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Virus phenotype switching and disease progression in HIV-1 infection

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One of the phenotypic distinctions between different strains of human immunodeficiency virus type 1 (HIV-1) has to do with the ability to cause target cells to form large multinucleate bodies known as syncytia. There are two phenotypes according to this characterization: syncytium-inducing (SI) and non-syncytium-inducing (NSI). NSI strains are usually present throughout infection, while SI strains are typically seen at the beginning of the infection and near the onset of AIDS. The late emergence of SI strains is referred to as phenotype switching. In this paper we analyse the factors that lead to phenotype switching and contribute to the dynamics of disease progression. We show that a strong immune system selects for NSI strains while a weak immune system favours SI strains. The model explicitly accounts for the fact that CD4+ cells are both targets of HIV infection and crucial for activating immune responses against HIV. In such a model, SI strains can emerge after a long and variable period of NSI dominated infection. Furthermore, versions of the model which do not explicitly account for HIV-specific, activated CD4+ cells do not exhibit phenotype switching, emphasizing the critical importance of this pool of cells.

Keywords: syncytium-inducing; non-syncytium-inducing; phenotype switch; HIV-1; mathematical model

1. INTRODUCTION

Acquired immunodeficiency syndrome (AIDS) is a disease caused by the human immunodeficiency virus (HIV). In the first few weeks after infection with HIV, patients experience a period of acute viraemia known as primary infection. The end of this phase is usually coincident with the first signs of an immune response against HIV (Koup *et al*. 1994; Safrit & Koup 1995; Price *et al*. 1997). An asymptomatic period follows primary infection, which can vary from two to 15 or more years (Pantaleo & Fauci 1996; Phair 1994). Although no visible symptoms are present, the replication kinetics of the virus are extremely fast during this period (Ho *et al*. 1995; Wei *et al*. 1995; Perelson *et al*. 1996). Still, virus levels change very little in this time, and the immune system is thought to be the mechanism which controls the virus (Haynes *et al*. 1996; Mackewicz *et al*. 1991; Haas *et al*. 1996; Goulder *et al*. 1997). At the onset of AIDS (when the CD4+ T-helper cell count falls below 200 cells μl^{-1}) there is an overall weakness of the immune system which allows the establishment of opportunistic diseases (Haynes *et al*. 1996). These diseases eventually lead to death.

Throughout infection the viral population is composed of a quasi-species of strains (Nowak *et al*. 1991; Coffin 1995) that can differ in, among other things, replication rate, cell tropism and the ability to cause target cells to form large multinucleate bodies (syncytia). It has been reported that in a vast majority of patients the strains recovered just after primary infection are of the non-syncytium-inducing (NSI) phenotype, and that this phenotype generally predominates in the asymptomatic phase (Roos *et al*. 1992; Zhu *et al*. 1993). This is true even in cases in which syncytium-inducing (SI) strains were contained in the infectious inoculum and observed to proliferate during primary infection (Cornelissen *et al*. 1995; Groenink *et al*. 1991; Baur *et al*. 1989). However, because HIV replicates quickly and mutates with a high probability, SI mutants can arise from the NSI population, and consequently in about 50% of patients, SI strains are seen to re-emerge late in infection (Miedema *et al*. 1994; Schuitemaker *et al*. 1992), a phenomenon sometimes referred to as phenotype switching. The presence of SI strains has been found to correlate with worsened prognosis and accelerated disease progression (Tersmette *et al*. 1989; Connor *et al*. 1993; Miedema *et al*. 1994).

The switch from an NSI to an SI phenotype has been mapped to as few as one or two amino-acid substitutions in the V3 loop of the virus envelope (de Jong *et al*. 1992; Fouchier *et al*. 1992). The question that has puzzled researchers for some time is why this change is not observed sooner *in vivo*. Indeed, due to the high replication rate of the virus and its mutation rate, those amino-acid changes, if non-deleterious, should be present soon after infection (Coffin 1995; Ribeiro *et al*. 1998). This is, for instance, why drug resistance arises so quickly in cases where very few mutations are needed for virus escape

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(Loveday *et al*. 1995; Richman 1996; Boucher & Reedjik 1995). This suggests that some other phenomenon must be hindering the SI strain's ability to appear in the population, and that at later stages of infection this phenomenon is less important or is absent (Wodarz & Nowak 1998; Wodarz *et al*. 1999). Evidence points to the possibility that the immune response against the SI strains is stronger than against the NSI strains (Schuitemaker *et al*. 1992; Bouhabib *et al*. 1994; Cornelissen *et al*. 1995; Fauci 1996; Koot *et al*. 1999). This has been further supported by measures of ds/dn ratios of SI and NSI strains (Bonhoeffer *et al*. 1995) and by other recent results (Herbein *et al*. 1998).

In this paper we present a model that addresses the question of phenotype switching during the asymptomatic phase of HIV infection. We will assume that the immune response against both strains is mediated by CD4+ T-helper cells specific for HIV (Kalams & Walker 1998; Rosenberg & Walker 1998; Rosenberg *et al*. 1997). We show that the progressive depletion of this cell pool can lead to the phenotype switch, and that such a model explains the variation among patients in the time to emergence of the SI strain.

2. THE MODEL

HIV infects a variety of cells that express both CD4 and an appropriate co-receptor (Berger 1997). In this paper we are primarily concerned with what happens to CD4+ T cells, which are considered to be the main target of HIV infection, as well as an essential population in mounting the anti-HIV immune response. It has been shown that HIV productively infects only activated CD4+ T cells, and it is not able to replicate in the resting pool of T cells (Bukrinsky *et al*. 1991; Stevenson 1996). On the other hand, not all activated CD4+ T cells are specific for HIV and only those that are specific will participate in the immune response against HIV.

