population, and then strain 2 invades it ($a_1/\beta_1<a_2/\beta_2<a_3/\beta_3$). The broken curve in Fig. 1 is for the population before the invasion including strains 1 and 3 only. It consists of three arcs connected by kinks. Two curves with negative slopes are Eq. (4) for different levels of λ. Both P and Q are the communities with two strains. Strain 2 with an intermediate value of a_2/β_2 is added to the population.

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 because the new cross-point is larger than a_i/β_i of these strains. In this case the outcome of invasion is simply the addition of a new strain 2 without extinction of the resident strains. 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 strain 3 to extinction—the replacement of strain 3 by strain 2 occurs. The new level of uninfected cells x is too low for the strain 3 to be maintained.

From these arguments, we can see the following: (1) The invasibility of a novel strain is determined by whether or not the equilibrium abundance of uninfected cells before the invasion is greater than a_i/β_i (invasible if $x^*_{before} > a_i/\beta_i$; not invasible otherwise). (2) As the result of a successful invasion, the location of the equilibrium would move upward and the abundance of uninfected cells downward ($x^*_{after} < x^*_{before}$). (3) If x^* moves less than the threshold for some resident species $x^*_{after} > a_j/\beta_j$, they should go extinct, while those species would remain positive if $x^*_{after} < a_j/\beta_j$ is satisfied. As a result of invasion, the equilibrium intensity of immune reaction Y increases, but the number of strains maintained in the system may increase or remain unchanged or decrease.

A more rigorous proof will be given in a later section. Before giving a formal proof, we would like to explain several different models of cross-immunity in which a similar evolutionary trend holds.

3. Alternative models of immune system interaction

In this section, we explain several alternative models in which immune reaction to different strains interact, for which we will later prove a statement similar to Proposition 1.

3.1. Model 2: same as model 1 but with a proportional activation term

We may consider the following dynamics of immune cells:

$$\frac{dz_i}{dt} = \left(c_i y_i - b_i \left(1 + u \sum_{j=1}^{n} \beta_j y_j \right) \right) z_i. \tag{5}$$

In this model, immune cells that are specific against virus mutant i are activated at a rate, $c_i y_i z_i$, which is proportional to the product of virus abundance and immune cell abundance (Nowak and Bangham, 1996). Müller et al. (2001) discussed the difference in behavior between the immune dynamics with Eq. (1c) and those with Eq. (5). The second term within the brackets of Eq. (5) implies that the mortality of immune cells increases with general activity of viral load ($u\sum_{i=1}^{n}\beta_i y_i$). In the absence of this effect ($u=0$), Eq. (5) is the same as model 3 in Iwasa et al. (2004). It is also similar to a model by Regoes et al. (1998), but they differ in two points: First, the impairment of immune reaction was assumed as a function of $\sum_{i=1}^{n} y_i$ in Regoes et al., but it is a function of $\sum_{i=1}^{n}\beta_i y_i$ in this model. Second, the parameters a_i, p_i, c_i were assumed common among strains (no suffix) in Regoes et al., but they can differ between strains in Eq. (5).

The equilibrium abundance of y_i can be expressed as a function of uninfected cell number x and the intensity of total immunity Y:

$$Case\,1: \quad \text{for} \quad x > \frac{a_i}{\beta_i}, \quad y_i = \frac{b_i}{c_i}(1+uY), \quad z_i = \frac{\beta_i}{p_i}\left(x - \frac{a_i}{\beta_i}\right), \tag{6a}$$

$$Case\,2: \quad \text{for} \quad x = \frac{a_i}{\beta_i}, \quad 0 < y_i < \frac{b_i}{c_i}(1+uY), \quad z_i = 0, \tag{6b}$$

$$Case\,3: \quad \text{for} \quad x < \frac{a_i}{\beta_i}, \quad y_i = z_i = 0. \tag{6c}$$

The graphical representation is useful. On a (x, y_i)-plane, with fixed Y, equilibrium condition Eq. (6) is represented as three straight lines with a step-like form. y_i is a continuous function of x except for a single point $x = a_i/\beta_i$, at which y_i can take any value within an interval $0 < y_i < (b_i/c_i)(1+uY)$, which appears as a vertical line. Fig. 2 illustrates an example. Eq. (3) now becomes

$$\frac{Y}{1+uY} = \sum_{i=1}^{n} \frac{\beta_i b_i}{c_i} H\left[x - \frac{a_i}{\beta_i}\right]_+, \tag{7}$$

where $H[x] = 1$, for $x \geqslant 0$ and $H[x] = 0$, for $x < 0$ is a Heaviside function. Eq. (7) can be used except for a_i/β_i ($i = 1, 2, \ldots, n$), at which one of y_i is discontinuous. When the right-hand side is discontinuous, we can interpret Eq. (7) as indicating that $Y/(1+uY)$ is between the limit from below and the limit from above of the right-hand side.

