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Evolutionary pattern of intra-host pathogen antigenic drift: effect of cross-reactivity in immune response

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SUMMARY

Several viruses are known to change their surface antigen types after infecting a host, thereby escaping the immune defence and ensuring persistent infection. In this paper, we theoretically study the pattern of intra-host micro-evolution of pathogen antigen variants under the antigen specific immune response. We assume that the antigen types of the pathogen can be indexed in one-dimensional space, and that a mutation can produce a new antigen variant that is one step distant from the parental type. We also assume that antibodies directed to a specific antigen can also neutralize similar antigen types with a decreased efficiency (cross-reactivity). The model reveals that the pattern of intra-host antigen evolution critically depends on the width of cross-reactivity. If the width of cross-reactivity is narrower than a certain threshold, antigen variants gradually evolve in antigen space as a travelling wave with a constant wave speed, and the total pathogen density approaches a constant. In contrast, if the width of cross-reactivity exceeds the threshold, the travelling wave loses stability and the distribution of antigen variants fluctuates both in time and in genotype space. In the latter case, the expected episodes after infection are a series of intermittent outbreaks of pathogen density, caused by distantly separated antigen types. The implication of the model to intra-host evolution of equine infectious anaemia virus and human immunodeficiency virus is discussed.

1. INTRODUCTION

Pathogens infecting their vertebrate host typically face severe immune defences directed to the cell-surface antigen, and are often eliminated from the host's body quickly. Some, however, ensure persistent infection for a prolonged period by repeatedly changing their antigen types after infecting a host (antigenic drift) – see Birbeck & Penn (1986) and Borst & Greaves (1987), for review. For instance, human immunodeficiency virus (HIV) is known to change its antigen types in a patient (Hahn *et al*. 1986; Wolfs *et al*. 1991). A horse infected by equine infectious anaemia virus (EIAV) experiences recurrent episodes of fevers caused by evolving antigen variants (Montelaro *et al*. 1986). Analogous to the intra-host antigenic drift listed above, predominant antigen types of influenza A virus have been gradually shifting year by year, although the pandemic outbreaks can be ascribed to genetic reassortment of genes from avian and human strains in the mixed infection case (Waddel *et al*. 1963; Laver & Webster 1973).

A pathogen that changes antigen type could escape immune defence because the host immune surveillance system fails to recognize a new antigen mutant until corresponding B lymphocytes are activated. Wolfs *et al*. (1991) studied the changes in amino acid sequences of envelope gene, *gp*120, of the inoculated HIV population in a patient, and found that amino acid substitutions rapidly accumulate over time. The fast rate of amino acid substitutions in a variable region, V3, of the envelope gene could therefore be regarded as an adaptive evolution driven by the dynamical antagonistic interaction between viral antigen variants and the antigen-specific immune response. Detailed molecular phylogenetic analyses provides supporting evidence for this view: The non-synonymous substitution rate of HIV in the putative antigen-determining sites is several times higher than the synonymous substitution rate in the same gene (Yamaguchi and Gojobori, personal communication), suggesting that the substitutions are promoted by positive Darwinian selection favouring new antigenic property.

A yet more interesting finding by Wolfs *et al*. (1991) and Yamaguchi & Gojobori is that the pattern of antigenic drift is not continuous but episodic: both the substitution rate and the clonal diversity of the HIV population fluctuate over time since infection, associated with the fluctuation in total viral density in a host. It appears that the evolution rate and clonal diversity are maximized in periods where viral density is expanding. Periodical appearance characterizing EIAV infection also suggests that the antigenic evolution in an infected horse might not be uniform in time and in antigen type space – indeed, the viral variants responsible for consecutive fibrile episodes differ in several amino acid residues in the epitope but there is no detectable increase of viral density by the intermediate types. In the present paper we model the dynamical interaction between viral antigen variants and the immune response in an infected host, and

attempt to clarify what factor is responsible for explaining the observed non-uniform patterns of antigenic drift.

The antigenic drift and switching is a subject attracting increasing theoretical attentions in evolutionary biology (Agur *et al*. 1989; Nowak & May 1991; Sasaki 1992, 1994; Nowak *et al*. 1995). Nowak & May (1991) studied the intra-host population dynamics of HIV with antigenic diversity, and showed that the immune defence fails to regulate the HIV population when the diversity of viral antigens exceeds a threshold. Agur *et al*. (1989) analysed a mathematical model of the antigen switching of trypanosome, by assuming that an individual trypanosome switches the cell-surface glycoprotein expressions through a short intermediate phase in which two glycoproteins are simultaneously expressed. They showed that this doubly expressed phase plays an important role in determining parasitaemia patterns of trypanosomiasis. Sasaki (1994) studied a model of antigen drift in a host and revealed that the distribution of antigen variants evolves as a travelling wave in the antigen space with a constant wave speed, by which the pathogen ensures persistent infection by continuously escaping the immune defence. None of the previously developed models, however, consider the effect of cross-reactivity in the immune response. In the present paper, we extend the model of Sasaki (1994) to take into account the effect of cross-reactivity. It will be shown below that the width of cross-reactivity is the most important factor among those considered in the model in determining the pattern of intra-host evolution of antigen variants.

A single amino acid substitution occurred in pathogen antigen-determining sites may completely change the antigenic property. If however the substitution fails to sufficiently alter the tertiary structure and chemical property of the epitope, the mutant protein should be still exposed to the immune response induced by the ancestral epitope. We model the effect of this distributed immune response (cross-reactivity) toward a range of antigen types. It is important to note here that in the immunological terminology, the cross-reactivity implies that the same antiserum, which might include a variety of antibody species, reacts to different antigens. In this paper, we restrict the meaning of cross-reactivity to mean that a single antibody can react to similar antigens. The effect of the distributed immune response is best characterized by the width of cross-reactivity, which is defined as the number of mutational steps needed to sufficiently alter the antigenic property.

In this paper, we ignored the mortality of B cells that at one time proliferated in the body, thereby preventing the reappearance of the same antigen type of viruses. This apparent simplification does not necessarily weaken the robustness of the result of our model, as long as memory B cells could effectively respond to the same antigen. We also ignored the complex processes of affinity maturation of immune system, which possibly broaden the range of cross-immunity in our model.

In the following, we first derive the intra-host population dynamics of the pathogen antigen variants and corresponding B cells, and then analyse the pattern of progression of antigenic drift. The implication of the model for the evolutionary patterns of HIV and EIAV antigenic drift will be discussed in light of the theoretical results.

