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Directional Evolution of Virus Within a Host Under Immune Selection

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Summary. Viruses, such as the human immunodeficiency virus, the hepatitis B virus, the hepatitis C virus, undergo many rounds of inaccurate reproduction within an infected host. They form a heterogeneous quasispecies and change their property following selection pressures. We analyze models for the evolutionary dynamics of viral or other infectious agents within a host, and study how the invasion of a new strain affects the composition and diversity of the viral population. We previously proved, under strain specific immunity, that (Addo et al. 2003) the equilibrium abundance of uninfected cells declines during viral evolution, and that (Bittner et al. 1997) the absolute force of infection increases during viral evolution. Here we extend the results to a wider class of models describing the interaction between the virus population and the immune system. We study virus induced impairment of the immune response and certain cross-reactive stimulation of specific immune responses. For nine different mathematical models, virus evolution reduces the equilibrium abundance of uninfected cells and increases the rate at which uninfected cells are infected. Thus, in general, virus evolution tends to increase its pathogenicity. Those trends however do not hold for general cross-reactive immune responses, which introduce frequency dependent selection among viral strains. Hence an idea for combating HIV infection is to construct a virus mutant that can outcompete the existing infection without being pathogenic itself.

7.1 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, and escape from immune responses and drug treatment (Hahn et al. 1986; Holmes 1992; Fenyo 1994; McMichael and Phillips 1997; Borrow et al. 1997). The continuous evolution of HIV within an infected individual over several years shifts the balance of power between the immune system and the virus in favor of the virus (Nowak et al. 1991). Virus evolution as mechanism of disease progression in HIV infection has been a common theme for the last 15 years (Nowak et al. 1990,

1995; DeBoer and Boerlijst 1994; Sasaki 1994; Nowak and May 2000). 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 responds 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 but becomes heterogeneous afterwards (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 (Ho et al. 1995; Perelson et al. 1996; Bonhoeffer et al. 1997); hence the HIV quasi-species can rapidly respond to selection pressure; (iii) HIV escapes from B-cell and T-cell mediated immune responses (Phillips et al. 1991; Wei et al. 2003; Addo et al. 2003).

In Iwasa et al. (2004), we analyze three models for the interaction between a virus population and immune responses (Perelson 1989; McLean and Nowak 1992; De Boer and Booerlijst 1994; Nowak and Bangham 1996; De Boer and Perelson 1998; Bittner et al. 1997; Perelson and Weisbuch 1997; Wodarz et al. 1999; 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, in which there are n virus strains (or mutants) which induce n immune responses that are directed at the strains that induce them. Virus mutants can differ in all virological and immunological parameters.

In the absence of immune responses only one virus strain with the maximum fitness can survive at equilibrium. However, in the presence of immune responses, multiple strains can coexist stably. Consider a population of viral strains at a stable equilibrium. Suppose that a new strain is generated by mutation. There can be several different outcomes: the new strain may simply be added to the existing population thereby increasing the number of strains by one; the new strain may invade the existing population and other strains may become extinct; or the new strain may not be able to invade.

We ask whether there are quantities that will consistently increase (or decrease) during such viral evolution. We can prove that neither viral load nor viral diversity increases monotonically with virus evolution (although they are likely to increase in a probabilistic sense). Iwasa et al. (2004) proved that any successful invasion of a new virus strain always decreases the total abundance of uninfected cells if the immune response is specific to the strain. Further we find that any successful invasion increases the total force of infection, denoted by $\sum_{i=1}^{n} \beta_i y_i$. In the present chapter, after summarizing Iwasa et al. (2004), we mathematically examine how the invasion of a new strain affects

the composition and diversity of viral population in a host for some classes of models with virus induced impairment of immune responses or cross-reactive immune stimulations. We can show that the same directional evolutionary trends as in the models without cross-immunity hold for a class of model with cross-reactive impairment or activation of immune response. Under these settings. pathogenicity always increases by evolution within a host individual.

However we can also illustrate that these unidirectional trends of virus evolution under immune selection do not hold for general cross-reactive immune responses, in which a new strain can increase the uninfected cell number.