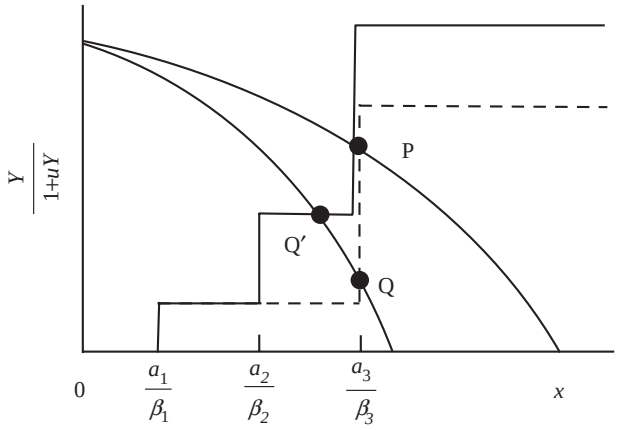

Fig. 2. Graphical representation of Eqs. (7) and (4) for a population before and after the invasion of a new strain. The model is given by Eq. (1a), (1b) and (5). Eq. (7) is a step-like function. Broken curve is for the population with strain 1 and strain 3. Solid curve for the population with strain 2 is added. The curves with negative slopes are Eq. (4), with different λ. Horizontal axis is the abundance of uninfected cells x. P and Q are for the equilibrium corresponding to different values of λ, both including two strains. After invasion of strain 2, Eq. (7) would change to a solid curve. The equilibrium P remains the same on this graph, but now includes three strains. But the uninfected cell number (horizontal axis x) does not change. In contrast Q will shift to Q′, and the strain 3 is replaced by strain 2 and the equilibrium number of uninfected cell x decreases (moves toward left) after invasion.

We assume that species differ in discontinuous points (a_i/β_i). Then there is at most one species that might cross the curve if Eq. (4) and vertical line of $x = a_i/\beta_i$, all the other species are either $x > a_i/\beta_i$ or $x < a_i/\beta_i$ at equilibrium. This makes a slight modification to Proposition 1. There can be the situation in which a new strain invades successfully and replace the resident, and yet the abundance of uninfected cells x remains exactly the same as before. Graphical representation of Eqs. (7) and (4) is shown in Fig. 2. Here equilibrium P did not change, and the equilibrium number of uninfected cells (x^*) remains the same as before. But a new strain is added without extinction of the residents. In contrast, equilibrium Q would shift to Q′ after the invasion of strain 2, which causes the extinction of strain 3 and x^* becomes smaller than before. Note that a similar situation was discussed in Iwasa et al. (2004). However, the equilibrium abundance of the uninfected cells should not increase after a successful invasion, it either decreases or remains unchanged. As a result, the value of $Y = \sum_{i=1}^{n} \beta_i y_i$ also either increases or remains unchanged after a successful invasion, respectively. We summarize the result as follows:

Proposition 2. *If the invasion of a new strain is successful, the equilibrium abundance of uninfected cells x never increases in the evolutionary change. It either decreases or remains the same as before. The equilibrium total force of infection $\sum_{i=1}^{n} \beta_i y_i$ either increases or remains the same as before, respectively.*

3.2. Model 3: impairment of immune cell activation

Regoes et al. (1998) also consider the case in which the immune system impairment appear as a factor reducing the rate of immune activation:

$$\frac{dz_i}{dt} = \left(\frac{c_i y_i}{1 + u \sum_{j=1}^{n} \beta_j y_j} - b_i \right) z_i. \tag{8}$$

In this model, all virus mutants contribute with different efficiency, β_j, to impairment of immune cell activation. For this model too, we can prove Proposition 2.

3.3. Model 4: cross-reactive immune activation

In all the models of interaction between immune systems to different strains so far, the presence of a strain impairs the immune reaction of other strains. This may be plausible for HIV infection because infection of one strain would impair the general immune system.

A common way of interaction between different immune reactions is cross-immunity, in which an antigen stimulates the immune reaction of other antigens that are similar to the original one. To represent this, we consider

$$\frac{dz_i}{dt} = c_i y_i \left(1 + u \sum_{j=1}^{n} \beta_j y_j \right) - b_i z_i. \tag{9}$$

Here, the presence of any strain would reduce the equilibrium abundance of all the other strains. For dynamics with Eq. (1a), (1b), and Eq. (9), Proposition 1 holds. In fact, as we show later, the proof of the proposition is easier for cross-immunity models than the models with immune impairment.