Accordingly, our model includes the concentrations of four populations of CD4+ T cells: resting cells both non-specific (R_1) and specific (R_2) for HIV epitopes, as well as the corresponding antigenically activated cells, A_1 and A_2 (McLean & Kirkwood 1990; McLean & Nowak 1992). The model incorporates cells infected by HIV strains exhibiting the two phenotypes: those infected by the NSI virus (I_n), and those infected by the SI virus (I_s). We define the rates of change of these six variables as follows: the resting cells (R_1 and R_2) are produced at constant rates (λ_1 and λ_2, respectively), and die with natural death rates $d_R R_1$ and $d_R R_2$, respectively. In the model, non-HIV-specific resting cells, R_1, encounter a constant background abundance of antigen, N, whereas the HIV-specific resting cells, R_2, are activated by the total amount of HIV-infected cells present, I_n+I_s. The resting cells and infected cells are considered to be well mixed and thus their activation is determined by a mass action term, i.e. activation occurs at a rate proportional to the abundance of the specific antigen and the number of resting cells, with a rate constant α; that is, $\alpha N R_1$ and $\alpha(I_n+I_s)R_2$. Upon activation, both specific and non-specific cells are assumed to proliferate by a factor s, as they move from the resting pool to the active pool. The activated cells have natural death rates $d_A A_1$ and $d_A A_2$. The infection of susceptible cells by free virus can be represented according to a mass action term proportional to the amount of virus. However, it is known that free virus dynamics are extremely fast (Ho *et al*. 1995; Wei *et al*. 1995; Perelson *et al*. 1996), and consequently it is valid to assume that virus concentration changes instantaneously with the concentration of infected cells. This allows us to represent free virus as a function of the infected cells, hence we do not include a separate equation for virus frequency (Bonhoeffer *et al*. 1997). In this way, the activated cells are infected by the two strains of virus according to a mass action term that is represented by $\beta_n I_n A_i$ or $\beta_s I_s A_i$, for NSI or SI virus respectively, and where A_i can represent either specific (A_2) or non-specific activated cells (A_1). Infected cells die at a rate aI_i. An obvious extension to this model would be to relax the assumption that SI- and NSI-infected cells die at the same rate. Finally, we include a specific immune response directed against HIV. Because successful immune responses against HIV (such as cytotoxic T lymphocytes and antibodies) require CD4+ T cells to function (Kalams & Walker 1998; Rosenberg & Walker 1998; Rosenberg *et al*. 1997), we consider the response to be proportional to the amount of HIV-specific, active CD4+ cells present, with rate constants u_n for NSI virus and u_s for SI virus; that is, $u_n I_n A_2$ and $u_s I_s A_2$, respectively. Taken together, the equations of the model are as follows:

$$\dot{R}_1 = \lambda_1 - d_R R_1 - \alpha N R_1,$$
$$\dot{R}_2 = \lambda_2 - d_R R_2 - \alpha(I_n + I_s)R_2,$$
$$\dot{A}_1 = s\alpha N R_1 - d_A A_1 - (\beta_n I_n + \beta_s I_s)A_1,$$
$$\dot{A}_2 = s\alpha(I_n + I_s)R_2 - d_A A_2 - (\beta_n I_n + \beta_s I_s)A_2,$$
$$\dot{I}_n = \beta_n I_n (A_1 + A_2) - aI_n - u_n I_n A_2,$$
$$\dot{I}_s = \beta_s I_s (A_1 + A_2) - aI_s - u_s I_s A_2.$$

The first equation, which describes the evolution in time of the frequency of resting cells not specific for HIV, is independent of all the others. If the frequency of these resting cells is in equilibrium, then $R_1 = \lambda_1/(d_R + aN)$. This expression can be substituted into the equation for the rate of change of A_1, and hence we can omit the first equation.

In this model we are principally interested in the behaviour of T cells, however HIV also infects other cell populations, such as macrophages (Gartner *et al*. 1986; Koenig *et al*. 1986). We have found that the main results shown in this paper do not differ when we include macrophages in the model. Thus, for simplicity, macrophages are omitted in the analysis and discussion of the model.

All strains of HIV, including both NSI and SI, infect primary T cells *in vitro* (Berger 1997). This suggests that these strains have a common target cell pool *in vivo*. However, it is now known that for the most part NSI and SI strains use different co-receptors for cell entry (Berger 1997). In this context, it is important to note that we chose to present a model which does not separate target cell populations into those displaying the CCR-5 co-receptor, which the NSI virus typically uses for entry, and the CXCR-4 co-receptor, which the SI virus typically uses (Berger 1997). Such a consideration

involves separating each population of resting and active cells described above into two distinct populations according to their co-receptor expression. Consequently, the number of equations describing resting and active cells increases from four to eight, resulting in a system of ten coupled differential equations. Our studies indicate that the main results we obtain from the model above hold for this more complicated model. Below we address more thoroughly the differences in behaviour between these two models.

3. ANALYSIS OF THE MODEL

An important feature of this model is that the equations governing the dynamics of both strains are the same; we differentiate between the two only in our choice of parameter values for β and u. The first determines the fitness of the virus as measured by R_0. This index (Anderson & May 1993) represents how many new infected cells are produced when one infected cell is put in a wholly susceptible population of uninfected cells. In our model, $R_{0i}=\beta_i\alpha\mathcal{N}\lambda_1/(d_A(d_R+\alpha\mathcal{N})a)$, where $i=n$ or s. Experimental evidence suggests that the SI virus is more virulent than the NSI virus (Tersmette *et al*. 1989; Connor *et al*. 1993; Fouchier *et al*. 1996; Koot *et al*. 1996), and so we will choose $R_{0s}>R_{0n}$ (or equivalently, $\beta_s>\beta_n$). On the other hand, it has been recently suggested that both NSI and SI are equally cytopathic (Grivel & Margolis 1999), but that the SI virus has a larger target cell population. Our model is consistent with this alternative theory, because a larger target cell population for SI would necessarily mean a larger R_0 for this strain. The second parameter, u, controls the strength of the immune response against the virus. It is likely that this response is stronger against the SI virus than against the NSI strains, that is, $u_s>u_n$, (Schuitemaker *et al*. 1992; Bouhabib *et al*. 1994; Fauci 1996; Cornelissen *et al*. 1995; Bonhoeffer *et al*. 1995; Herbein *et al*. 1998).