2. MODEL

Let us consider a pathogen infecting a host with a given antigen type. We assume that the antigen type i of the pathogen can be indexed in one-dimensional space: $i \in \{0, \pm 1, \pm 2, \dots\}$. We also assume that a given antigen type i can mutate to one of the neighbouring types $i-1$ and $i+1$ with the equal probability $\mu Q/2$ in the unit time interval, where μ is the total mutation rate and Q is the fraction of antigen-changing mutants. The residual fraction $(1-Q)$ of mutation is assumed to be lethal. Let us denote the intra-host density of pathogen with an antigen type i by $N_i(t)$ and the density of the corresponding B-cell population by $B_i(t)$. Then the densities change with time as

$$\frac{\mathrm{d}N_i}{\mathrm{d}t} = rN_i - \sum_j \beta_{i-j} N_i B_j + \frac{\mu Q}{2}(N_{i+1} + N_{i-1}) - \mu N_i, \quad (1a)$$

$$\frac{\mathrm{d}B_i}{\mathrm{d}t} = \sum_j \alpha_{i-j} N_j B_i, \quad (1b)$$

with the initial conditions $B_i(0) = B_0$ (implying that the density of any B-cell species is at a low level at the beginning of infection) and $N_i(0) = N_0\delta_{i0}$, where $\delta_{ij} = 1$ if $i = j$ and $d_{ij} = 0$ if $i \neq j$ (i.e. the inoculated pathogen population is monomorphic with the antigen type 0). Here r represents the per capita growth rate of a pathogen; β_{i-j}, the rate of destruction of pathogen type i by antibodies produced by B-cell type j; α_{i-j} the rate of increase of activated B-cell type i by the unit dose of pathogen antigen type j. β_d and α_d are monotonically decreasing with the genotypic distance δ between two types. Non-zero β_d and α_d for $\delta > 0$ represent that there is cross-reactivity.

Specifically, we assume that the intensity of cross-reactivity decreases with genotypic distance following the Gaussian density function,

$$\beta_d = \beta_{\max} \exp(-d^2/2l_\beta^2), \quad \alpha_d = \alpha_{\max} \exp(-d^2/2l_\alpha^2), \quad (2)$$

where l_α and l_β are the characteristic genotypic distance within which the immune response acts as strongly as that for the completely matched genotype. Other choices of kernel functions (equation (2)): e.g. geometrically decreasing and triangular kernels, give qualitatively similar results.

The dilution term of the B cells is ignored in equation (1) – the density of once-activated B cells therefore never decreases. This is not just for mathematical simplicity but is necessary for taking into account the irreversibility in the immune response: we would like to see the dynamics if once-manifested antigen variants never break out again in the same host.

We approximate the antigen types i by continuous variable x to have partial differential equations. By

denoting $N(t, x) = N_i(t)$ as the density of pathogen type x, and $B(t, x) = B_i(t)$ as the density of activated B-cell type x at time t, we have from equation (1)

$$\frac{\partial N}{\partial t} = (R-\beta^*B)N+D\frac{\partial^2 N}{\partial x^2}, \tag{3a}$$

$$\frac{\partial B}{\partial t} = (\alpha^*N)B, \tag{3b}$$

where $R = r-(1-Q)\mu$ and $D = \mu Q/2$. The intensity of the immune defence towards pathogen antigen type x is proportional to the weighted average B-cell density around the type x:

$$\beta^*B = \int_{-\infty}^{\infty}\beta(x-y)B(t,y)\,\mathrm{d}y,$$

where f^*g denotes the convolution of functions f and g. Likewise, the growth rate of B-cell type x is proportional to the weighted average density of cognate pathogen antigens around the type x:

$$\alpha^*N = \int_{-\infty}^{\infty}\alpha(x-y)N(t,y)\,\mathrm{d}y.$$

The tails of kernel function α and β characterizes how far each B-cell type responds to antigens distant from the completely matched genotype. Analogous to equation (2) in the discrete version, we assume that the intensity of immune defence/activation decreases with genotypic distance following Gaussian distribution with the characteristic distance σ_β and σ_α:

$$\beta(x) = \frac{\beta_0}{\sqrt{(2\pi)}\sigma_\beta}\mathrm{e}^{-x^2/2\sigma_\beta^2}, \quad \alpha(x) = \frac{\alpha_0}{\sqrt{(2\pi)}\sigma_\alpha}\mathrm{e}^{-x^2/2\sigma_\alpha^2}, \tag{4}$$

where β_0 and α_0 measure the total intensity of immune response, and σ_β and σ_α respectively give the width of cross-immunity and cross-activation, i.e. the characteristic genotypic distance by which immune response (B-cell growth enhancement) is considerably reduced from the maximum rate. The maximum rate of destruction by a B cell for a completely matched antigen is given by $\beta_{\max} = \beta_0/\sqrt{(2\pi)}\sigma_\beta$. Likewise, the maximum growth rate of B cells by the unit dose of a completely matched antigen is given by $\alpha_{\max} = \alpha_0/\sqrt{(2\pi)}\sigma_\alpha$.

If a single-step mutation is enough to change the antigenic property, we set both σ_α and σ_β smaller than 1; whereas, if multiple substitutions are necessary to alter the antigenic property, we should have both σ_α and σ_β larger than 1. Specifically, σ_α and σ_β give the approximate number of substitutional steps in the antigen-determining sites necessary to change the antigenic variability enough to escape the currently predominant immune defence.

(*a*) *Persistence of pathogen*

The initial rapid expansion of pathogen with a single antigen type should be followed by the activation of corresponding B cells; this will end by their elimination from the body. The persistence of pathogen should therefore depend on whether the pathogen can produce sufficient number of escaping mutants before the immune defence is activated.

According to the numerical simulations of equation (3), the distributions of both antigen variants and B cells *at the frontal part* converge to travelling waves with a constant speed (though the behaviour behind the front shows diverse patterns depending on parameters, as will be discussed later). The condition for pathogen persistence is then examined by linearizing the system (equation (3)) at the frontal end of the travelling wave in the antigen space, where both the pathogen density and B-cell density are small: The pathogen density then changes as

$$\frac{\partial N}{\partial t} = (R-\beta_0 B_0)N+D\frac{\partial^2 N}{\partial x^2}, \tag{5}$$

with the B-cell densities kept almost constant: $B(t, x) = B_0$. From equation (5) we can see that the pathogen will become extinct if the initial growth rate is negative, $R-\beta_0 B_0 < 0$, and will persist otherwise. In the latter case, the frontal part of the wave shift with a wave speed,

$$v = 2\sqrt{((R-\beta_0 B_0)D)} \tag{6}$$

(Kolmogorov *et al.* 1937; Fisher 1937). These results are the same as that of Sasaki (1994). Recalling that $D = \mu Q/2$ and $R = r-(1-Q)\mu$, we have $v = \sqrt{(2\{r-\beta_0 B_0-(1-Q)\mu\}\mu Q)}$. Consequently the wave speed, or the evolution rate of mean antigenic types, is the fastest when the mutation rate is at an intermediate between 0 and the threshold $\mu_c = (r-\beta B_0)/(1-Q)$ for persistence. Specifically the evolution rate is maximized if the mutation rate is half of the threshold $\mu = \mu_c/2$. Sasaki (1994) found that the pathogen mutation rate evolves to a value close to $\mu_c/2$ (about half of the error-catastrophe mutation threshold, or 0.5 per replication per genome) even if most mutations are lethal ($Q \ll 1$). Implication of these results to the evolution of viral mutation rate is discussed in Sasaki (1994). To conclude, the cross-reactivity in the immune response does not change the condition for pathogen persistence by antigenic drift. The way of persistence, however, drastically depends on the extent of cross-reactivity, as shown in the following.