3.4. Model 5: cross-immunity with an alternative form

We can also consider the following form:

$$\frac{dz_i}{dt} = \left(c_i y_i \left(1 + u \sum_{j=1}^{n} \beta_j y_j \right) - b_i \right) z_i, \tag{10}$$

which is an alternative form of cross-immunity. For model with Eqs. (1a), (1b), and (10), we can prove Proposition 2.

4. Proof of directional evolution

To prove the directionality of the evolutionary process, as stated in Propositions 1 and 2, we consider the following general model in which immune reaction to different strains interact. Let $Y = \sum_{i \in A} \beta_i y_i$:

$$\frac{dx}{dt} = \lambda - dx - xY, \tag{11a}$$

$$\frac{dy_i}{dt} = y_i f_i(x, y_i, Y, z_i), \quad i = 1, 2, \ldots, n, \tag{11b}$$

$$\frac{dz_i}{dt} = g_i(x, y_i, Y, z_i), \quad i = 1, 2, \ldots, n. \tag{11c}$$

Let A be a set of strains ($A \subset \{1, 2, 3, \ldots, n\}$). Suppose there is an equilibrium formed by a group of strains in set A. Let x^* and Y^* be the equilibrium number of uninfected cells and the total force of immunity. We further assume that, starting from any point in which all the strains in A have a positive abundance, it will converge to the equilibrium (i.e. it is globally stable).

From the equilibrium condition of the dynamics equations (11b) and (11c), we can calculate y_i and z_i as a function of x and Y. In the situation for Proposition 1 to hold, such as the model given by Eq. (1), the equilibrium is a continuous function of x and Y. Here we first concentrate on such a situation (the cases in which y_i is a step function of x will be handled later). We denote the equilibrium abundance of cells infected by strain i by

$$y_i = \phi_i(x, Y), \tag{12}$$

which is calculated from Eqs. (11b) and (11c). In the equilibrium of the whole system Eq. (11), we have:

$$Y^* = \sum_{i\in A} \beta_i \phi_i(x^*,\ Y^*) \tag{13}$$

from the definition of Y. From Eq. (11a), we also have

$$Y^* = \frac{\lambda}{x^*} - d \tag{14}$$

at equilibrium.

Strain i has a positive abundance at equilibrium if x^* is greater than a_i/β_i, the minimum x for strain i to maintain. If the level of x^* is too high, some of the strains in set A may go extinct in the equilibrium. We have

Strain i has a positive abundance at equilibrium, if

$$\phi_i(x^*, Y^*) > 0. \tag{15a}$$

Strain i is absent at equilibrium, if

$$\phi_i(x^*, Y^*) = 0. \tag{15b}$$

In a similar manner, we can express the invasion condition in terms of ϕ. When a strain k which is not in A invades the equilibrium, whether or not it increases can be judged by the sign of $\phi_k(x^*,\ Y^*)$:

Strain k can invade the equilibrium, if

$$\phi_k(x^*,\ Y^*) > 0, \tag{16a}$$

Strain k fails to invade the equilibrium, if

$$\phi_k(x^*,\ Y^*) = 0 \tag{16b}$$

To discuss the outcome of a successful invasion, we assume the following two conditions:

Condition 1: $\phi_i(x,\ Y)(1/Y)$ is a decreasing function of Y if $\phi_i(x,\ \ Y) > 0$.

Condition 2: $\phi_i(x,\ \ Y)$ is continuous and non-increasing function of x.

In Appendix A, we can prove the following Theorem 1.

Theorem 1. *If Conditions* 1 *and* 2 *are satisfied, after a successful invasion of a strain, the equilibrium abundance of uninfected cells x becomes smaller than the level before the invasion. The total rate of infection,* $\sum_{i\in A}\beta_i y_i x$, *increases by invasion.*

Note that the increase in $\sum_{i\in A}\beta_i y_i x$ implies the increase of per capita rate of infection $Y = \sum_{i\in A}\beta_i y_i$, because x decreases by the invasion. Hence from Theorem 1, we can conclude Proposition 1. Eqs. (1c) and (9) satisfy the conditions above, and hence Proposition 1 holds (see Appendix B).