The parameters β_s, β_n, u_s, and u_n completely determine the differences between the two virus populations, and can be used to find the conditions for emergence of the SI virus. Consider the last two equations of the model, which can be rewritten as follows:

$$\dot{I}_n = f(A_1, A_2)I_n,$$
$$\dot{I}_s = g(A_1, A_2)I_s.$$

If $g(A_1, A_2)>f(A_1, A_2)$ for all values of A_1 and A_2, then the per cell growth rate will always be greater for SI-infected cells than for NSI-infected cells. Hence, if both strains are present during primary infection, the SI strain will eventually outgrow the NSI strain, without a period of prolonged suppression. It is easily seen that $g(A_1, A_2)>f(A_1, A_2)$ for all A_1 and A_2 if $\beta_s>\beta_n$ and $u_s<u_n$. This is a sufficient condition for the persistence of SI strains throughout infection. Alternatively, this result shows that $u_s>u_n$ is a necessary condition for the early dominance of the NSI strain. We now address the conditions for invasion of the NSI dominated equilibrium by the SI strain.

When the system is in a steady state with only the NSI virus present, as is the case in many patients during the asymptomatic phase, the condition for invasion of the SI virus is derived from the $\dot{I}_s$ equation, as follows:

$$\beta_s(A_1^* + A_2^*) - a - u_sA_2^* > 0,$$

where the $(^*)$ denotes equilibrium values. Since the system is in a steady state, $I_n=0$, and we are able to reach the following condition:

$$\begin{cases} \dot{I}_n = 0 \\ \dot{I}_s > 0 \end{cases} \Leftrightarrow \begin{cases} \beta_n(A_1^* + A_2^*) = a + u_nA_2^* \\ \beta_s(A_1^* + A_2^*) > a + u_sA_2^* \end{cases}$$

$$\Leftrightarrow a(\beta_s - \beta_n) + A_2^*(\beta_su_n - \beta_nu_s) > 0.$$

We have made the assumption that $\beta_s>\beta_n$. If, in addition, $\beta_su_n-\beta_nu_s>0$ (i.e. $u_s/u_n<\beta_s/\beta_n$) then the above condition is always fulfilled. Thus, the SI strain will always invade the NSI-only equilibrium, regardless of the amount of HIV-specific activated CD4+ cells remaining at this equilibrium. Though this condition is sufficient for invasion, it is not necessary since it is possible that the SI strain could emerge before the NSI virus attains equilibrium.

Alternatively, for $\beta_su_n-\beta_nu_s<0$ (i.e. $u_s/u_n>\beta_s/\beta_n$), the condition for invasion is fulfilled only if

$$A_2^* < a\frac{\beta_n - \beta_s}{\beta_su_n - \beta_nu_s}.$$

Since we assume $\beta_s>\beta_n$, this invasion criterion is relevant only when the immune response against the SI strains is stronger than against the NSI strains ($u_s>u$), as might be expected from the experimental evidence cited above.

We have determined that a necessary condition for suppression of the SI strain after primary infection is $u_s>u_n$. Under this condition, if $u_s/u_n<\beta_s/\beta_n$, the SI virus will invade the NSI equilibrium independently of the amount of HIV-specific activated cells. Alternatively, if $u_s/u_n>\beta_s/\beta_n$, these cells must be depleted below a certain level in order for the SI virus to invade. One way by which the SI virus might be introduced into the population during NSI dominance is via mutation from the NSI virus. This is plausible because, as stated above, NSI and SI variants may differ by only one or two amino acids (de Jong *et al*. 1992; Fouchier *et al*. 1992). It is also for this reason that in the simulations of the model we include mutation between NSI and SI variants at a rate μ. Phenotype switching is observed for a large range of mutation rates, specifically for any $0<\mu<0.001$.

4. RESULTS

Motivated by this analysis, we now turn to numerical simulations to explore the case where $u_s>u_n$. Several important features of the model's dynamics for this case are evident in figure 1. After initial transient dynamics, the number of NSI-infected cells slowly approaches a set point, and the SI-infected cells are maintained by mutation from the NSI pool at very low levels. As the HIV infection persists, the number of active, HIV-specific CD4+ T cells gradually declines (figure 1*b*). As this pool decreases in size, the strength of the immune response against HIV declines. When that cell population falls

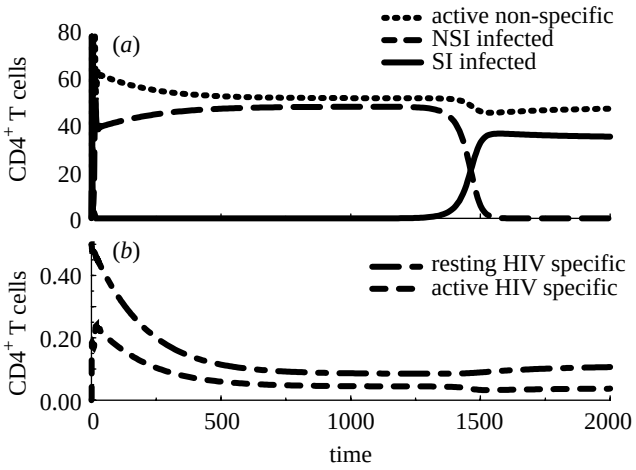

Figure 1. Example of phenotype switching in the minimal model. After primary infection, a long period of NSI dominance ensues. This is followed by a rapid emergence of the SI strain (*a*). The HIV-specific host response decreases slowly over time as in (*b*), until a threshold is reached and the SI virus gains a selective advantage. Parameters are $\lambda_1 = 0.9995$, $\lambda_2 = 0.0005$, $d_R = 0.001$, $\mathcal{N} = 10$, $\alpha = 10^{-4}$, $s = 50$, $d_A = 0.05$, $\beta_n = 0.0086$, $\beta_s = 0.013$, $a = 0.433$, $u_n = 0.5$, $u_s = 5.3$, $\mu = 10^{-8}$.