(*b*) *Pattern of propagation*

Here we examine how the pattern of pathogen antigen evolution depends on various parameters. To this purpose we reduce the number of parameters by rescaling the variables. It will be shown below that only three dimensionless parameters are sufficient to determine the qualitative behaviour of the system. Let us denote the time, antigen type, pathogen density and B-cell density in the original scale by t', x', N', and B', and define new variables as

$$t = Rt', x = \frac{x'}{d_c}, N(t,x) = \frac{\alpha_0}{R}N'(t',x')$$

$$\text{and} \quad B(t,x) = \frac{\beta_0}{R}B'(t',x'), \tag{7}$$

where $d_c = \sqrt{(D/R)}$ is the characteristic genotypic distance by which progeny antigen types diffuse in genotype space by mutation in one reproductive cycle $(1/R)$. Defining the width of the cross-reactivity in units of this characteristic distance:

$$d_c = \sqrt{(D/R)} \quad \text{and} \quad S_\alpha = \sigma_\alpha/d_c \quad S_\beta = \sigma_\beta/d_c. \tag{8}$$

The kernel functions in equation (3) are accordingly redefined as

$$\alpha(x) = \frac{1}{\sqrt{(2\pi)}S_\alpha} e^{-x^2/2S_\alpha^2}, \quad \beta(x) = \frac{1}{\sqrt{(2\pi)}S_\beta} e^{-x^2/2S_\beta^2}. \tag{9}$$

Therefore equation (3) becomes

$$\frac{\partial N}{\partial t} = (1-\beta^* B)N + \frac{\partial^2 N}{\partial x^2}, \tag{10a}$$

$$\frac{\partial B}{\partial t} = (\alpha^* N)B. \tag{10b}$$

The dynamical behaviour of equation (10) is determined by only three parameters: the rescaled width of cross-reactivity, S_α and S_β, and the rescaled initial level of the B cells, $B_0^* = \beta_0 B_0/R$. The analysis of the following therefore focuses on mapping the patterns of antigen variants propagation in the space of these three parameters.

The equilibrium patterns of outbreaks for different parameters can be classified into three categories. (i) Pathogen extinction – the pathogen will be eliminated from the body. This occurs if B_0^* is larger than 1 as shown before (i.e. the initial rate of destruction of the pathogens exceeds the pathogen growth rate: $R < \beta_0 B_0$). (ii) Stable travelling wave – both pathogen antigen variants and B cells converge to travelling waves with a constant wave speed. The total density of the pathogen converges to a constant. This occurs if B_0^* is smaller than 1 (R larger than $\beta_0 B_0$) and the width of cross-reactivity S_β is smaller than a certain threshold that will be determined later. (iii) Discontinuous shift with intermittent outbreaks – the mean antigen type shows a stair-like increase with time, and the total density of the pathogen fluctuates periodically. This occurs if B_0^* is smaller than 1 and the width of the cross-reactivity S_β is larger than the threshold. In the following we examine patterns (ii) and (iii) in detail.

If there is no cross-immunity or if the width of cross-immunity is smaller than the threshold, the pattern of disease progression is a stable travelling waves of both the pathogen antigen variants and B cells. Figure 1 demonstrates this pattern of antigenic drift observed in numerical simulations. The initial distribution was concentrated at the antigen type 0, and the B cells were uniformly distributed in antigen space with a small initial density, B_0. The antigen type continuously shifts towards the direction in which the B cells are not yet activated, and the total pathogen density converges to a constant.

For S_β just above the threshold, the peak height of the constantly shifting unimodal distribution begins periodical fluctuation with a small amplitude (figure 2*a*), i.e. the travelling wave loses stability. The total pathogen density also fluctuates periodically (upper panel). If S_β is further increased past the threshold the antigen variants propagate with intermittent bursts in total density fluctuation (figure 2*b*). The distribution of the antigen variants therefore shows large fluctuations in both time and antigen space (figure 2*b* and figure 3).

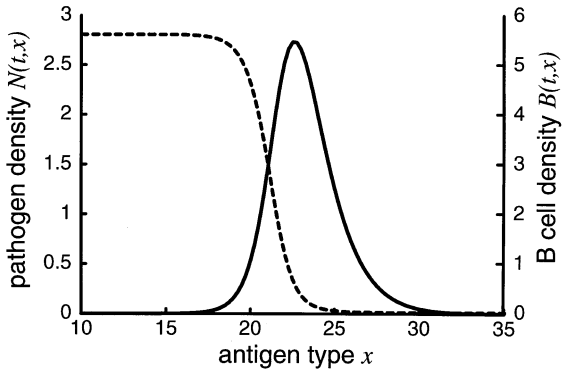

Figure 1. Distribution of pathogen antigen variants density (solid curve) and B-cell density (dotted curve) at time $t = 21$ from infection. There is no cross-immunity or cross-activation ($S_\beta = 0$, $S_\alpha = 0$). Both distributions converge to stable travelling waves with a constant wave speed ($v = 2$). The distribution of pathogen antigen variants is unimodal, and continuously shifts to the right with the shape remaining the same. The distribution of B-cell density is monotone and synchronously chases the pathogen density distribution. Since we ignore the dilution of B cells to take into account the irreversibility of immune response, B-cell types once activated never decrease. The total pathogen density approaches a constant. $B_0^* = 0.0022$.

This pattern of antigenic drift can be explained as follows. After the infection of a host by a given antigen type, the pathogen density first rapidly increases, but then declines when the corresponding B cell is activated. The activated B cells not only suppress the propagation of the inoculated antigen type, but also the antigen mutants derived from the inoculated one. After a long time, mutations accumulate at the antigen-determining sites to produce finally the next escaping mutant, which is sufficiently different from the type of the previous outbreak. The next episode of outbreak starts with the appearance of such an escaping mutant, and this again initiates a long-lasting period of suppressed evolution.