7.2 Model of cytotoxic immunity

We start with a model in which cytotoxic immune responses reduce the lifetime of infected cells (Iwasa et al. 2004). Let x be the abundance of uninfected target cells, and y_i be the abundance of cells infected with virus strain i. Let z_i be the abundance of immune cells specific to strain i. Consider the following system of ordinary differential equations:

[Model 1] : Strain specific immunity

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

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

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

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 $b_i z_i$. We do not model the dynamics of free viral particles explicitly, but we simply assume that the number of free viral particles is proportional to the number of cells infected. This is valid because the number of free vial particles changes at a much shorter time scales than those variables in (1) (Regoes et al. 1998; Iwasa et al. 2004).

The equilibrium

The model given by (1) has a stable equilibrium. The equilibrium values of y_i and z_i can be written as functions of x, derived from (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.

$$y_i = \frac{b_i}{c_i p_i}[\beta_i x - a_i]_+ , \quad \text{and} \quad z_i = \frac{1}{p_i}[\beta_i x - a_i]_+ , \tag{2}$$

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

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

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

The right hand side of (3) is a sum of increasing functions, and hence it is also an increasing function of x. Incontrast $Y = \lambda/x - d$ is a decreasing function of x. Hence there is always a single positive solution x^* at which (3) is equal to $Y = \lambda/x - d$. x^* is the equilibrium number of uninfected cells. Figure 7.1 plotted (3) and $Y = \lambda/x - d$, in which the horizontal axis is x,

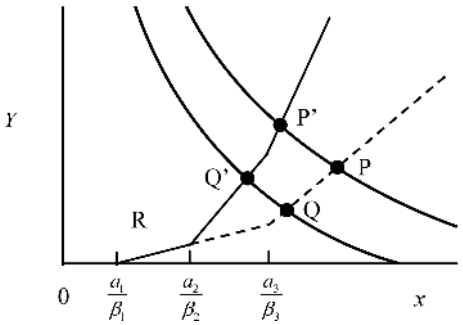

Fig. 7.1. Graphical representation of (3) and $Y = \lambda/x - d$ for a population before and after the invasion of a new strain. The model is given by (1). *Broken curve* is for the population with strain 1 and strain 3. *Solid curve* is for the population with strain 2 is added. *Three arcs connected by kink* is (3), indicating per capita risk of uninfected cells. The curves with negative slopes are $Y = \lambda/x - d$, with different value of λ. Horizontal axis is the abundance of uninfected cells x. P and Q are for the equilibrium corresponding to different λ, both including two strains. After invasion of strain 2, (3) would change to a solid curve and the equilibria would shift to P' and Q'. All three strains coexist in P'. But strain 3 is replaced by strain 2 in Q'

and the vertical axis is Y. Equation (3) is a piecewise straight line with a positive slope. $Y = \lambda/x - d$ is a curve with a negative slope. The equilibrium solution x^* is given by their cross point.

As explained in Iwasa et al. (2004), the model given by (1) has a Lyapunov function and hence the equilibrium calculated in this way is globally stable.

The possibility of invasion of a new strain into the population and its outcome is also known from a figure such as Fig. 7.1. After invasion, (3) increases by $\beta_j y_j(x)$. If, before the invasion of strain j, the population 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 a_j/β_j, then strain j can increase. As an outcome of invasion, the cross–point would shift to above and toward left. The level of uninfected cells x becomes smaller than before the invasion, and Y is larger than before the invasion, and hence $Y = \sum_{i=1}^{n} \beta_i y_i$ should increase.

Figure 7.1 illustrates the situation where two strains (strain 1 and strain 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. 7.1 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 $Y = \lambda/x - d$ 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, and 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: (Addo et al. 2003) 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 $x^*_{\text{before}} > a_i/\beta_i$; not invasible otherwise). (Bittner et al. 1997) As the result of a successful invasion, the location of the equilibrium would move upward and the abundance of uninfected cells downward ($x^*_{\text{after}} < x^*_{\text{before}}$). (Bonhoeffer and Nowak 1994) If x^* moves less than the threshold for some resident species $x^*_{\text{after}} < a_j/\beta_j$, they should go extinct, while those species would remain positive if $x^*_{\text{after}} > a_j/\beta_j$ 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. To clarify, we state this as the following proposition:

Proposition 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.*

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

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.