4.1. When equilibrium y_i is a step function of x

For the model Eq. (1a), (1b) combined with immunity dynamics given by Eqs. (5), (8), or (10), y_i is not a continuous function of x, and hence Condition 2 is not satisfied. However y_i is expressed as Eq. (12) except for a single point $x = a_i/\beta_i$, at which y_i is not specified but takes any value between the maximum and the minimum, exemplified by Eq. (6b). We here assume that a_i/β_i differ between species. At $x = a_i/\beta_i$ $(i = 1, 2, \ldots, n)$, the right hand of Eq. (13) is discontinuous. Then, we use the following inequality instead of Eq. (13):

$$\sum_{i\in A} \beta_i \phi_i(x-0,\ Y) \leqslant Y \leqslant \sum_{i\in A} \beta_i \phi_i(x+0,\ Y). \tag{17}$$

We summarize these as follows:

Condition 3: $\phi_i(x, Y)$ is continuous and non-increasing function of x except for a single point $x = a_i/\beta_i$, in which it is not defined. We have $\phi_i(x, Y) = 0$ for $x < a_i/\beta_i$, and $\phi_i(x, Y) > 0$ for $x > a_i/\beta_i$. At $x = a_i/\beta_i$, we have Eq. (17).

In Appendix A, we can prove the following Theorem 2.

Theorem 2. *If Conditions* 1 *and* 3 *are satisfied, after a successful invasion of one or more strains, the equilibrium abundance of uninfected cells x either decreases from the level before the invasion or remains the same. The equilibrium rate of infection,* $\sum_{i\in A}\beta_i y_i x$, *increases or remain the same, respectively.*

In Appendix B, we can show that these conditions are met for the models with Eqs. (1a) and (1b), together with the immunity dynamics given by Eqs. (5), (8), or (10). For these models, Theorem 2 holds, and hence Proposition 2 holds, because the increase in $Y = \sum_{i\in A}\beta_i y_i$ is derived from the increase in $\sum_{i\in A}\beta_i y_i x$.

5. Target cells are helper T-cells

HIV infects $CD4^+$ T helper cells. By depleting this target cell population, HIV impairs immune responses. In this section, we therefore assume that uninfected target cells, x, are needed for immune activation (Wodarz et al., 1999; Wodarz and Nowak, 2000; Wahl et al., 2000). We consider models in which the dynamics of specific immune cells depends directly on the number of uninfected cells. Suppose immune activation requires the presence of a sufficiently many helper T-cells in the tissue but the shortage of uninfected helper T would cause the general decrease in the immune activity for all the antigens. This can be expressed as the immune activation rate dependent directly on the uninfected cell number x.

5.1. Model 6

$$\frac{dz_i}{dt} = z_i(c_i y_i x - b_i), \quad i = 1, 2, \ldots, n. \tag{18}$$

In Eq. (18) the stimulation of immune reaction is proportional to the abundance of uninfected cells x. This was called "target cell dependence in immune activation" by Regoes et al. (1998). If a strain is abundant, it infects and reduces uninfected cell number x, which causes the decrease of the immune activation for all the other strains. Hence Regoes et al. regarded this as a way of introducing immune impairment, and also called it "indirect impairment model". We can prove that, for the model with immune dynamics Eq. (18), Proposition 2 holds.

We may also think the system in which Eq. (18) is replaced by the following:

5.2. Model 7

$$\frac{dz_i}{dt} = c_i y_i x - b_i z_i, \quad i = 1, 2, \ldots, n. \tag{19}$$

The model, given by Eqs. (1a), (1b) and (19), satisfies the condition for Theorem 1, and hence we have Proposition 1. The equilibrium abundance of uninfected cells decreases and the $Y = \sum_{i\in A}\beta_i y_i$ increases after a successful invasion of a mutant.

5.3. Bistability

In contrast, consider the case in which the target cell dependence is of impairment type, and the degree of the dependence is stronger than the one assumed by Eq. (18). For example,

$$\frac{dz_i}{dt} = z_i(c_i y_i x^2 - b_i), \quad i = 1, 2, \ldots, n, \tag{20}$$

instead of Eq. (18). The equilibrium number of cells infected by strain i is:

$$y_i = \begin{cases} \frac{b_i}{x^2 c_i} & \text{for } x > a_i/\beta_i, \\ 0 & \text{for } x < a_i/\beta_i. \end{cases} \tag{21}$$

The equilibrium is determined by a solution of the following equality:

$$\lambda - dx = \frac{1}{x}\left(\frac{\beta_1 b_1}{c_1} H\left[x - \frac{a_1}{\beta_1}\right] + \frac{\beta_2 b_2}{c_2} H\left[x - \frac{a_2}{\beta_2}\right]\right), \tag{22}$$

where H is the Heaviside function. In the case illustrated in Fig. 3, there are three equilibria—the one in the middle is unstable, and the smallest possible and the largest possible equilibria are both stable. Hence the model constituting Eqs. (1a), (1b), and (20) is bistable. This makes global stability impossible. Note that bistability in immune system due to a different mechanism was reported (Altes et al., 2003).