(*c*) *Mean antigen type divergence and clonal diversity*

The pattern of antigen evolution in a host is well characterized by the time series data for the sequence of divergence of antigen-determining sites from the inoculated sequence, and by the sequence diversity within population (e.g. Wolfs *et al.* 1992). Here we summarize how the mean genotypic distance from the infected one and antigenic variance (clonal diversify)

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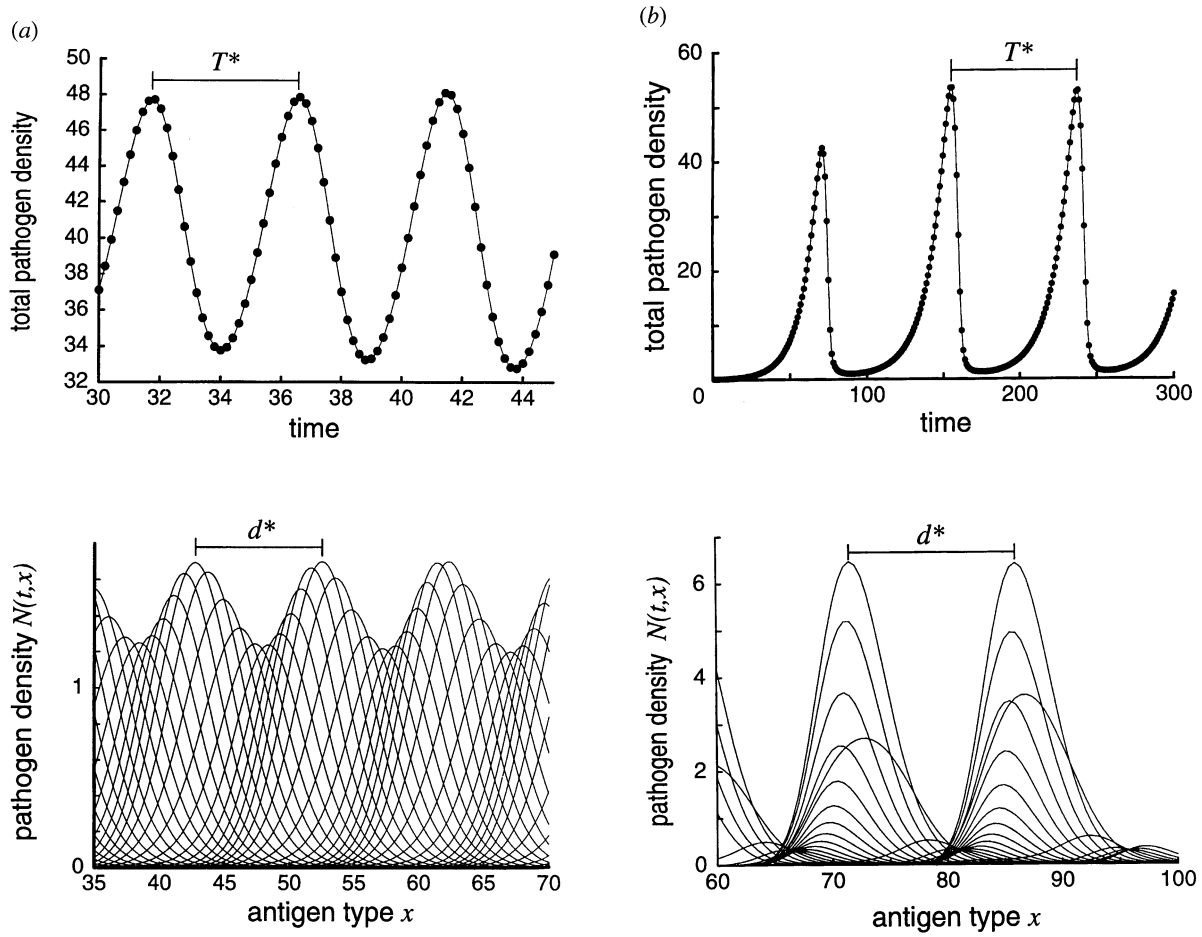

Figure 2. (*a*) Lower panel: The pattern of the change in the pathogen antigen variants distribution $N(t, x)$ (distributions overlaid with the interval of 0.5 time step), when the width of cross-immunity mildly exceeds the threshold: $S_\beta = 3.5$ (the threshold is $(S_\beta)_c = 2.6$). The unimodal distribution continues to move to the right-hand side with the peak heights fluctuating periodically. Upper panel: time change in the total pathogen density for the same simulation. Note the scale in the vertical axis. Other parameters: $S_\alpha = 0$, $B_0^* = 0.0022$. (*b*) The same as (*a*) but the width of the immune response is well above the threshold: $S_\beta = 4.5 > (S_\beta)_c = 2.9$. The distribution of the antigen variants in each time step is unimodal and still continuously moves to right, but the peak height shows extreme fluctuation in time (over 30-fold difference between the minimum and maximum heights). The distance, d^*, between the genotypic positions of two adjacent outbreaks is illustrated (numerically, we define d^* as the lowest positive period of the power spectrum estimate (Press *et al.* 1992) for the distribution of the maximum density, $N_{\max}(x) \equiv \max_{t \geq 0} N(t, x)$, reached at each antigen type in the simulations).

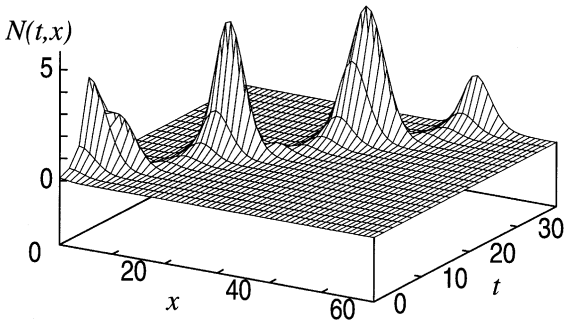

Figure 3. The density of the pathogen antigen variants plotted in time and antigen type space. Parameters are the same as in figure 2*b*.

in the intra–host population should change with time, according to the present model.