7.3 Cytotoxic immunity with proportional activation term

Next, we study another model for strain specific immunity, given by (1) in which (1c) is replaced by the following:

[Model 2]:

$$\frac{\mathrm{d}}{\mathrm{d}t} z_i = (c_i y_i - b_i) z_i \,, \quad i = 1, 2, 3, \ldots, n \,. \tag{4}$$

Here the immune response reduces the life-time 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 (4) is given by $c_i y_i z_i$ instead of $c_i y_i$ as in (1c). If viral abundance is kept constant, the immune activity shows an exponential increase in (4), but a linear increase in (1c). Again, there is a single, globally stable equilibrium (see appendix A of Iwasa et al. 2004). It is also similar to a model by Regoes et al. (1998), but 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 (4).

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

$$\text{(Case 1) for } x > \frac{a_i}{\beta_i} \,, \quad y_i = \frac{b_i}{c_i} \,, \quad z_i = \frac{\beta_i}{p_i} \left(x - \frac{a_i}{\beta_i} \right) \,, \tag{5a}$$

$$\text{(Case 2) for } x = \frac{a_i}{\beta_i} \,, \quad 0 < y_i < \frac{b_i}{c_i} \,, \quad z_i = 0 \,, \tag{5b}$$

$$\text{(Case 3) for } x < \frac{a_i}{\beta_i} \,, \quad y_i = z_i = 0 \,. \tag{5c}$$

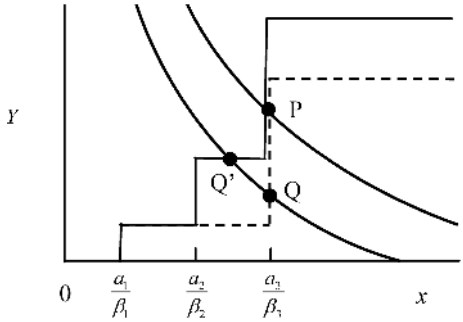

Fig. 7.2. Graphical representation of (6) and $Y = \lambda/x - d$ for a population before and after the invasion of a new strain. The model is given by (1a), (1b) and (4). Equation (6) is a step like function. *Broken curve* is for the population with strain 1 and strain 3. *Solid curve* is for the population with strain 2 is added. The curves with negative slopes are $Y = \lambda/x - d$ with different λ. Horizontal axis is the abundance of uninfected cells x. P and Q are for the equilibrium corresponding to different λ, both including two strains. After invasion of strain 2, (6) 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

On a (x, y_i)–plane, with fixed Y, equilibrium condition (5) 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 $0 < y_i < \frac{b_i}{c_i}$, which appears as a vertical line. Figure 7.2 illustrates an example. Equation (3) now becomes

$$Y = \sum_{i=1}^{n} \frac{\beta_i b_i}{c_i} H\left[x - \frac{a_i}{\beta_i}\right], \tag{6}$$

where $H[x] = 1$, for $x \geq 0$ and $H[x] = 0$, for $x < 0$ is a Heaviside function. Equation (6) can be used except for $a_i/\beta_i (i = 1, 2, \ldots, n)$, at which one of y_i is discontinuous. When the right hand side is discontinuous ($x = a_i/\beta_i$), we can interpret (6) as indicating that Y is between the limit from below and the limit from above of the right hand side.

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 (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 requires 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 (6) and $Y = \lambda/x - d$ is shown in Fig. 7.2. 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. (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 $Y = \sum_{i=1}^{n} \beta_i y_i$ 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 decreases in the evolutionary change. It never increases. The equilibrium total force of infection $\sum_{i=1}^{n} \beta_i y_i$ either increases or remains the same as before, respectively.*

A formal proof of this proposition 2 will be given later.