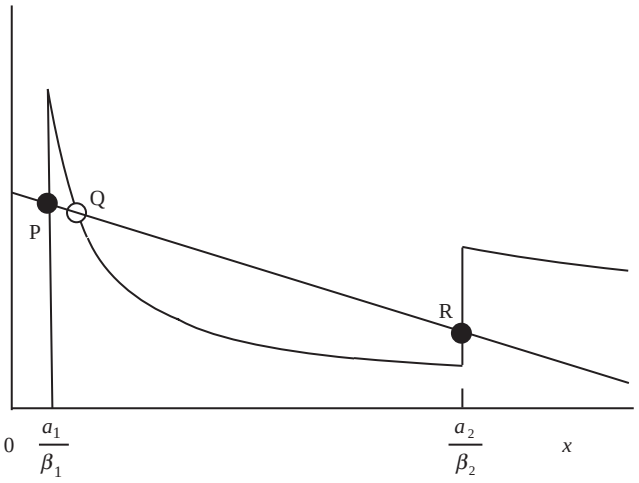

Fig. 3. Graphical representation of both sides of Eq. (22), in which cross-points are for the equilibria of dynamics Eqs. (1a), (1b), and (20). There are three equilibria. Numerical analysis shows that P and R are locally stable and Q with an intermediate x^* is unstable. The system is bistable, and the evolutionary trends suggested by Propositions 1 and 2 do not hold for this system.

6. Discussion

In this paper, we studied the evolution of virus within a patient by analysing a series of models for the dynamics of multiple strains of virus and the immune activities of the host corresponding to those strains. In all of these, the immune activities to different antigens interact. We study both the case in which immune reaction to an antigen impairs the immune reaction to other antigens and the case in which the presence of an antigen stimulates the immune activity to other antigens (cross-immunity). In all the models studied in the present paper, the directional trends of virus evolution is proved, which were shown previously for the models without cross-immunity (Iwasa et al., 2004).

The result suggests that the equilibrium abundance of uninfected cells decreases monotonically in the viral evolution occurring within a host if controlled by immune selection. It also suggests that the total force of infection increases monotonically with the evolutionary changes of viral strain composition. The strain diversity may increase and the mean virulence of the virus may increase statistically, but the two tendencies we proved in the twin paper and the present one are the changes that always occur in those directions.

Regoes et al. (1998) studied by computer simulation of several different models in which the presence of a virus strain impair or suppress the immune reaction on other strains. For all the models studied by Regoes et al., we study a slightly modified version in the present paper. The modification is on the assumption of impairment function—the rate of immune activation or decay is a function of the total number of infected

cells ($\sum_{i=1}^{n} y_i$) in Regoes et al., but the total force of infection ($\sum_{i=1}^{n} \beta_i y_i$) in the present paper. In addition, several parameters fixed by Regoes et al. can differ between strains in this paper.

Although Regoes et al. (1998) focused the case with immunity impairment type, we can extend our result to the case with cross-immunity—in which the presence of one strain activates, rather than impairs, the immune reaction to other strains. When cross-immunity is at work, the increase of general viral abundance should reduce the increase rate of each viral strain, and hence $y_i = \phi_i(x, Y)$ is likely to be a decreasing function of Y. Hence Condition 1 is likely to satisfy cross-immunity models. In contrast, models with immune impairment has $y_i = \phi_i(x, Y)$ an increasing function of Y, as exemplified by Eqs. (2a) and (6). If the impairment effect is very strong, Condition 1 is not satisfied, and we will not obtain the directional evolution suggested by Propositions 1 and 2. This is shown by the case with Eq. (20), which has bistability (see Fig. 3). Hence the condition for Propositions is easier to satisfy in the models with cross-immunity than in the ones with immune impairment.

Whether or not the conditions required for Propositions 1 and 2 are sufficiently close to those observed in real immune systems is certainly an important question to study in the future in theoretical immunology. However, given that there is a group of models describing the interaction between immune reaction to different strains, in which the evolution of virus population within a single patient is the monotonic increase in pathogenicity, we may be able to have a simple picture of viral evolution as a first-step approximation to reality. After the infection to a host, the virus might be suppressed by the immune system to a sufficiently low level, but as the evolution progresses, the viral strains would be replaced by different strains that would cause increasingly smaller abundance of uninfected cells, thus increasing higher total force of infection. Such a gloomy picture of viral evolution might be the mainstream path of the things occurring within a patient of HIV.