Under the condition of weak or no cross-reactivity (i.e. for S_β smaller than the threshold), the clonal mean antigen type

$$\bar{x}(t) = \int_{-\infty}^{\infty} xN(t, x)\mathrm{d}x \Big/ \int_{-\infty}^{\infty} N(t, x)\,\mathrm{d}x, \qquad (11)$$

linearly diverges from the original one after the initial transient (figure 4*a*). The slope, which is regarded as the evolution rate of the antigen-determining sites, is given by the wave speed v defined in equation (6). The intra-host antigenic variance (clonal diversity)

$$V(t) = \int_{-\infty}^{\infty} \{x - \bar{x}(t)\}^2 N(t, x)\mathrm{d}x \Big/ \int_{-\infty}^{\infty} N(t, x)\,\mathrm{d}x, \qquad (12)$$

approaches a constant after the initial transient increase. These results can be derived from the fact that the pathogen density converges to a travelling wave.

The evolutionary pattern is very different if S_β is larger than the threshold. The mean sequence divergence $\bar{x}(t)$ shows a stair-like increase with time (figure 4*b*). This implies that the long stasis in antigen evolution will be broken by a sudden shift – the

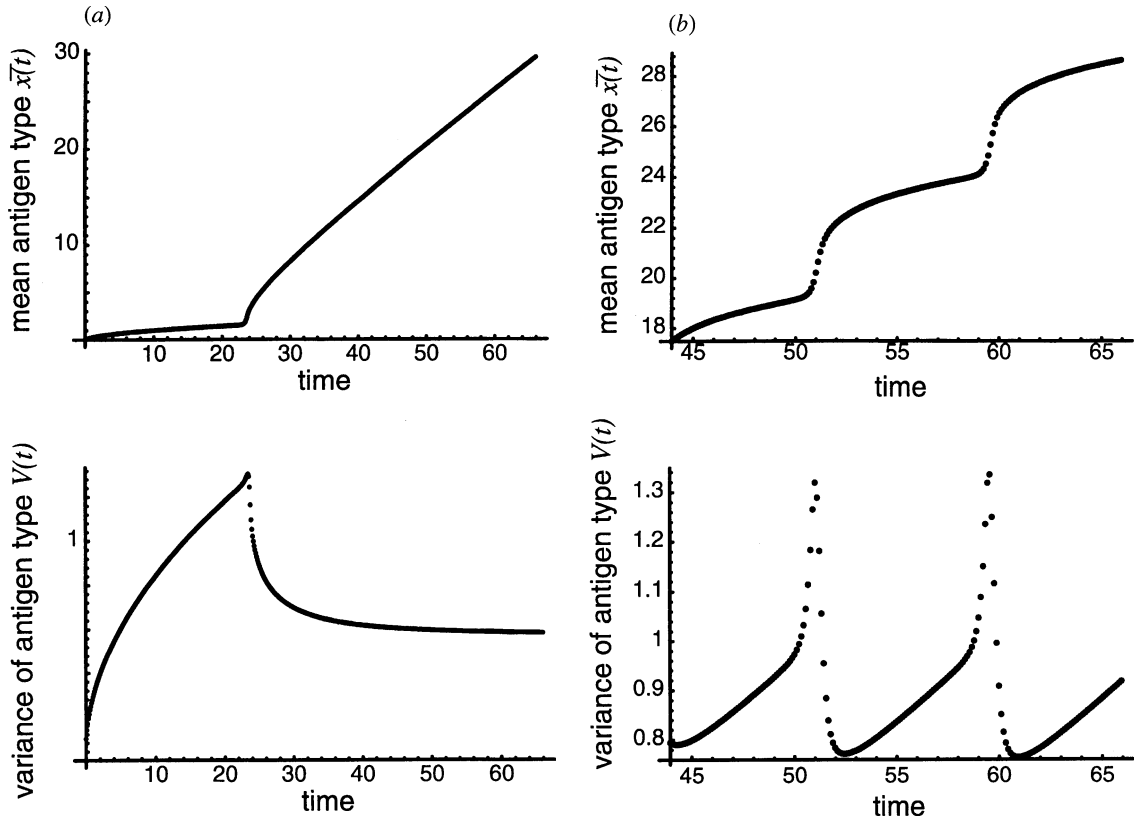

Figure 4. The clonal mean antigen type $\bar{x}(t)$ and the clonal diversity $V(t)$ as a function of time. The clonal mean antigen type and clonal diversity is zero at the start of the simulation, because the infection starts from the monomorphic pathogen population with antigen type 0. (*a*) Time change in $\bar{x}(t)$ and $V(t)$ if the width of cross-immunity is smaller than the threshold: $S_\beta = 0.099$ and $S_\alpha = 0.066$. (*b*) Time change in $\bar{x}(t)$ and $V(t)$ for the width of cross-immunity greater than the threshold: $S_\beta = 4.5$, $S_\alpha = 2.4$. $B_0^* = 0.0022$.

appearance of the escaping mutant that accumulated substitutions in epitope sufficiently to evade the current antiserum. The total viral density is maximum just after the sudden shift in divergence.

The clonal diversity $V(t)$ also fluctuates periodically (figure 4*b*). The diversity is maximum during the short phase of sudden rise in $\bar{x}(t)$, indicating that a new escaping mutant far separated from the current mean appears and replaces the others with an overwhelming advantage. The clonal diversity is minimum just after the transitions, because the population almost entirely consists of the single escaping mutant, and will be gradually restored by the accumulation of mutations.

(*d*) *Genotypic distance d* and time interval T* between successive outbreaks*

We can define the genotypic distance d^* between two predominant antigen types that caused the successive peaks of pathogen outbreaks, and the period T^* in the total density fluctuation. Figure 5 shows how d^* and T^* depend on the scaled width of cross-immunity S_β: For S_β below the threshold, there is no periodicity of outbreaks in genotype space and time, because the distribution of the antigen variants converges to stable travelling waves. A finite period $d^*(T^*)$ in genotype space (in total density change) appears when S_β exceeds the threshold. Both periods

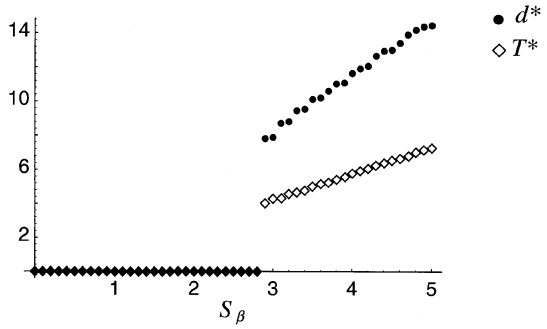

Figure 5. The genotypic distance d^* between antigen types that caused the successive peaks of outbreaks, and the period T^* in the total pathogen density fluctuation as a function of S_β. See the legend of figure 2 for the way to calculate d^*. T^* is determined from the power spectrum density estimate of time series data (Press *et al.* 1992) for the total densities observed in numerical simulations. Other parameters: $S_\alpha = 0$ and $B_0^* = 0.0022$. Note that there is the relationship $d^* = \nu T^*$, with $\nu = 2$.

