Superinfection with a heterologous HIV strain *per se* does not lead to faster progression

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**ABSTRACT**

**Background:** It has been suggested that superinfection of HIV positive individuals with heterologous HIV strains could lead to faster progression to AIDS, generating concern over the risks of exposure to new infections in those already infected.

**Methods:** A mathematical model of the within-host dynamics of two sequential infections with strains of HIV describing activation and infection of immune cells was developed. Multiple stochastic realisations describing progression to AIDS in the individual were generated, comparing the situation with and without superinfection.

**Results:** It was found that the susceptibility of immune cells to dual infection is crucial to the outcome of HIV superinfection. A low susceptibility leads to competitive exclusion between the strains and a high susceptibility may lead to co-existence if the superinfecting strain is sufficiently fit. It was also found that only superinfection with a fitter strain leads to faster progression to AIDS, rather than superinfection per se.

**Conclusion:** In theory, a superinfection event with a heterologous strain of HIV does not lead to faster progression to AIDS. Unless superinfection allows the spread of fitter virus, it should not be of concern for public health.

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**1. Introduction**

HIV dual infection, including both coinfection and superinfection, is an important issue as it may lead to recombination of HIV genomes that in turn facilitates the emergence of resistant strains [1]. It also affects our understanding of the human immune response against HIV infection, as the occurrence of superinfection events imply that the anti-HIV immune responses in the HIV-positive individual are insufficient to protect against a second infection [1–4], which has substantial implications for the development of an HIV vaccine [3]. (For a recent review on HIV dual infection, see [5].)

Early reports of HIV superinfection suggested that it may hasten the progression to AIDS [6,7]. However, some patients with long-term non-progressive HIV-1 infection have experienced co-infection or superinfection without clinical manifestations [8,9]. Thus it is uncertain whether superinfection *per se* is associated with disease progression. This has public health implications since there is concern over whether those already infected with HIV need to protect themselves from superinfection when in contact with known HIV infected partner [1,10]. Transmission of drug-resistant strains in the event of superinfection has also been documented [11–14].

The implications of HIV dual infections are to some extent dependent upon the ability of the virus to co-infect cells, which is a prerequisite to HIV genome recombination at a cellular level. Mechanisms of down-modulation of CD4 receptors by HIV protein nef, vpu and env have been discovered [15,16]. Studies also found that coinfection of cells was more frequent than expected in random events [17–19]. A scaling law kinetics has been identified regarding HIV superinfection of cells and it was suggested that most infected cells are being infected and reinfected simultaneously, as a consequence of infection kinetics and target cell depletion [20, erratum in [21]]. To what extent an HIV-infected target cell is resistant to being superinfected with another virus genome will have an impact, not only upon the frequency of genome recombination, but to the whole viral and cellular profile of HIV dual infection (reviewed in Nethe et al. [22]).

Mathematical models have been used to help elucidate the mechanisms of HIV pathogenesis [23–25]. Some of them were tailored to focus on specific problems, e.g. residual replication [26], viral latent reservoirs [27] and transient viraemia [28,29].
Complementary to both clinical and epidemiological studies on HIV superinfection, mathematical models may serve as a starting point for us to probe in greater detail the within-host dynamics of HIV in the case of superinfection with a heterologous virus.

In order to investigate how the cellular and viral profiles of HIV superinfection would be affected by a varying degree of HIV superinfection of cells, and to elucidate whether superinfection per se leads to faster progression to AIDS, a mathematical model has been developed. The model will be presented in Section 2. In Section 3, the model behaviour – quasi-equilibrium values and basic reproductive number – will be analysed. To investigate (a) how viral fitness and cellular susceptibility to co-infection affects the outcome of superinfection of a heterologous HIV strain in an HIV positive individual, which can be defined as co-existence of both strain or competitive exclusion of either strain, and (b) whether HIV superinfection leads to faster progression to AIDS, numerical solutions of the model were obtained through computer simulations and presented in Section 4.