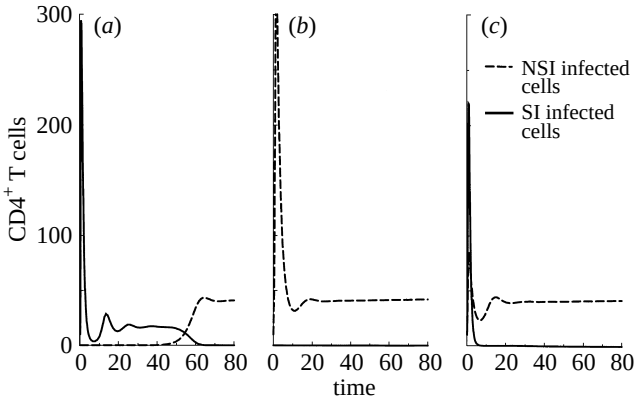

Figure 2. Initial conditions do not affect long-term dynamics. Inoculation with SI alone (*a*), NSI alone (*b*), or both virus strains together (*c*), results in dominance of the NSI virus. The ensuing dynamics are identical to those observed in figure 1. Parameters chosen as in figure 1.

below a critical threshold, the immune response is no longer strong enough, and the SI strain gains a selective advantage and becomes the dominant strain. Results from the more complete model, with sub-populations expressing CCR-5 and CXCR-4, confirm the general behaviour described above, i.e. there is a large range of parameters for which the SI strain does not emerge until after a period of NSI dominance. However, there are two important differences observed in the larger model. First, since the SI and NSI strains no longer compete for the same populations of target cells, we do not observe competitive exclusion. Consequently, after the emergence of the SI variant, the NSI strain remains in the population and, in fact, it might even be more prevalent than the SI strain, because the relative amounts of each strain depend on the parameters. This result is supported by clinical observations, in which both strains are shown to co-exist late in infection (Koot *et al*. 1996). Also, during the period of dominance of the NSI strain, the SI strain replicates at very low levels. This has been recently confirmed (H. Schuitemaker, personal communication). Second, since the SI strain is either more virulent (Tersmette *et al*. 1989; Connor *et al*. 1993; Fouchier *et al*. 1996; Koot *et al*. 1996) or has a larger pool of target cells (Grivel & Margolis 1999), when it finally emerges there is a significant depletion of the total CD4+ T-cell population. Again this is in agreement with experimental results (Koot *et al*. 1996).

While these are important results, the addition of distinct target cell populations does not alter the more general feature of phenotype switching observed in the simpler model. It is because we wish to understand specifically this feature of HIV disease progression that we have focused our efforts on the more minimal model which does not account for co-receptor expression. However, the remainder of our results hold for both models.

We find that phenotype switching is not dependent on initial conditions. Figure 2 shows that inoculating with SI virus only (figure 2*a*), NSI virus only (figure 2*b*), or equal amounts of both (figure 2*c*) always leads to the same result: early in infection both strains can be present, but after a short period of time the NSI strain dominates and the SI strain is driven to minute levels. From this point on, the dynamics are identical to those seen in figure 1. Perhaps the most interesting case is shown in figure 2*a*, where the NSI virus is not present in the initial inoculum; it emerges after the SI virus establishes a transient infection and produces NSI mutants.

The time elapsed from primary infection to the phenotype switch is highly variable, and depends on parameters such as immune response to the SI virus, background activation, and virulence of the SI virus. Figure 3*a* shows that a large immune response results in a long time to emergence. Increasing u_s strengthens the selective pressure against the SI virus; consequently fewer HIV-specific active cells (A_2) are needed to keep the virus strain from emerging, and hence the frequency of A_2 cells must drop to a lower level, requiring a greater amount of time, before the SI virus can escape immune control. For sufficiently large values of u_s, the SI virus never re-emerges, and for small values it dominates the population immediately.

In figure 3*b*, a larger amount of background activation results in a shorter time to SI emergence. By increasing the amount of background antigen present (determined by the parameter $\mathcal{N}$), the number of non-specific activated cells responding to infection, A_1, is increased. This expands the size of the target pool for both virus strains, but because the SI strain is more virulent than the NSI strain (i.e. $\beta_s > \beta_n$), the SI virus infects these cells more efficiently. Thus if A_1 is large, more HIV-specific immune cells are needed to contain the SI virus, and as the number of HIV-specific cells decays, the point at which the SI virus can emerge is reached earlier.