then increase approximately linearly with S_β. We can see the linear relationship

$$d^* = \nu T^*, \tag{13}$$

where $\nu = 2$ is the scaled wave speed. This means that although the parasitaemia is highly localized in both

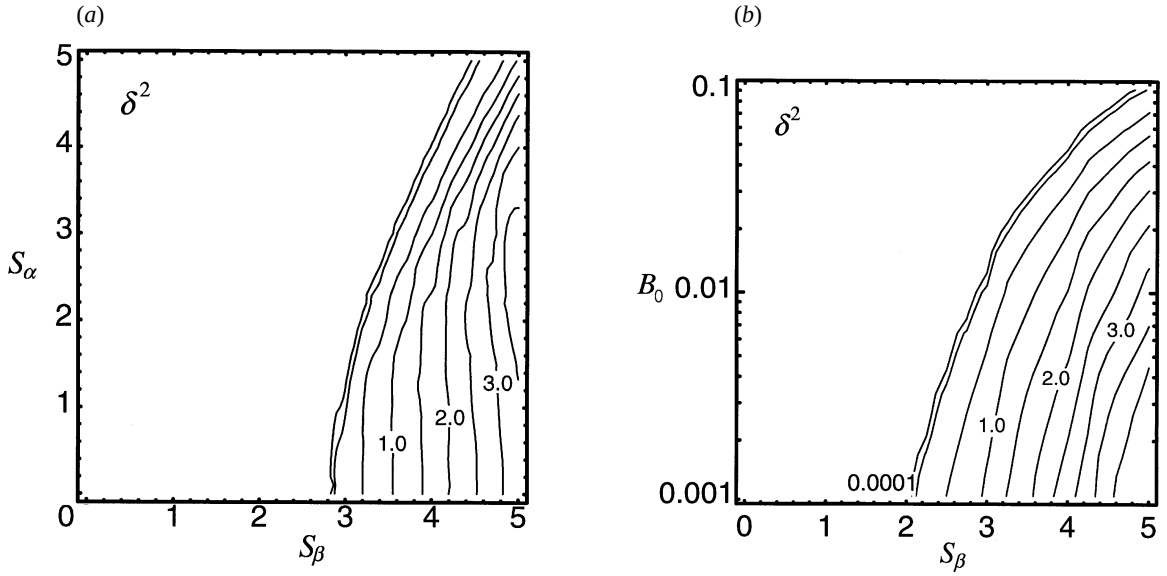

Figure 6. (*a*) The contours of the non-uniformity index, δ^2, observed in numerical simulations in the parameter space of S_α and S_β. The index δ^2 is defined as the mean square deviation of the linear regression applied for the time series data for clonal mean antigen type $(t, \bar{x}(t))$: i.e. $\delta^2 = (1/n)\sum_{i=n_0}^{n_0+n}|\bar{x}(t_i) - (at_i + b)|^2$, where a and b are determined by least-square fitting of the data (truncated that of the transient phase $i < n_0$). The thick curve represents the set of bifurcation points for asymptotic states from the stable travelling wave to episodic jumps. $B_0^* = 0.0022$. (*b*) The same as (*a*), but the contours are plotted in $S_\beta - B_0^*$ parameter space. $S_\alpha = 0$. Note that the vertical axis is logarithmic.

time and antigen type axes, these isolated peaks appear periodically along the straight line in the space-time space with the slope the same as the constant speed of the wave front (see figure 3).

(*e*) *Bifurcation diagram in parameter space*

To map the asymptotic patterns of antigenic drift in the parameter space, we used three indices for the non–uniformity of propagation: d^* and T^* defined above, and δ^2, which is defined as the mean square deviation of the linear regression, applied for the time series data $(t, \bar{x}(t))$ after initial transient. Since the mean antigen type $\bar{x}(t)$ continuously drifts away with a constant speed for S_β below the threshold (figure 4*a*), and it periodically steps away with fixed intervals in time and space for S_β above the threshold (figure 4*b*), this index measures how the antigen evolution is episodic in time and genotype space. The index δ^2 increases approximately linearly from zero as the parameter S_β increases past the threshold.

There are two surfaces dividing qualitatively different asymptotic states of the system in the three-dimensional rescaled parameter space of $(S_\alpha, S_\beta, B_0^*)$. The first surface, defined as $B_0^* = 1$, divides the persistence and eventual extinction of the pathogens. The second surface divides two different patterns of persistent antigenic drift, simple travelling waves and intermittent outbreaks, which depend mainly on the width of the cross-immunity S_β. Figure 6 illustrates the contours of the non–uniformity index δ^2 in $S_\beta - S_\alpha$ space (figure 6*a*) and in $S_\beta - B_0^*$ space (figure 6*b*). Using the other indices, d^* and T^*, gives the identical results for the bifurcation points. We can see from figure 6 that the condition for non–uniform outbreak can be written as

$$S_\beta \equiv \sigma_\beta / d_c > C(S_\alpha; B_0^*). \tag{14}$$

The threshold value C for S_β mildly depends on the other two scaled parameters S_α and B_0^*, varying as $C = 3 \sim 4$ in figure 6. Returning to the original scale, the threshold width of cross-immunity σ_β is proportional to the characteristic genotypic distance $d_c = \sqrt{(D/R)}$, the mean mutational steps of progeny produced in a unit duration of reproductive cycles $(1/R)$.

It is interesting to note that, although the dependence is weak, increasing the width of the cross-activation of the B cells, S_α, tends to stabilize the dynamics, in spite of the fact that increasing S_β destabilizes it. The stable travelling wave turns out to be stable, instead of the episodic jumps as S_α – increases for a fixed S_β (figure 6). The influence of the initial B-cell density B^{*0} is similar to that of S_α – increasing B_0 tends to stabilize the pathogen population dynamics.

3. DISCUSSION

This paper examined the evolution of pathogen antigen variations in a host. The analysis of the model reveals that the cross-immunity is very important in determining the micro-evolutionary pattern of antigen evolution: We observe dichotomous patterns of antigenic drift depending on whether the width of cross-immunity is above a certain threshold. The results are summarized as follows.

1. For the width of cross-immunity below the

threshold, the distribution of antigen variations converges to a travelling wave, and the clonal mean antigen type linearly diverges with time.

2. For the width of cross-immunity above the threshold, the travelling wave loses stability and the total pathogen density fluctuates periodically. The clonal mean antigen type shows a stair-like increase with time, and the clonal diversity will periodically rise at times of large shifts in the mean antigen type. The intuitive reason why this intermittent periodical outbreak occurs is that if the range of cross-immunity is sufficiently wide, the immune response to a predominant antigen type suppresses the growth of neighbouring antigen types as well. This leads to a long stasis in antigen evolution until it is finally broken by the appearance of the next escaping mutant that accumulates enough substitutions to evade the current immune response. The whole process then repeats itself.