7.4 Models of immune impairment

Before explaining the proof of the two propositions, we would like to explain other models that behave in a similar manner. We examine the models including the interaction between the immune reaction to different strains, such as cross-reactive immune impairment and cross–reactive immune activation, which were not covered in Iwasa et al. (2004).

[Model 3]: Cross-reactive immune impairment

Consider the model of the virus-immunity dynamics, which is composed of (1a) and (1b), but using the following, instead of (1c):

$$\frac{\mathrm{d}z_i}{\mathrm{d}t} = c_i y_i - b_i z_i \left(1 + u \sum_{j=1}^{n} \beta_j y_j\right) . \tag{7}$$

Equation (7) indicates that the decay rate is not a constant but an increasing function of the total abundance of virus, $b_i \left(1 + u \sum_{j=1}^{n} \beta_j y_j\right)$. This assumption represents that any viral strain impairs immune activity against other viral strains. Based on a similar logic, we can prove Proposition 1 the same evolutionary trend to hold for the model given by (7), which includes cross-immunity ($u > 0$). Hence the successful invasion of a new strain always decreases the equilibrium abundance of uninfected cells, and always increases the total force of infection $\sum_{i=1}^{n} \beta_i y_i$.

[Model 4]:Same as Model 3 but with a proportional activation term

We may consider the following dynamics of immune cells,

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

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). The second term within brackets of (8) implies that the mortality of immune cells increases with general activity of viral load ($u \sum_{i=1}^{n} \beta_i y_i$). This is also similar to a model by Regoes et al. (1998), but 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 (8).

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

$$\text{(Case 1) for } x > \frac{a_i}{\beta_i}\,, \quad y_i = \frac{b_i}{c_i}(1+uY)\,, \quad z_i = \frac{\beta_i}{p_i}(x - \frac{a_i}{\beta_i}) \tag{9a}$$

$$\text{(Case 2) for } x = \frac{a_i}{\beta_i}\,, \quad 0 < y_i < \frac{b_i}{c_i}(1+uY)\,, \quad z_i = 0 \tag{9b}$$

$$\text{(Case 3) for } x < \frac{a_i}{\beta_i}\,, \quad y_i = z_i = 0 \tag{9c}$$

The graphical representation is useful. On a (x, y_i)-plane, with fixed Y, equilibrium condition (9) is represented as three straight lines with a step-like form, similar to (6). (3) now becomes

$$\frac{Y}{1+uY} = \sum_{i=1}^{n} \frac{\beta_i b_i}{c_i} H\left[x - \frac{a_i}{\beta_i}\right]_+ . \tag{10}$$

For this model we can prove Proposition 2. 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.

[Model 5]: 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:

$$\frac{\mathrm{d}z_i}{\mathrm{d}t} = \left(\frac{c_i y_i}{1 + u \sum_{j=1}^{n} \beta_j y_j} - b_i \right) z_i \,. \tag{11}$$

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.

[Model 6]: Cross-reactive immune activation

In all the models of interaction between immune systems to different strains studied 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

$$\frac{\mathrm{d}z_i}{\mathrm{d}t} = c_i y_i \left(1 + u \sum_{i=1}^{n} \beta_i y_i\right) - b_i z_i \,. \tag{12}$$

Here, the presence of any strain would reduce the equilibrium abundance of all the other strains. For dynamics with (1a), (1b), and (12), 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.

[Model 7]: Cross-immunity with an alternative form

We can also consider the following form:

$$\frac{\mathrm{d}z_i}{\mathrm{d}t} = \left(c_i y_i \left(1 + u \sum_{i=1}^{n} \beta_i y_i\right) - b_i\right) z_i \,. \tag{13}$$

which is an alternative form of cross-immunity. For model with (1a), (1b), and (13), we can prove Proposition 2.