But the mathematical result can also be used to change the direction of viral evolution, as is demonstrated by two examples of general cross-reactivity studied by Iwasa et al. (2004). To do so, we need to produce a vaccination of a novel strain that can cause strong activation of the immune reaction, but not so much to itself. After receiving such a strain, the total force of infection by viruses would be reduced and the number of uninfected cells would recover (see Iwasa et al., 2004).

Finally, we may speculate the application of the current analysis of viral evolution to cancer. Tumorigenesis is also the evolutionary process by accumulating mutations within a host individual. The role of immune system to suppress the cancer, and the escape of cancer by mutation are aspects common to the virus evolution studied in the present paper. However, cancer would require a more careful treatment on the effect of spatial pattern because those cells normally stick to each other forming a clumped colony, which might make modeling based on ordinary differential equations less accurate than the virus dynamics.

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Appendix A.

Proof of Theorem 1. Let A be a group of strains with a positive abundance in the equilibrium. Let x^* and Y^* be the uninfected cell number and the total force of infection at the equilibrium. Then from Eq. (15a): $\phi_i(x^*, Y^*) > 0$ for all $i \in A$. We also have

$$1 = \sum_{i \in A} \frac{1}{Y^*} \beta_i \phi_i(x^*, Y^*), \tag{A.1}$$

from Eq. (13). We consider strain k, which is not in A, invades the equilibrium. From Eq. (16b), if $\phi_k(x^*, Y^*) = 0$, the invasion attempt fails. If instead

$$\phi_k(x^*, Y^*) > 0, \tag{A.2}$$

strain k increases when rare. It can invade A (see, Eq. (16a)). Then how does the abundance of uninfected cell number change after such a successful invasion? We denote $B = A \cup \{k\}$. Let x^B and Y^B be values in the new equilibrium after the invasion. Note that some of the strains in set B may go extinct in the new equilibrium. In the new equilibrium, Eq. (13) becomes

$$1 = \sum_{i \in A} \frac{1}{Y^B} \beta_i \phi_i(x^B, Y^B) + \frac{1}{Y^B} \beta_k \phi_k(x^B, Y^B). \tag{A.3}$$

From Eq. (14), we have $Y^B = \lambda / x^B - d$. From Eqs. (A.2) and (A.3), we have

$$1 > \sum_{i \in A} \frac{1}{Y^B} \beta_i \phi_i(x^B, Y^B). \tag{A.4}$$

Now we can prove $x^B < x^*$, implying that the equilibrium number of uninfected cells should decrease after a successful invasion. The proof is done by assuming the opposite inequality $x^B \geqslant x^*$ and deriving the contraction. If $x^B \geqslant x^*$, we have $Y^B \leqslant Y^*$ from Eq. (14). From

Conditions 1 and 2,

$$\left[\begin{array}{l}\text{The right-hand}\\ \text{side of Eq. (A.4)}\end{array}\right] = \sum_{i\in A}\frac{1}{Y^B}\beta_i\phi_i(x^B, Y^B) \geqslant \sum_{i\in A}\frac{1}{Y^*}\beta_i\phi_i(x^*, Y^*) = 1, \quad \text{(A.5)}$$

where we used Eq. (A.1) for the last equality. Combing this and Eq. (A.4), we reach $1>1$, which is a contradiction. Hence we cannot assume $x^B \geqslant x^*$, and hence we conclude $x^B < x^*$.

From Eq. (14), $Yx = \lambda - dx$ holds at equilibrium. Hence the product of Y and x must increase when x decreases after the invasion of k. □

Proof of Theorem 2. Let A be a group of strains with a positive abundance in the equilibrium. Let x^* and Y^* be the uninfected cell number and the total force of infection at equilibrium. Then there are two situations:

Case 1: For all i in A, $x^* > a_i/\beta_i$, and hence $\phi_i(x^*, Y^*)>0$.

Case 2: There is one strain j in A, at which $x^* = a_j/\beta_j$ holds. For all the other trains in A, $x^* > a_i/\beta_i$ and hence $\phi_i(x^*, Y^*)>0$.

For Case 1, we can apply the same argument the used to prove Theorem 1 concerning the shift in the equilibrium when an invader succeeds. Hence Theorem 1 holds, which implies Theorem 2 holds. In the following we focus on Case 2.

We denote the set of all the strains in A except for j by A'. Hence $A = A' \cup \{j\}$.

We assume a similar setting as Theorem 1. Then concerning the abundance of the "boundary strain" j, we have

$$\sum_{i\in A}\frac{1}{Y^*}\beta_i\phi_i(x^*, Y^*) < 1 < \sum_{i\in A}\frac{1}{Y^*}\beta_i\phi_i(x^*, Y^*) + \frac{1}{Y^*}\beta_j\phi_j(x^*+0, Y^*). \quad \text{(A.6)}$$

Note that $\phi_j(x, Y^*)$ is discontinuous at $x = x^*$, and we need to keep x^*+0 symbol indicating the limit from above. But for all the strains i in A', $\phi_i(x, Y^*)$ is continuous, which removes symbol for the limit from below in (A.6).