3. The threshold for the width of cross-immunity is proportional to the mean mutational steps by which the progeny antigen type diverges from that of the parent. Thus the intermittent outbreak is expected if the immune response, induced by a predominant antigen variant, is still effective against the mutants produced in the next few replication cycles, and the stable travelling wave is expected if otherwise.

The importance of cross-immunity on the pattern of antigenic drift progression has been ignored by the previously developed models of antigen drift and switching (Agur *et al*. 1989; Nowak & May 1991, 1992; Nowak *et al*. 1991; Sasaki 1994). In a series of papers by Nowak and his colleagues (Nowak & May 1991, 1992; Nowak *et al*. 1991), the antigen types are defined as being equally different from each other (the Island model in the population genetical literature). Under this assumption, one is unable to describe the process of sequence divergence, because the genotypic distance cannot be defined. Sasaki (1994) considered the antigenic drift in one–dimensional antigen type space and discussed the pattern by which clonal mean antigen type shifts in time. However, the cross-reactivity is neglected in Sasaki (1994) by regarding the antigenic variations, which allow the cross-reactions by the same B cell, as belonging to the same antigen type. This approximation is in some sense supported by the present results. The qualitative behaviour is essentially the same as that of Sasaki (1994) if σ_β is below the threshold, and even above the threshold, the locations of successive outbreaks in time-antigen type space can be plotted on the line with the slope ν. However, if we focus on more detailed scales in time and antigen types, the pattern of propagation is very different from what the model without any cross-reactivity predicts.

Agur *et al*. (1989) have modelled the antigen switching of trypanosome, and concluded that the presence of a doubly expressed switch-intermediate phase of individual trypanosome is necessary to account for the observed patterns of trypanosome infections. When the fraction of indirect switch via the doubly expressed intermediate is larger than 0.9, among the total rate including direct switches between single expressors, the parasitaemia waves with roughly regular time intervals can be generated. These patterns, however, can also be generated by the cross-immunity, as our model suggests.

Now we briefly discuss the patterns of EIAV and HIV antigenic drift in relation to our model.

(*a*) *Recurrent febrile episodes in EIAV infection*

Montelaro *et al*. (1986) studied the antigenic drift of EIAV in a horse. In the case studied by Montelaro *et al*. (1986), there were four fibrile episodes with an interval of about 4–5 weeks during the chronic stage of the infection. By examining the antigenicity of viral variants sampled in each febrile episode, they showed that the antisera that was effective to the virus in the first episode is unable to neutralize the variants sampled from the later fibrile episodes, supporting the antigenic drift hypothesis.

They compared two glycoproteins gp90 and gp45 of each isolate sampled from four fibrile episodes. Multiple substitutions appeared to be responsible for the marked difference observed between the antigenicities of two isolates. This pattern of antigenic drift is consistent with what our model predicts when the width of cross-immunity is above the threshold – periodical bursts of viral density, associated with a large shift of antigen types between successive peaks. On the other hand, the observed pattern of antigenic drift is more complicated than our model predicts. There may be multiple major epitopes and the detailed analyses suggest that the recombination between and within epitopes would play a role in the antigenic drift of EIAV (Montelaro *et al*. 1986).

(*b*) *Antigen drift of HIV*

By censoring HIV disease progression in two patients, Wolfs *et al*. (1991) have studied the nucleotide sequences of part of the envelope coding region during five years. The extent of genomic diversity of the intra-host viral population (clonal diversity) varies during the course of the HIV infection (Wolfs *et al*. 1991). In both patients, there were two phases of massive HIV replications: the first peak immediately following the infection, and the second peak about five years after the infection. The number of amino acid differences between the clonal sequences and the founder sequence increases with time, with the rate varying with time. On the other hand, the clonal diversity (the mean hamming distance between sequences at each time of census) first increases at the phase of massive HIV replication described above, but then declines. The clonal diversity again increases at the final stage of the productive phase. This result can be compared to our results for the clonal diversity evolution (see figure 4).

In the cases studies by Wolf *et al*., the mean sequence difference from the inoculated consensus increases with time with a diminishing rate. There are no available data after the second productive phase of viral growth, so it is not clear whether the difference again increases rapidly after the plateau, as predicted from our model. There may not be the second rapid increase at all if the

number of sites responsible for the antigen-determining epitope is sufficiently large. This is because if the antigen space is highly dimensional, there are many *new* directions for the progression of antigenic drift, and new genotypes are not necessarily more distant from the founder sequence than their predecessor. The result is the saturating divergence from the founder sequence, even if the predominant antigen type continues to drift away from the current type. These conjectures will be examined in the accompanying paper (A.S., Y.H. and N. Gonda in preparation).

According to more detailed studies on the intra-host evolution of the HIV envelope gene hypervariable V3 region, one of the major epitope of HIV, the evolution rate of both synonymous and non-synonymous sites are found to vary with time (Yamaguchi and Gojobori, personal communication). Moreover, the rate of non-synonymous substitution is several times higher than that of the synonymous one at some periods of time since infection, indicating the predominance of positive selection in antigen-determining sites. The fact that the evolution rate varies with time could again be compared to the predicted pattern of antigen evolution when the width of cross-immunity is above the threshold (figure 3).

(*c*) *Antigenic switching of trypanosome*

Some parasitic protozoa and bacteria (e.g. *Trypanosoma brucei*, *Neiseria gonorrhoeae*, *Boleria* bacteria) also switch their antigen type to evade the host immune defence (see, for example, Birkbeck & Penn (1986) and Borst & Greaves (1987)). Although the underlying molecular mechanism of the antigen switching is quite different from that of viral antigen drift that primarily relies on point mutations, it is tempting to apply our model to these cases. A trypanosome parasite stores a large number of silent antigenic variations in its genome, and hence the expressed antigen type would change to a very different one by a single switching event. If, however, there is a tendency that switching most probably occurs between homologous sequences, the population/genotypic dynamics of antigenic switching would be very similar to the analysis by the present model.

Although we failed to derive an analytical result for the threshold dividing two qualitatively different asymptotic states, the results are parallel to the analytical results obtained for a single-species competition model (Sasaki 1997): Assuming that the members of a single species in the neighbourhood compete for spatially distributed resource, it can be shown analytically that a highly clumped distribution, triggered by tiny spatial heterogeneity in resource level, is generated if the width of cross-interaction σ between neighbours is greater than the characteristic migrational distance (Sasaki 1997), analogous to the results obtained here. It can also be shown that the characteristic period in spatial distribution should increase approximately linearly as σ increases past the threshold, as is numerically confirmed in the present model.