2. The model

The model described below is an extension of the model of Fraser et al. of long-term HIV within-host dynamics that encompasses both primary infection and the long progression to AIDS [30]. Additional insights from the extended version of Fraser et al.’s model [30] by Griffin and colleagues (James T.A. Griffin, unpublished Ph.D. Thesis, Imperial College London, 2006, and Griffin et al. [31]), Fraser et al.’s model of short-term HIV within-host dynamics [32] and Kim and Perelson’s model on viral and latent reservoir persistence [27] were added to the model. Fig. 1 is a flowchart representing the model. The model parameters and variables are described in Tables 1 and 2, respectively.

2.1. Healthy T cells

The model describes uninfected quiescent T cells, $x_i$, where $i = 4$ represents CD4 cells and $i = 8$ represents CD8 cells,

$$\frac{dx_i}{dt} = \lambda_i - a_i + 2\rho_i\mu x_i - \theta x_i (x_4 + x_8).$$

(1)

CD4 and CD8 cells are supplied from a thymic source at a constant rate, $\lambda_4$ and $\lambda_8$, respectively, and go through non-antigen-driven homeostatic cell division at a rate of $\mu$. Their deaths are density-dependent, at rates of $\theta x_i (x_4 + x_8)$ and $\theta x_i (x_4 + x_8)$, respectively, where $\theta = \lambda_4 + \lambda_8 + \mu + 2\rho_0(2\rho_1 + 1)$, chosen to ensure at steady state $x_4 + x_8 = 1$ on average. They are activated at rates $a_4$ and $a_8$, a stochastic process that will be discussed below, and become activated cells, $x_{4A}$ and $x_{8A}$:

$$\frac{dx_{4A}}{dt} = a_4 - \mu x_{4A} - \beta_1(1 - \text{drug}_{RT1}) \nu_1 x_{4A} - \beta_2(1 - \text{drug}_{RT2}) \nu_2 x_{4A},$$

(2)

$$\frac{dx_{8A}}{dt} = a_8 - \mu x_{8A}.$$  

(3)

Activated cells undergo divisions at a per cell rate of $\mu_A$ and become quiescent cells at a per cell rate of $2\rho_A\mu_A$, where $\rho_A$ is the average probability of an activated T cell successfully dividing in an HIV-negative individual. In the presence of virus, where $v_1$ and $v_2$ denoting the first strain and the superinfecting strain, respectively, activated CD4 cells are infected at a rate of $\beta_1 v_1$ and $\beta_2 v_2$, denoting fitness of the two viral strains, respectively. In the presence of reverse transcriptase inhibitor (RTI), the rate of infection will be reduced by a proportion, the RTI drug efficacy with respect to each virus strain, denoted by $\text{drug}_{RT1}$ and $\text{drug}_{RT2}$, respectively.

2.2. Infection of CD4 cells

Infected cells are divided into categories according whether they are activated ($y_i$), latent infected ($y_{li}$) or virally productive ($y_{pi}$) and with which virus are the cells infected ($i = 1$ or $2$):

$$\frac{dy_{i}}{dt} = (1 - f_i)\beta_i(1 - \text{drug}_{RT}) v_i x_{4A} + a_y y_i - a_y y_i - 3y_i$$

$$- \alpha_y v_i \beta_i(1 - \text{drug}_{RT}) v_i y_i;$$

(4)

$$\frac{dy_{li}}{dt} = f_i \beta_i(1 - \text{drug}_{RT}) v_i x_{4A} - a_y y_{li};$$

(5)

$$\frac{dy_{pi}}{dt} = a_y y_i - 3y_i - \alpha_y v_i \sigma z_{pi} y_{pi},$$

(6)

where $i, j = 1$ or $2$, and $i \neq j$, representing two strains of virus.