Figure 3*c* shows that SI strains which are more virulent will emerge sooner. This parameter of the strain is increased by changing its reproductive ratio, R_{0s}. In this case, more HIV-specific active cells (A_2) are required to

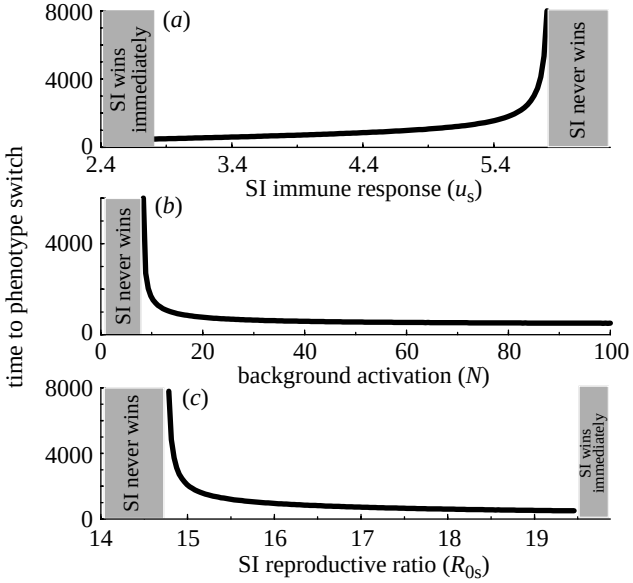

Figure 3. Effect of parameters on time to SI emergence. (*a*) Increasing u_s results in greater selective pressure against the SI virus strain; this increases the time to phenotype switch. Beyond a certain value of u_s, the NSI virus persists for the duration of the infection. Similarly, below a certain threshold, the NSI virus never dominates, and SI is the dominant virus strain from primary infection onwards. (*b*) As background activation increases, the time required for the SI virus to shift into dominance decreases. As in (*a*), there is a threshold value below which the SI virus can never establish infection. (*c*) Increasing the reproductive ratio, R_{0s}, results in a shorter time to SI emergence. Once again there are thresholds, below which the SI virus never wins and above which the SI virus wins immediately and for all time. With the exception of the parameter being varied in each figure, all parameters are defined as in figure 1.

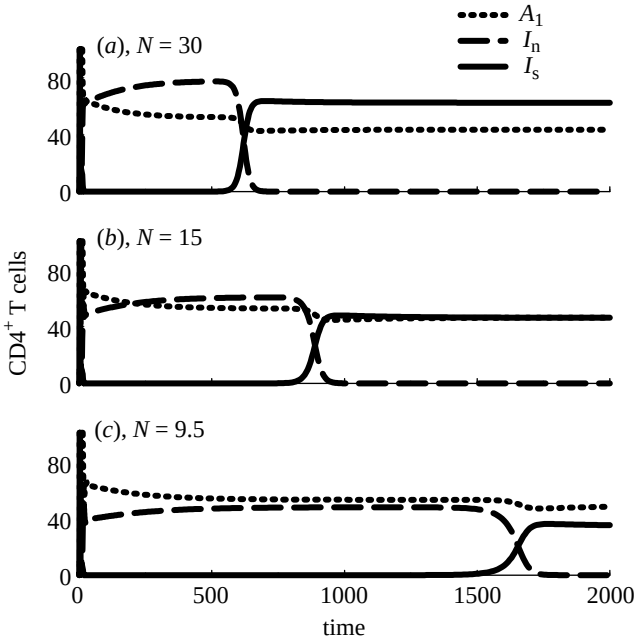

Figure 4. Changing background activation, N, shows that virus load can increase as the time to SI emergence simultaneously decreases. (*a*) $N=30$. Before SI emergence, $I_n \approx 80$; SI emerges near $t=600$. (*b*) $N=15$. Before, $I_n \approx 60$; SI emerges near $t=800$. (*c*) $N=9.5$. $I_n \approx 50$ before emergence; SI emerges near $t=1600$. With the exception of N, all parameters are as in figure 1.

contain a strain of higher virulence. Thus the threshold of A_2, below which the SI strain can emerge, is higher and consequently reached sooner. Note that the parameters varied in figure 3 were chosen because their function is considered to be particularly important in determining the progression of HIV (Pantaleo & Fauci 1996).

The results in figure 4 show that a negative correlation exists between the number of infected cells prior to phenotype switching and the length of time between primary infection and emergence of the SI strain. Parameters for each case are identical with the exception of N, the background activation. Similar results were obtained by varying α, the activation coefficient (not shown).

To test whether phenotype switching is a general feature of the model, we ran simulations which sampled a much broader range of the parameter space than in figures 3 and 4. Indeed, phenotype switching was still a commonly observed feature (data not shown), demonstrating the robustness of the model and that its behaviour is not the consequence of a degenerate choice of parameters.

A central feature of this model is the incorporation of a population of CD4+ cells which is both susceptible to infection by HIV and, when active, crucial to the immune response against HIV. In order to assess the importance of these active CD4+ cells in the emergence of the SI strain late in infection, we constructed two additional models: one which treated the HIV-specific and non-specific populations as one while still separating resting and active cells, and another which distinguished between HIV-specific and non-specific cells, but did not distinguish between resting and activated cells. In the first model, phenotype switching did not occur. In the second model phenotype switching occurred, but only for a very narrow range of parameters, specifically ones which made the active cell average lifetime unreasonably long. These results suggest that the phenotypic switch is heavily dependent upon the active, HIV-specific CD4+ population.

5. CONCLUSIONS

In this paper we have presented a model which exhibits the phenomenon of phenotype switching in a manner which is consistent with clinical and experimental observations. Specifically, after primary infection, in which both strains can be present, the model exhibits a period of NSI dominance which is variable in length and can be followed by emergence of the SI strain, as is common in HIV-infected individuals who progress to AIDS (Tersmette *et al*. 1989; Connor *et al*. 1993; Miedema *et al*. 1994; Pantaleo & Fauci 1996). In the model, this behaviour does not depend upon the amount of each virus strain initially present, as is the case in clinical studies of primary infection (Cornelissen *et al*. 1995; Pratt *et al*. 1995; Roos *et al*. 1992; Van't Wout *et al*. 1994). Furthermore, as in these studies, though the SI virus can replicate in primary infection it is not seen again until much later in the progression of the disease. The most important parameters in the model which affect the duration of the

NSI-dominated phase are those which determine the basic reproductive ratio, background activation, and the immune response directed against the SI strain. Studies which show that patients with higher levels of immune system activation, such as infected persons in regions of the world where general health conditions are poor, progress more quickly to AIDS (Anzala *et al*. 1995; Nagelkerke *et al*. 1990; Quinn *et al*. 1987; Ostrowski *et al*. 1998) concur with our finding that a higher amount of background activation results in a shorter NSI-dominated period, and consequently faster progression to disease. Furthermore, our model demonstrates that higher amounts of background activation lead to higher amounts of infected cells and consequently a shorter time until switch (figure 4), and this result is in accord with a study which shows that individuals with high virus loads progress to disease faster (Mellors *et al*. 1996).