In this paper, we assume that the antigen type can be indexed in one–dimensional space. In other words, we assume that a pathogen antigen type has only two ways to evade the immune defence. This assumption seems to be too restrictive to apply to the antigenic drift of viruses including HIV, EIAV and influenza. However, if only a small number of paths, among all possible ones, are taken during the course of antigenic drift progressions (i.e. if the branching pattern along the phylogenetic tree of antigenic variants is rather simple), then the model might capture the essential characteristic of the antigen evolution.

In deriving the results, we assumed a specific form, Gaussian, for the immune response, decreasing its strength with genotypic distance. However, we conjecture that the qualitative results remain the same for influential kernels other than Gaussian, as long as it declines with distance monotonically and sufficiently steeply. Indeed, we numerically confirmed the existence of the same kind of threshold for the cases of geometrically decreasing and linearly decreasing (triangular) kernels, though the values of the threshold should change accordingly.

We also assumed that a single mutation can change the type of antigen only to one of the nearest neighbours. This assumption, however, is by no means restrictive, because progeny that accumulated several substitutions will appear, though not many, in a given time interval. A single-point mutation may radically change the antigenic property. If this is common among spontaneous mutations at antigen-determining sites, the scaled width S_β of cross-reactivity must be smaller than the threshold, and we expect the stable travelling wave to be the pattern of antigen evolution.

To simplify the analysis of the model, we ignored several potentially important factors of the immunological processes. First, we ignored the mortality of the B lymphocytes, which therefore dictates that the once-activated immune response will not decline in the host individual. Alternatively and preferably, we could model the dynamics of memory B cells, instead of assuming immortal B cells. Second, we ignored the fact that B-cell population also diffuses in their genotype space by the somatic mutation on the DNA sequence encoding the variable regions of the immunoglobulin and T-cell receptor. This might enable the immune system to respond more rapidly to the antigenic drift of pathogens, and might have similar effect on increasing the width of cross-activation σ_α in our model. The somatic mutation rate varies during the process of a B-cell proliferation, which motivated the models of the optimal mutation schedule of the immune system (Agur *et al*. 1991; Kepler & Perelson 1995). This might suggest that adding a diffusion process in B-cell genotype dynamics would not be enough to take into account the effect of affinity maturation.

Our model would make a major contribution in revealing that the extent of cross-reactivity primarily determines the pattern of antigenic drift and disease progressions. However, real evolutionary processes must be more complicated as discussed in the case of EIAV and HIV infections, and the model no doubt needs further extensions. For example, the present model cannot be applied if there are more than two

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epitopes (see Nowak *et al*. (1995) for the interplay between intra-host dynamics and immunodominance which arises if there are more than two epitopes). In the accompanying paper, we attempt to model the antigenic drift in which genotypes are defined as the set of amino acid residues at multiple sites of the epitope (A.S., Y.H. and N. Gonda in preparation), hoping that it would capture another aspect of the antigenic drift observed in nature.

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REFERENCES

Agur, Z., Abiri, D. & Van der Ploeg, L. H. T. 1989 Ordered appearance of antigenic variants of African trypanosomes explained in a mathematical model based on a stochastic switch process and immune-selection against putative switch intermediates. *Proc. natl. Acad. Sci., U.S.A.* **86**, 9626–9630.

Agur, Z., Mazor, G., & Meilijson, I. 1991 Maturation of the humoral immune response as an optimization problem. *Proc. R. Soc. Lond.* B. **245**, 147–150.

Birkbeck, T. H. & Penn, C. W. 1986 *Antigenic variation in infectious diseases*. Oxford: IRL Press.

Borst, P. & Greaves, D. R. 1987 Programmed gene rearrangements altering gene expression. *Science* **235**, 658–667.

Fisher, R. A. 1937 The wave of advance of advantageous genes. *Ann. Eugenics* **7**, 353–369.

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

Kepler, T. & Perelson A. 1995 Modeling and optimization of populations subject to time-dependent mutation. *Proc. natl. Acad. Sci. U.S.A.* **92**, 8219–8223.

Kolmogorov, A. N., Petrovskii, I. G. & Piskunov, N. S. 1937 A study of the equation of diffusion with increase in the quantity of matter, and its application to a biological problem. *Bjul. Moskovskovo Gos. Univ.* **17**, 1–72

Laver, W. G. & Webster, R. G. 1973 Studies on the origin of pandemic influenza. III. Evidence implicating duck and equine influenza viruses as possible progenitors of the Hong Kong strain of human influenza. *Virology* **51**, 383–391.

Montelaro, R. C., Issel, C. J., Payne, S. & Salinovich, O. 1986 Antigenic variation during persistent infectious by equine infectious anemia virus. In *Antigenic variation in infectious disease* (eds T. H. Birkbeck & C. W. Penn), pp. 41–56. Oxford: Washington DC.

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. & May, R. M. 1991 Mathematical biology of HIV infections: antigenic variation and diversity threshold. *Math. Biosci.* **106**, 1–21.

Nowak, M. & May, R. M. 1992 Coexistence and competition in HIV infections. *J. theor. Biol.* **159**, 329–342.

Nowak, M. A., May, R. M. & Sigmund, K. 1995 Immune response against multiple epitopes. *J. theor. Biol.* **175**, 325–353.

Press, W. H., Teukolsky, S. A., Betterling, W. T. & Flannery, B. P. 1992 *Numerical recipes in C*, 2nd edn, Cambridge: Cambridge University Press.

Sasaki, A. 1992 The evolution of host and pathogen genes under epidemiological interaction. In *Population pareogenetics. Proceeding of the seventeenth Taniguchi international symposium on biophysics* (ed. N. Takahata), pp. 247–263. Tokyo: Japan Scientific Society Press.

Sasaki, A. 1994 Evolution of antigen drift and switching: Continuously evading pathogens. *J. theor. Biol.* **168**, 291–308.

Sasaki, A. 1997 Clumped distribution by neighborhood competition. *J. Theor. Biol.* (In the press.)

Waddel, G. H., Teigland, M. B. & Sigel, M. M. 1963 A new influenza virus associated with equine respiratory disease. *J. Am. vet. Med. Ass.* **143**, 587–590.

Wolfs, T. F. W., Zwart, G., Bakker, M., Valk, M., Kuiken, C. L. & Goudsmit, J. 1991 Naturally occurring mutations with HIV-1 V3 genomic RNA lead to antigenic variation dependent on a single amino acid substitution. *Virology* **185**, 195–205.

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