If invader k satisfies $a_k/\beta_k > x^*$, the invasion should fail (see Eqs. (16)). Invasion would be successful when $a_k/\beta_k < x^*$ and hence $\phi_k(x^*, Y^*)>0$.

After such a successful invasion, strain j may still remain the system at a positive abundance, or strain j may go extinct. This can be distinguished into the following two cases:

Case 2a: If the following inequality holds,

$$\sum_{i\in A}\frac{1}{Y^*}\beta_i\phi_i(x^*, Y^*) + \frac{1}{Y^*}\beta_k\phi_k(x^*, Y^*) < 1, \quad \text{(A.7)}$$

strain j still remains in the system in the new equilibrium keeping a reduced but positive abundance. Then the number of uninfected cells remains x^*, the same value as before the invasion. The outcome of the invasion is simply addition of strain k to the community. The abundances of different strains in the new equilibrium are:

$$y_i = \phi_i(x^*, Y^*) > 0 \quad \text{for} \quad \text{all } i \in A', \quad \text{(A.8a)}$$

$$y_k = \phi_k(x^*, Y^*) > 0, \quad \text{(A.8b)}$$

$$y_j = \frac{1}{\beta_j}\left(Y^* - \sum_{i\in A'}\beta_i\phi_i(x^*, Y^*) - \beta_k\phi_k(x^*, Y^*)\right) > 0. \quad \text{(A.8c)}$$

Case 2b: In contrast, if

$$\sum_{i\in A}\frac{1}{Y^*}\beta_i\phi_i(x^*, Y^*) + \frac{1}{Y^*}\beta_k\phi_k(x^*, Y^*) > 1, \quad \text{(A.9)}$$

strain j cannot be maintained after the invasion of strain k. In this case, we can apply a similar logic as used in deriving Theorem 1. Let $B = A' \cup \{k\}$. We assume the contrary to the inequality to prove. Suppose $x^B \geqslant x^*$. From Eq. (14), this leads to $Y^B \leqslant Y^*$. Then, using Conditions 1 and 3 we have

$$[\text{The left-hand side of Eq. (A.8)}] = \sum_{i\in B}\frac{1}{Y^*}\beta_i\phi_i(x^*, Y^*) \leqslant \sum_{i\in B}\frac{1}{Y^B}\beta_i\phi_i(x^B, Y^B) = 1,$$

which combined with Eq. (A.9) leads us to $1>1$, which is a contradiction. Hence we conclude $x^B < x^*$. From Eq. (14), we have $Y^B x^B > Y^* x^*$. □

Appendix B

Here we show $\phi_i(x, Y)$ for the models discussed in this paper. In all the models, Eq. (1a) is used for the dynamics of uninfected cells, and Eq. (1b) is for the dynamics of cells infected by strain i. They differ in the dynamics of z_i immune activity specific to strain i.

Model 1 (Eq. (1c)):

$$\phi_i(x, Y) = \frac{b_i\beta_i}{c_i p_i}\left[x - \frac{a_i}{\beta_i}\right]_+ (1+uY). \quad \text{(B.1)}$$

Model 2 (Eq. (5)), and Model 3 (Eq. (8)):

$$\phi_i(x, Y) = \frac{b_i}{c_i}H\left[x - \frac{a_i}{\beta_i}\right](1+uY). \quad \text{(B.2)}$$

Model 5 (Eq. (10)):

$$\phi_i(x, Y) = \frac{b_i}{c_i} H\left[x - \frac{a_i}{\beta_i}\right] \frac{1}{1 + uY}. \quad \text{(B.3)}$$

Model 4 (Eq. (9)):

$$\phi_i(x, Y) = \frac{b_i \beta_i}{c_i p_i} \left[x - \frac{a_i}{\beta_i}\right]_+ \frac{1}{1 + uY}. \quad \text{(B.4)}$$

Model 6 (Eq. (18)):

$$\phi_i(x, Y) = \frac{b_i}{c_i x} H\left[x - \frac{a_i}{\beta_i}\right]. \quad \text{(B.5)}$$

Model 7 (Eq. (19)):

$$\phi_i(x, Y) = \frac{b_i \beta_i}{c_i p_i x} \left[x - \frac{a_i}{\beta_i}\right]_+. \quad \text{(B.6)}$$

For Models 1, 4, and 7, we can prove Theorem 1. In contrast, for Models 2, 3, 5, and 6, together with the convention of Eq. (17) at the point of discontinuity ($x = a_i/\beta_i$), we can prove Theorem 2.