7.5 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 $Y = \sum_{i \in A} \beta_i y_i$.

$$\frac{\mathrm{d}x}{\mathrm{d}t} = \lambda - dx - xY \,, \tag{14a}$$

$$\frac{\mathrm{d}y_i}{\mathrm{d}t} = y_i f_i(x, y_i, Y, z_i) \,, \quad i = 1, 2, 3, \ldots, n \,. \tag{14b}$$

$$\frac{\mathrm{d}z_i}{\mathrm{d}t} = g_i(x, y_i, Y, z_i) \,, \quad i = 1, 2, 3, \ldots, n \,. \tag{14c}$$

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 dynamics given by (14b) and (14c), 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 (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

$$y_i = \phi_i(x, Y)\,, \tag{15}$$

which is calculated from (14b) and (14c). In the equilibrium of the whole system (14), we have:

$$Y^* = \sum_{i \in A} \beta_i \phi_i(x^*, Y^*)\,, \tag{16}$$

from the definition of Y. From (14a), we also have

$$Y^* = \frac{\lambda}{x^*} - d\,, \tag{17}$$

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

$$\text{Strain } i \text{ has a positive abundance at equilibrium, if } \phi_i(x^*, Y^*) > 0\,, \tag{18a}$$
$$\text{Strain } i \text{ is absent at equilibrium, if } \phi_i(x^*, Y^*) = 0\,. \tag{18b}$$

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 $\phi_k(x^*, Y^*)$:

$$\text{Strain } k \text{ can invade the equilibrium, if } \phi_k(x^*, Y^*) > 0\,, \tag{19a}$$
$$\text{Strain } k \text{ fails to invade the equilibrium, if } \phi_k(x^*, Y^*) = 0. \tag{19b}$$

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

[Condition 1] $\phi_i(x, Y)\frac{1}{Y}$ is a decreasing function of Y if $\phi_i(x, Y) > 0$.
[Condition 2] $\phi_i(x, Y)$ is a continuous and non–decreasing function of x.

All the models we have been discussed have the unique positive equilibrium satisfying (16) and (17). This can be shown, as follows: We define: $\psi(x) = (1/Y(x)) \sum_{i=1} \beta_i \phi_i(x, Y(x))$. If Y is replaced by $Y(x) = \lambda/x - d$, (16) becomes $1 = \psi(x)$. $\psi(x)$ is an increasing function of x, because $Y(x)$ is a decreasing function, and that $(1/Y) \sum_{i=1} \beta_i \phi_i(x, Y)$ is a decreasing function of Y. Note $\psi(x) = 0$ for $x \leq \min_i(a_i/\beta_i)$ because $\phi_i(x, Y) = 0$ for $x \leq a_i/\beta_i$. Also note $\lim_{Y \to +0}(1/Y) \sum_{i=1} \beta_i \phi_i(x, Y) = \infty$ for $x > \min_i(a_i/\beta_i)$. Combining these, we can conclude that there is the unique solution with $x > 0$

which satisfies both (16) and (17). Using this, we can calculate all the other variables (y_i and z_i for all i).

The global stability of this positive equilibrium is proved for models 1 and 2 in Iwasa et al. (2004), using a Lyapunov function. But for other models, we simply assume the global stability. When an invasion of mutant is successful, the positive equilibrium satisfying (16) and (17) would shift to a new positive equilibrium that is unique. This conjecture is supported by all the simulations we have done.

Under this stability assumption, we calculate the directionality of the evolution as follows (see appendix A for proof):

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.

When equilibrium y_i is a step function of x

For the model (1a), (1b) combined with immunity dynamics given by (4), (8), (11) or (13), y_i is not a continuous function of x, and hence Condition 2 is not satisfied. However y_i is expressed as (15) 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 (5b). 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 (16) is discontinuous. Then, we use the following inequality instead of (16):

$$\sum_{i\in A}\beta_i\phi_i(x-0,Y) \le Y \le \sum_{i\in A}\beta_i\phi_i(x+0,Y)\,. \tag{20}$$

We summarize these as follows:

[Condition 3] $\phi_i(x,Y)$ is a continuous and non–decreasing 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 (20).

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 (1a) and (1b), together with the immunity dynamics given by (4), (8), (11), or (13). 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 $\sum_{i \in A} \beta_i y_i x$.