We have assumed that, whatever the important effector mechanisms of the immune response, their function is proportional to the amount of T-helper cells present which are specific to HIV (Kalams & Walker 1998; Rosenberg & Walker 1998; Rosenberg *et al*. 1997). Consequently, the balance of virus production and removal is determined by the availability of all active CD4+ T cells for production, and of HIV-specific cells only for removal. The factors that determine the dynamics of the two virus populations are identical; the only differences between them are in parameters which define efficiency of infection and susceptibility to the immune response (Nowak & May 1991). In our numerical simulations, it is clear that there is a threshold value of the immune response against the SI virus, u_s, below which this strain emerges immediately (figure 3*a*). Furthermore, our analytical results show that a necessary condition for the late emergence of the SI virus is $u_s > u_n$. These results, which indicate the importance of the strength of the immune response in the phenomenon of phenotype switching and therefore disease progression, are also supported by experimental evidence (Schuitemaker *et al*. 1992; Bouhabib *et al*. 1994; Fauci 1996; Cornelissen *et al*. 1995; Bonhoeffer *et al*. 1995; Herbein *et al*. 1998). Another suggested mechanism for progression of disease is based on the generation of diversity (Nowak *et al*. 1991; Nowak & May 1991). Although in this paper we only treat a specific aspect of disease progression, without evolution of the viral strains or the immune system, the emergence of SI strains is probably related with the phenomenon of diversity generation.

The model's dynamics depend crucially on the population of active, HIV-specific CD4+ T cells. Our results indicate that this population of cells decays slowly, and once it falls low enough the SI virus emerges. There is a potential for this conclusion to be accurately and directly tested: techniques using HLA class I–peptide tetrameric complexes to quantify HIV-specific CD8+ T-cell levels are currently being perfected (Ogg *et al*. 1998; McMichael & O'Callaghan 1998), and hopefully soon it will be possible to do the same for CD4+ T cells using HLA class II. Such a technique would enable researchers to very accurately monitor HIV-specific CD4+ T cells and determine if indeed levels become low before any SI variants are detected.

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REFERENCES

Anderson, R. A. & May, R. M. 1993 *Infectious diseases of humans*, 3rd edn. London: Oxford University Press.

Anzala, O. A., Nagelkerke, N. J., Bwayo, J. J., Holton, D., Moses, S., Ngugi, E. N., Ndinya-Achola, J. O. & Plummer, F. A. 1995 Rapid progression to disease in African sex workers with human immunodeficiency virus type 1 infection. *J. Infect. Dis.* **171**, 686–689.

Baur, A., Scwarz, N., Ellinger, S., Korn, K., Harrer, T., Mang, K. & Jahn, G. 1989 Continuous clearance of HIV in a vertically infected child. *Lancet* **ii**, 1045.

Berger, E. A. 1997 HIV entry and tropism: the chemokine receptor connection. *AIDS* **11**, S3–S16.

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

Bonhoeffer, S., Coffin, J. M. & Nowak, M. A. 1997 Human immunodeficiency virus drug therapy and virus load. *J. Virol.* **71**, 3275–3278.

Boucher, C. A. B. & Reedjik, M. 1995 Viral resistance: a major challenge in managing HIV disease. *J. Biol. Regul. Homeo. Agents* **9**, 91–94.

Bouhabib, D. C., Roderiquez, G., Oravecz, T., Berman, P. W., Lusso, P. & Norcross, M. A. 1994 Cryptic nature of envelope V3 region epitopes protects primary monocytotropic human-immunodeficiency-virus type-1 from antibody neutralization. *J. Virol.* **68**, 6006–6013.

Bukrinsky, M. I., Stanwick, T. L., Dempsey, M. P. & Stevenson, M. 1991 Quiescent lymphocytes-T as an inducible virus reservoir in HIV-1 infection. *Science* **254**, 423–427.

Coffin, J. M. 1995 HIV population dynamics *in vivo*: implications for genetic variation, pathogenesis, and therapy. *Science* **267**, 483–489.

Connor, R. I., Mohri, H., Cao, Y. & Ho, D. D. 1993 Increased viral burden and cytopathicity correlate temporally with CD4+ T-lymphocyte decline and clinical progression in human immunodeficiency virus type-1 infected individuals. *J. Virol.* **67**, 1772–1777.

Cornelissen, M. (and 10 others) 1995 Syncytium inducing (SI) phenotype suppression at seroconversion after intramuscular inoculation of a non-syncytium inducing/SI phenotypically mixed human immunodeficiency population. *J. Virol.* **69**, 1810–1818.

de Jong, J. J., de Ronde, A., Keulen, W., Tersmette, M. & Goudsmit, J. 1992 Minimal requirements for the human immunodeficiency virus type 1 V3 domain to support the syncitium-inducing phenotype: analysis by single amino acid substitution. *J. Virol.* **66**, 6777–6780.

Fauci, A. S. 1996 Host factors and the pathogenesis of HIV-induced disease. *Nature* **384**, 529–534.