When activated CD4 cells are infected by virus 1 and 2, a small fraction $f_i$ of them become latently infected cells $y_{li}$, and $y_{pi}$, while the majority of them, $1 - f_i$, become activated infected cells, $y_{i1}$ and $y_{i2}$. Latently infected cells are activated at a per cell rate of $a_y$. Activated infected cells die at a per cell rate of $\alpha_y$ and become productively infected cells, $y_{pi}$ and $y_{pi}$, at a per cell rate of $a_y$. Productively infected cells die at a per cell rate of $\gamma$ and were killed by cytotoxic lymphocyte (CTL) action at a rate of $\sigma z_{pi}$, where $\sigma$ is the maximum rate of CTL killing of HIV-infected cells and

![Fig. 1. Flowchart representing the mathematical model. Adapted from James T.A. Griffin (Ph.D. Thesis, Imperial College London, 2006).](image-url)
As we shall see below, because the growth of the activated CTLs
depends on the total number of productively infected cells:

\[ \frac{dy_j}{dt} = \alpha_{yj12}(1 - f_j) \beta_j(1 - \text{drug}_{j/2}) y_j y_{12} + \alpha_{yj21}(1 - f_j) \beta_j(1 - \text{drug}_{j/1}) y_j y_{12} - a_{yj} y_j y_{12} \]

Likewise, a small fraction of these doubly infected cells becomes
latent, \( y_{12/1} \), while the rest are activated, \( y_{12/2} \). The latter will become
virally productive, \( y_{12/2} \). \( \xi \) is a parameter that allows for the intensity