7.6 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.

[Model 8]:

$$\frac{\mathrm{d}z_i}{\mathrm{d}t} = z_i(c_i y_i x - b_i) \,, \quad i = 1, 2, \ldots, n \,. \tag{21}$$

In (21) 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 by cross–immunity, and also called it "indirect impairment model". We can prove that, for the model with immune dynamics (21), Proposition 2 holds.

We may also think of the system in which (21) is replaced by the following:

[Model 9]:

$$\frac{\mathrm{d}z_i}{\mathrm{d}t} = c_i y_i x - b_i z_i \,, \quad i = 1, 2, \ldots, n \,. \tag{22}$$

The model, given by (1a), (1b) and (22), 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.

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 (21). For example,

$$\frac{\mathrm{d}z_i}{\mathrm{d}t} = z_i(c_i y_i x^2 - b_i)\,, \quad i = 1, 2, \ldots, n\,. \tag{23}$$

instead of (21). The equilibrium number of cells infected by strain i is:

$$Y = \begin{cases} \frac{b_i}{x^2 c_i} & \text{for} \quad x > a_i/\beta_i \\ 0 & \text{for} \quad x < a_i/\beta_i \end{cases}\,. \tag{24}$$

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

$$\lambda - dx = \frac{1}{x}\left(\frac{\beta_i b_i}{c_i} H\left[x - \frac{a_i}{\beta_i}\right] + \frac{\beta_2 b_2}{c_2} H\left[x - \frac{a_2}{\beta_2}\right]\right)\,, \tag{25}$$

where $H[\cdot]$ is the Heaviside function. 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 (1a), (1b), and (23) is bistable.

7.7 General cross–immunity violates the fundamental theorem

We have been studied the evolutionary trends of virus within a host individual for a particular model of interaction between immunity to different strains. However in general cases of the cross–immunity, the decrease in the equilibrium abundance of uninfected cells no longer holds, as illustrated by two examples in Iwasa et al. (2004). One of the examples was

$$\frac{\mathrm{d}x}{\mathrm{d}t} = \lambda - dx - \sum_{i=1}^{n} \beta_i x y_i\,, \tag{26a}$$

$$\frac{\mathrm{d}}{\mathrm{d}t} y_i = \left(\beta_i x - a_i - p_i \sum_{j=1}^{m} c_{ij} z_j\right) y_i\,, \quad i = 1, 2, 3, \ldots, n\,, \tag{26b}$$

$$\frac{\mathrm{d}}{\mathrm{d}t} z_j = \sum_{j=1}^{m} y_i c_{ij} - b_j z_j\,, \quad i = 1, 2, 3, \ldots, m\,. \tag{26c}$$

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 (26), 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 (26b), which indicates that a strain

stimulating an epitope is more likely to be suppressed by the corresponding immunity.

Iwasa et al. (2004) discussed a case of two strains and 1 epitope ($n = 2, m = 1$) with the following parameters: $\beta_1 = \beta_2 = a_1 = a_2 = 1$, $d = 0$, $c_1 = 1$, $c_2 = 5$, $p_1 = 10$, $p_2 = 1$. There is no equilibrium in which both strain 1 and strain 2 coexist. The equilibrium with strain 1 only is invaded by mutant strain 2 which replaces strain 1. The evolutionary change makes the number of uninfected cells at equilibrium 5 times greater than before. Hence the conjectured statement of monotonic decrease in uninfected cell number does not hold.

7.8 Discussion

In this paper, we studied the evolution of virus within a patient by analyzing a series of models for the dynamics of multiple strains of virus and the immune activities of the host corresponding to those strains. The immune activities to different antigens may interact with each other. 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 most cases 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 equilibrium abundance of uninfected cells decreases monotonically in the viral evolution occurs 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 and the mean virulence of the virus may increase statistically, but can decrease for a particular situation. In contrast the two tendencies we proved 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 slightly modified versions 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 uninfected 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, we also studied cases with cross–immunity in which a presence of one strain activate, rather than impair, 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 cross-immunity models, [Condition 1] is likely to satisfy. In contrast, for models with immune impairment has $y_i = \phi_i(x, Y)$ an increasing function of Y. 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 (23), which has bistability. 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 proposition 1 and 2 are sufficiently close to those observed in real immune systems is an important question to study in immunology. However given that there are 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, and increasing higher total force of infection. Such a gloomy picture of viral evolution might be the mainstream path of the things occurring within patient of HIV.