Fouchier, R. A. M., Groenink, M., Kootstra, N. A., Tersmette, M., Huisman, H. G., Miedema, F. & Schuitemaker, H. 1992 Phenotype-associated sequence variation in the third variable domain of the human immunodeficiency virus typel gp120 molecule. *J. Virol.* **66**, 3183–3187.

Fouchier, R. A. M., Meyaard, L., Brouwer, M., Hovenkamp, E. & Schuitemaker, H. 1996 Broader tropism and higher cytopathicity for CD4+ T-cells of a syncytium-inducing compared to a non-syncytium-inducing HIV-1 isolates a mechanism for accelerated CD4+ T cell decline *in vivo*. *Virology* **219**, 87–95.

Gartner, S., Markovits, P., Markovits, D. M., Kaplan, M. H., Gallo, R. C. & Popovic, M. 1986 The role of mononuclear phagocytes in HTLV-III/LAV infection. *Science* **233**, 215–219.

Goulder, P. J. R. (and 11 others) 1997 Late escape from an immunodominant cytotoxic T-lymphocyte response associated with progression to AIDS. *Nature Med.* **3**, 212–217.

Grivel, J.-C. & Margolis, L. B. 1999 CCR5- and CXCR4-tropic HIV-1 are equally cytopathic for their T-cell targets in human lymphoid tissue. *Nature Med.* **5**, 344–346.

Groenink, K. M., Fouchier, R. A. M., de Goede, R. E. Y., de Wolf, F., Gruters, R. A., Cuypers, T. M., Huisman, H. G. & Tersmette, M. 1991 Phenotypic heterogeneity in a panel of infectious molecular human immunodeficiency virus type-1 clones derived from a single individual. *J. Virol.* **65**, 1968–1975.

Haas, G. (and 12 others) 1996 Dynamics of viral variants in HIV-1 nef and specific cytotoxic T lymphocytes in vivo. *J. Immunol.* **157**, 4212–4221.

Haynes, B. F., Pantaleo, G. & Fauci, A. S. 1996 Toward an understanding of the correlates of protective immunity to HIV infection. *Science* **271**, 324–328.

Herbein, G., Mahlknecht, U., Batliwalla, F., Gregersen, P., Pappas, T., Butler, J., O'Brien, W. A. & Verdin, E. 1998 Apoptosis of CD8+ T cells is mediated by macrophages through interaction with HIV gp120 with chemokine receptor CXCR4. *Nature* **395**, 189–194.

Ho, D. D., Neumann, A. U., Perelson, A. S., Chen, W., Leonard, J. M. & Markowitz, M. 1995 Rapid turnover of plasma virions and CD4 lymphocytes in HIV-1 infection. *Nature* **373**, 123–126.

Kalams, S. A. & Walker, B. D. 1998 The critical need for CD4 help in maintaining effective cytotoxic T lymphocyte responses. *J. Exp. Med.* **188**, 2199–2204.

Koenig, S., Gendelman, H. E., Orenstein, J. M., Dal Canto, M. C., Pezeshpour, G. H., Yungbluth, M., Janotta, F., Aksamit, A., Martin, M. A. & Fauci, A. S. 1986 Detection of AIDS virus in macrophages in brain tissue from AIDS patients with encephalopathy. *Science* **233**, 1089–1093.

Koot, M., van't Wout, A. B., Kootstra, N. A., de Goede, R. E., Tersmette, M. & Schuitemaker, H. 1996 Relation between changes in cellular load, evolution of viral phenotype, and the clonal composition of virus populations in the course of human immunodeficiency virus type 1 infection. *J. Infect. Dis.* **173**, 349–354.

Koot, M., van Leeuwen, R., de Goede, R. E., Keet, I. P., Danner, S., Eeftinck Schattenkerk, J. K., Reiss, P., Tersmette, M., Lange, J. M. & Schuitemaker, H. 1999 Conversion rate towards a syncytium-inducing (SI) phenotype during different stages of human immunodeficiency virus type 1 infection and prognostic value of SI phenotype for survival after AIDS diagnosis. *J. Infect. Dis.* **179**, 254–258.

Koup, R. A., Safrit, J. T., Cao, Y. Z., Andrews, C. A., McLeod, G., Borkowsky, W., Farthing, C. & Ho, D. D. 1994 Temporal association of cellular immune-responses with the initial control of viremia in primary human-immunodeficiency-virus type-1 syndrome. *J. Virol.* **68**, 4650–4655.

Loveday, C., Kay, S., Tenant-Flowers, M., Semple, M., Ayliffe, U., Weller, I. V. D. & Tedder, R. S. 1995 HIV-1 RNA serum-load and resistant viral genotypes during early zidovudine therapy. *Lancet* **345**, 820–824.

Mackewicz, C. E., Ortega, H. W. & Levy, J. A. 1991 CD8+ cell anti-HIV activity correlates with the clinical state of the infected individuals. *J. Clin. Invest.* **87**, 1462–1466.

McLean, A. R. & Kirkwood, T. B. L. 1990 A model of human immunodeficiency virus infection in T helper cell clones. *J. Theor. Biol.* **147**, 177–203.

McLean, A. R. & Nowak, M. A. 1992 Models of interaction between HIV and other pathogens. *J. Theor. Biol.* **155**, 69–86.

McMichael, A. J. & O'Callaghan, C. A. 1998 A new look at T cells. *J. Exp. Med.* **187**, 1367–1371.

Mellors, J. W., Rinaldo Jr, C. R., Gupta, P., White, R. M., Todd, J. A. & Kingsley, L. A. 1996 Prognosis in HIV-1 infection predicted by the quantity of virus in plasma. *Science* **272**, 1167–1170.