### Table 1

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
<th>Value in simulation</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \lambda ) and ( \lambda_b )</td>
<td>Daily rate of thymic production of new CD4 and CD8 T cells (^{-1} )</td>
<td>( \lambda_4 + \lambda_b = 10^{-4} ) and ( \lambda_4 = 5 \lambda_b )</td>
<td>Chosen so that the pre-infection CD4/CD8 ratio = 2 ([30])</td>
</tr>
<tr>
<td>( a_0 )</td>
<td>Average rate of T cells activation per antigenic exposure (^{-1} )</td>
<td>( 10^{-4} ) day (^{-1} )</td>
<td>Chosen to match the observed rate of disease progression ([55])</td>
</tr>
<tr>
<td>( \mu )</td>
<td>Daily rate of non-antigen-driven homeostatic T cell division (^{-1} )</td>
<td>0.01</td>
<td>Chosen to match turn-over rates directly observed in ([56])</td>
</tr>
<tr>
<td>( x_e )</td>
<td>Relative T cell pool size below which T cell activation fails due to exhaustion of repertoire (^{-1} )</td>
<td>0.05</td>
<td>Value at which T helper competence starts to decline ([30])</td>
</tr>
<tr>
<td>( \mu_a )</td>
<td>Activated T cell division rate (^{-1} )</td>
<td>1 day (^{-1} )</td>
<td>([30])</td>
</tr>
<tr>
<td>( p_x )</td>
<td>Average probability of an activated T cell successfully dividing in an individual free of HIV (^{-1} )</td>
<td>0.55</td>
<td>([30])</td>
</tr>
<tr>
<td>( \beta )</td>
<td>Average infection rate of an activated CD4 T cell per virion</td>
<td>754 (to be varied in the experiment)</td>
<td>Cf. ([30])</td>
</tr>
<tr>
<td>( a_p )</td>
<td>Activated infected cells become virally productive (^{-1} )</td>
<td>1 day (^{-1} )</td>
<td>([57])</td>
</tr>
<tr>
<td>( \sigma )</td>
<td>Death rate of productively infected cell in the absence of ( \text{CTL} ) (^{-1} )</td>
<td>1 day (^{-1} )</td>
<td>([57])</td>
</tr>
<tr>
<td>( \rho )</td>
<td>Rate of reactivation of latent infected cells (^{-1} )</td>
<td>0.01 day (^{-1} )</td>
<td>Assuming this process is similar to the non-antigen-driven homeostatic T cell division and therefore assuming ( q_1 = \mu ) ([30])</td>
</tr>
<tr>
<td>( f_i )</td>
<td>Proportion of successful infections that result in latency (^{-1} )</td>
<td>( 10^{-5} )</td>
<td>([30])</td>
</tr>
<tr>
<td>( a_{21} )</td>
<td>Rate of CTL activation per infected cell</td>
<td>( 1.3334 	imes 10^{-8} ) per CTL per infected cell per day</td>
<td>([32])</td>
</tr>
<tr>
<td>( p_z )</td>
<td>Maximum proliferation of anti-HIV CTLs (^{-1} )</td>
<td>1 day (^{-1} )</td>
<td>1% of CTL are activated ([57])</td>
</tr>
<tr>
<td>( z_0 )</td>
<td>Max. proliferation of anti-HIV CTLs (^{-1} )</td>
<td>0.01 day (^{-1} )</td>
<td>([58])</td>
</tr>
<tr>
<td>( y_T )</td>
<td>Pre-infection frequency of anti-HIV CTL (^{-1} )</td>
<td>( 10^{-6} )</td>
<td>([30])</td>
</tr>
<tr>
<td>( b/c )</td>
<td>Ratio of viral production rate in productively infected cells and viral clearance rate (^{-1} )</td>
<td>292</td>
<td>([31])</td>
</tr>
<tr>
<td>( \sigma )</td>
<td>Maximum rate of CTL killing of HIV-infected cells (^{-1} )</td>
<td>( 10^{5} ) day (^{-1} )</td>
<td>([30])</td>
</tr>
<tr>
<td>( N_{PB} )</td>
<td>Typical pre-infection T cell count (^{-1} )</td>
<td>1500 mm (^{-3} )</td>
<td>The peripheral blood (PB) CD4 count is ( N_{PB}(x_4 + x_8 + y + y_L) ) and the PB CD8 count is ( N_{PB}(x_8 + x_4 + z + 2) )</td>
</tr>
<tr>
<td>( \alpha ) and ( \alpha_b )</td>
<td>Average clearance rate in antigenic exposure model (^{-1} )</td>
<td>0.02 day (^{-1} )</td>
<td>([30])</td>
</tr>
<tr>
<td>( \tau_4 ) and ( \tau_8 )</td>
<td>Average exposure rate in antigenic exposure model (^{-1} )</td>
<td>0.1 day (^{-1} )</td>
<td>The average number of concurrent antigenic exposures = 5 ([30])</td>
</tr>
<tr>
<td>( \alpha_{yj12} )</td>
<td>The ratio of the rate of infection by virus 2 of a cell already infected by virus 1 to that of an uninfected cell by virus 2 (^{-1} )</td>
<td>([10-0.0001])</td>
<td>It is set to vary within this range or fix at a particular value</td>
</tr>
<tr>
<td>( \alpha_{yj21} )</td>
<td>The ratio of the rate of infection by virus 1 of a cell already infected by virus 2 to that of an uninfected cell by virus 1 (^{-1} )</td>
<td>([10-0.0001])</td>
<td>It is set to vary within this range or fix at a particular value</td>
</tr>
<tr>
<td>( \xi )</td>
<td>The ratio of CTL action against doubly infected cells to that against singly infected cells</td>
<td>1</td>
<td>Assumed that CTL action against infected cells regardless of whether they are singly or doubly infected</td>
</tr>
<tr>
<td>( \lambda_1 )</td>
<td>Efficacy of reverse transcriptase against virus 1 (^{-1} )</td>
<td>0.9</td>
<td>Set at this value to achieve suppression of virus 1</td>
</tr>
<tr>
<td>( \lambda_2 )</td>
<td>Efficacy of reverse transcriptase against virus 2 (^{-1} )</td>
<td>([0.0-1.0])</td>
<td>It is set to vary within this range or fix at a particular value</td>
</tr>
<tr>
<td>( \lambda_3 )</td>
<td>Efficacy of protease inhibitor against virus 1 (^{-1} )</td>
<td>0.9</td>
<td>Set at this value to achieve suppression of virus 1</td>
</tr>
<tr>
<td>( \lambda_4 )</td>
<td>Efficacy of protease inhibitor against virus 2 (^{-1} )</td>
<td>([0.0-1.0])</td>
<td>It is set to vary within this range or fix at a particular value</td>
</tr>
<tr>
<td>( \lambda_5 )</td>
<td>Half-life of reverse transcriptase inhibitor</td>
<td>0.5 day</td>
<td>Chosen to match the dose interval</td>
</tr>
<tr>
<td>( \lambda_6 )</td>
<td>Half-life of protease inhibitor</td>
<td>0.5 day</td>
<td>Chosen to match the dose interval</td>
</tr>
<tr>
<td>( \lambda_7 )</td>
<td>Interval between each dose of drug</td>
<td>0.5 day</td>
<td>Two doses per day</td>
</tr>
<tr>
<td>( \omega_{yj} )</td>
<td>denote the cellular and viral resistance of ( y_j ) to superinfection by ( y_j ), respectively ((i, j = 1 \text{ or } 2, \text{ and } i \neq j) ), and become doubly infected cells,</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\[ e = \tilde{y}_P / (\tilde{y}_P + y_T) \] \( \tilde{y}_P = (y_{P1} + y_{P2}) \) and \( y_T \) is the threshold value of productively infected cells for the logistic proliferative response of CTL to HIV. When the total number of productively infected cells \( \tilde{y}_P \) is very small, we can assume \( y_{P1} \approx \tilde{y}_P \) and \( y_T \ll y_{P1} \). Then, \( e \approx \tilde{y}_P / y_T \approx y_{P1} / y_T \), and therefore, \( \omega_{yj12} = \omega_{yj} / y_T \). As we shall see below, because the growth of the activated CTLs \( (\lambda_x) \) depends on the total number of productively infected cells; \( z_0 \) will be very small. As both \( y_{P1} \) and \( y_{P2} \) are very small, the term \( \sigma z_0 y_T \) tends towards zero, i.e. CTL action is minimal. The death of productively infected cells will then depend only on \( \gamma \). When the total number of productively infected cells is big, \( \tilde{y}_P \gg y_T \). Then, \( e \approx 1 \) and the term \( \sigma z_0 y_T \) becomes \( \sigma z_0 y_{P1} \), which is a mass action term for CTL action.