But the mathematical result can also be used to change the direction of viral evolution. 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). 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).

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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 (15): $\phi_i(x^*, Y^*) > 0$ 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 (16). We consider strain k, which is not in A, invades the equilibrium. From (19b), 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, (19a)). 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 have gone extinct in the new equilibrium. In the new equilibrium, (16) 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 (17), we have $Y^B = \lambda / x^B - d$. From (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 $x^B \geq x^*$ and deriving the contraction. If $x^B \geq x^*$, we have $Y^B \leq Y^*$ from (17). From Conditions 1 and 2,

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

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

From (17), $Yx = \lambda - dx$ holds at equilibrium. Hence the product of Y and x must increase when x decreases after the invasion of k. (End of proof of Theorem 1).

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 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

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

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 limit from below in (A.6).

If invader k satisfies $a_k/\beta_k > x^*$, the invasion should fail (see (19)). 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 in 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,

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

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:

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

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

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

[Case 2b] In contrast, if

$$\sum_{i\in A}\frac{1}{Y^*}\beta_i\phi_i(x^*,Y^*)+\frac{1}{Y^*}\beta_k\phi_k(x^*-0,Y^*)>1\,, \tag{A.9}$$

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 $x^B\geq x^*$. From (17), this leads to $Y^B\leq Y^*$. Then, we have

$$\begin{aligned}[\text{The left hand side of Eq.(A.7)}]&=\sum_{i\in B}\frac{1}{Y^*}\beta_i\phi_i(x^*,Y^*)\\&\leq\sum_{i\in B}\frac{1}{Y^B}\beta_i\phi_i(x^B,Y^B)=1\,,\end{aligned}$$

which combined with (A.9) leads us to $1>1$, which is the contradiction. Hence we conclude $x^B<x^*$. From (17), we have $Y^Bx^B>Y^*x^*$.

(End of Proof of Theorem 2)

Appendix B

Here we show $\phi_i(x,Y)$ for all the models discussed in this paper. In all the models, (1a) is used for the dynamics of uninfected cells, and (1b) is adopted 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 (1c):

$$\phi_i(x,Y)=\frac{b_i\beta_i}{c_ip_i}\left[x-\frac{a_i}{\beta_i}\right]_+\,. \tag{B.1}$$

Model 2 (4):

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

Model 3 (7):

$$\phi_i(x,Y)=\frac{b_i\beta_i}{c_ip_i}\left[x-\frac{a_i}{\beta_i}\right]_+(1+uY)\,. \tag{B.3}$$

Model 4 (8), and Model 5 (11):

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

Model 6 (12):

$$\phi_i(x,Y) = \frac{b_i\beta_i}{c_i p_i}\left[x - \frac{a_i}{\beta_i}\right]_+ \frac{1}{1+uY}\,. \tag{B.5}$$

Model 7 (13):

$$\phi_i(x,Y) = \frac{b_i}{c_i} H\left[x - \frac{a_i}{\beta_i}\right] \frac{1}{1+uY}\,. \tag{B.6}$$

Model 8 (21):

$$\phi_i(x,Y) = \frac{b_i}{c_i x} H\left[x - \frac{a_i}{\beta_i}\right]\,. \tag{B.7}$$

Model 9 (22):

$$\phi_i(x,Y) = \frac{b_i\beta_i}{c_i p_i x}\left[x - \frac{a_i}{\beta_i}\right]_+\,. \tag{B.8}$$

For models 1, 3, 6, and 9, we can prove Theorem 1. For model 2, 4, 5, 7 and 8, together with the convention (20) at $x = a_i/\beta_i$, we can prove Theorem 2.

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