Miedema, F. (and 10 others) 1994 Changing virus–host interactions in the course of HIV-1 infection. *Immunol. Rev.* **140**, 35–72.

Nagelkerke, N. J., Plummer, F. A., Holton, D., Anzala, A. O., Manji, F., Ngugi, E. N. & Moses, S. 1990 Transition dynamics of HIV disease in a cohort of African prostitutes: a Markov model approach. *AIDS* **4**, 743–747.

Nowak, M. A. & May, R. M. 1991 Mathematical biology of HIV infections—antigenic variation and diversity thresholds. *Math. Biosci.* **106**, 1–21.

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.

Ogg, G. S. (and 14 others) 1998 Quantitation of HIV-1-specific cytotoxic T lymphocytes and plasma load of viral RNA. *Science* **279**, 2103–2106.

Ostrowski, M. A., Krakauer, D. C., Li, Y., Justement, S. J., Learn, G., Ehler L. A., Stanley, S. K., Nowak, M. A. & Fauci, A. S. 1998 Effect of immune activation on the dynamics of human immunodeficiency virus replication and on the distribution of viral quasi-species. *J. Virol.* **72**, 7772–7784.

Pantaleo, G. & Fauci, A. S. 1996 Immunopathogenesis of HIV infection. *A. Rev. Microbiol.* **50**, 825–854.

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

Phair, J. P. 1994 Keynote address: variations in the natural history of HIV infection. *AIDS Res. Hum. Retroviruses* **10**, 883–885.

Pratt, R. D., Shapiro, J. F., McKinney, N., Kwok, S. & Spector, S. A. 1995 Virologic characterization of primary human immunodeficiency virus type 1 infection in a health care worker following needlestick injury. *J. Infect. Dis.* **172**, 851–854.

Price, D. A., Goulder, P. J. R., Klenerman, P., Sewell, A. K., Easterbrook, P. J., Troop, M., Bangham, C. R. M. & Phillips, R. E. 1997 Positive selection of HIV-1 cytotoxic T lymphocyte escape variants during primary infection. *Proc. Natl Acad. Sci. USA* **94**, 1890–1895.

Quinn, T. C., Piot, P., McCormick, J. B., Feinsod, F. M., Taelman, H., Kapita, B., Stevens, W. & Fauci, A. S. 1987 Serologic and immunologic studies in patients with AIDS in North America and Africa. The potential role of infectious agents as cofactors in human immunodeficiency virus infection. *J. Am. Med. Assoc.* **257**, 2617–2621.

Ribeiro, R. M., Bonhoeffer, S. & Nowak, M. A. 1998 The frequency of resistant mutant virus before antiviral therapy. *AIDS* **12**, 461–465.

Richman, D. D. 1996 HIV drug resistance—an overview. *AIDS* **10**, S121.

Roos, M. T. L., Lange, J. M. A., de Goede, R. E. Y., Coutinho, R. A., Schellekens, P. T. A., Miedema, F. & Tersmette, M. 1992 Viral phenotype and immune response in primary human immunodeficiency virus type-1 infection. *J. Infect. Dis.* **165**, 427–432.

Rosenberg, E. S. & Walker, B. D. 1998 HIV type 1-specific helper T-cells: a critical host defense. *AIDS Res. Hum. Retroviruses* **14**, S143–S147.

Rosenberg, E. S., Billingsley, J. M., Caliendo, A. M., Boswell, S. L., Sax, P. E., Kalams, S. A. & Walker, B. D. 1997

Vigorous HIV-1-specific CD4+ T cell responses associated with control of viremia. *Science* **278**, 1447–1450.

Safrit, J. T. & Koup, R. A. 1995 The immunology of primary HIV infection—which immune responses control HIV replication. *Curr. Opin. Immunol*. **7**, 456–461.

Schuitemaker, H., Koot, M., Kootstra, N. A., Dercksen, M. W., de Goede, R. E., van Steenwijk, R. P., Lange, J. M., Schattenkerk, J. K., Miedema, F. & Tersmette, M. 1992 Biological phenotype of human immunodeficiency virus type 1 clones at different stages of infection: progression of disease is associated with a shift from monocytotropic to T-cell-tropic virus population. *J. Virol*. **66**, 1354–1360.

Stevenson, M. 1996 Portals of entry—uncovering HIV nuclear transport pathways. *Trends Cell Biol*. **6**, 9–15.

Tersmette, M., Gruters, R. A., de Wolf, F., de Goede, R. E. Y., Lange, J. M. A., Schellekens, P. T. A., Goudsmit, J., Huisman, H. G. & Miedema, F. 1989 Evidence for a role of virulent human immunodeficiency virus (HIV) variants in pathogenesis of acquired immunodeficiency syndrome: studies on sequential HIV isolates. *J. Virol*. **63**, 2118–2125.

Van't Wout, A. B., Kootstra, N. A., Mulder-Kampinga, G. A., Albrecht-van Lent, N., Scherpbier, H. J., Venstra, J., Boer, K., Coutinho, R. A., Miedema, F. & Schuitemaker, H. 1994 Macrophage-tropic variants initiate human immunodeficiency virus type 1 infection after sexual, parenteral and vertical transmission. *J. Clin. Invest*. **94**, 2060–2067.

Wei, X. (and 11 others) 1995 Viral dynamics in human immunodeficiency type-1 infection. *Nature* **373**, 117–122.

Wodarz, D. & Nowak, M. A. 1998 The effect of different immune responses on the evolution of virulent CXCR4-tropic HIV. *Proc. R. Soc. Lond*. B **265**, 2149–2158.

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

Zhu, T., Mo, H., Wang, N., Nam, D., Cao, Y., Koup, R. & Ho, D. D. 1993 Genotypic and phenotypic characterization of HIV-1 patients with primary infection. *Science* **261**, 1179–1181.

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