Reference

Addo, M.M., Yu, X.G., Rathod, A., Cohen, D., Eldridge, R.L., Strick, D., Johnston, M.N., Corcoran, C., Wurcel, A.G., Fitzpatrick, C.A., Feeney, M.E., Rodriguez, W.R., Basgoz, N., Draenert, R., Stone, D.R., Brander, C., Goulder, P.J., Rosenberg, E.S., Altfeld, M., Walker, B.D., 2003. Comprehensive epitope analysis of human immunodeficiency virus type 1 (HIV-1)-specific T-cell responses directed against the entire expressed HIV-1 genome demonstrate broadly directed responses, but no correlation to viral load. J. Virol. 77, 2081–2092.

Altes, H.K., Ribeiro, R.M., De Boer, R.J., 2003. The race between initial T-helper expansion and virus growth upon HIV infection influences polyclonality of the response and viral set-point. Proc. R. Soc. London B 270, 1349–1358.

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

Bonhoeffer, S., Holmes, E.G., Nowak, M.A., 1995. Causes of HIV diversity. Nature 376, 125.

Bonhoeffer, S., May, R.M., Shaw, G.M., Nowak, M.A., 1997. Virus dynamics and drug therapy. Proc. Natl Acad. Sci. USA 94, 6971–6976.

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., 1995. Towards a general function describing T cell proliferation. J. Theor. Biol. 175, 567–576.

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.

Goulder, P.J., Brander, C., Tang, Y., Tremblay, C., Colbert, R.A., Addo, M.M., Rosenberg, E.S., Nguyen, T., Allen, R., Trocha, A., Altfeld, M., He, S., Bunce, M., Funkhouser, R., Pelton, S.I., Burchett, S.K., McIntosh, K., Korber, B.T., Walker, B.D., 2001. Evolution and transmission of stable CTL escape mutations in HIV infection. Nature 412, 334–338.

Ho, D.D., Neumann, A.U., Perelson, A.S., Chen, W., Leonard, J.M., Markowitz, M., 1995. Rapid turnover of plasma virions and CO4 lymphocytes in HIV-1 infection. Nature 373, 123–126.

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.

Iwasa, Y., Michor, F., Nowak, M.A., 2004. Some basic properties of immune selection. J. Theor. Biol. 229, 179–188.

Müller, V., Maree, A.F.M., De Boer, R.J., 2001. Small variations in multiple parameters account for wide variations in HIV-1 set-points: a novel modelling approach. Proc. R. Soc. London B 268, 235–242.

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., May, R.M., Anderson, R.M., 1990. The evolutionary dynamics of HIV-1 quasispecies and the development of immunodeficiency disease. AIDS 4, 1095–1103.

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

Perelson, A.S., Neumann, A.U., Markowitz, M., Leonard, J.M., Ho, D.D., 1996. HIV-1 dynamics in vivo: virion clearance rate, infected cell life-span, and viral generation time. Science 271, 1582–1586.

Phillips, R.E., Rowland-Johnes, S., Nixon, D.F., Gotch, F.M., Edwards, J.P., Ogunlesi, A.O., Elvin, J.G., Rothbard, J.A., Bangham, D.R.M., Rizza, C.R., McMichael, A.J., 1991. Human-immunodeficiency-virus genetic-variation that can escape cytotoxic T-cell recognition. Nature 354, 453–459.

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

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

Wahl, L.M., Bitter, B., Nowak, M.A., 2000. Immunological transitions in response to antigenic mutation during viral infection. Int. Immunol 12, 137–180.

Wei, X.P., Ghosh, S.K., Taylor, M.E., Johnson, V.A., Emini, E.A., Deutsch, P., Lifson, J.D., Bonhoeffer, S., Nowak, M.A., Hahn, B.H., Saag, M.S., Shaw, G.M., 1995. Viral dynamics in human-immunodeficiency-virus type-1 infection. Nature 373, 117–122.

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., Nowak, M.A., 2000. CD8 memory, immunodominance and antigenic escape. Eur. J. Immunol. 30, 2704–2712.

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.

Wolinsky, S.M., Korber, B.T.M., Neumann, A.U., Daniels, M., Kunstman, K.J., Whetsell, A.J., Furtado, M.R., Cao, Y.Z., Ho, D.D., Safrit, J.T., Koup, R.A., 1996. Adaptive evolution of human immunodeficiency virus-type 1 during the natural course of infection. Science 272, 537–542.