### 2.3. Superinfection of CD4 cells

Activated infected cells may be infected a second time by a heterologous virus at a per cell rate of \( \alpha_{yj12} \beta_j(1 - \text{drug}_{j/1}) y_j \), where

\[ \frac{dy_j}{dt} = \alpha_{yj12}(1 - f_j) \beta_j(1 - \text{drug}_{j/2}) y_j y_{12} + \alpha_{yj21}(1 - f_j) \beta_j(1 - \text{drug}_{j/1}) y_j y_{12} - a_{yj} y_j y_{12} \]
and become two resting CTLs at a per cell rate of
on the density of CD4 cells. An activated CTL will undergo division
where
v
bers of infected cells. This assumes that this process is rapid and at
resting CTLs at a constant rate of
infected by the same viral strain.
Therefore, no separate equations are made for cells super-
from the point of view of viral production as that infected by only
one virus. Therefore, no separate equations are made for cells super-
infected by two virus of the same strain would be the same
against singly infected cells. As viral production is limited by cellu-
of CTL action against doubly infected cells to be different from that
against singly infected cells. As viral production is limited by cellular
factors rather than number of viral genomes present in a cell, a
cell co-infected by two virus of the same strain would be the same
from the point of view of viral production as that infected by only
one virus. Therefore, no separate equations are made for cells super-
infected by the same viral strain.

2.4. Cytotoxic lymphocyte

CTLs play a certain role in clearing productively infected cells
[33] (but not the activated or latently infected cells in the model).
They are either resting, zR, or activated, zA:

\[
\frac{dz_R}{dt} = d_z(z_0 - z_R) + 2p_z z_R - a_z(y_{p_1} + y_{p_2} + y_{p12}) z_R,
\]

(10)

\[
\frac{dz_A}{dt} = a_z(y_{p_1} + y_{p_2} + y_{p12}) z_R - p_z z_A.
\]

(11)

The homeostasis of resting CTLs is maintained by a supply of new
resting CTLs at a constant rate of \(d_z\cdot z_0\) and a death rate of \(d_z\cdot z_R\). Resting CTLs are activated at a rate of \(a_z(y_{p_1} + y_{p2} + y_{p12})\) dependent on the density of CD4 cells. An activated CTL will undergo division and become two resting CTLs at a per cell rate of \(p_z\).

2.5. Virus

The creation and destruction of virus is not explicitly followed
in the model but the population of virus is a function of the num-
bers of infected cells. This assumes that this process is rapid and at
equilibrium coupled to the cell dynamics.

\[
v_i = \frac{b}{c} (1 - drug_{Pi}) \left( \frac{y_{ni} + y_{ni}}{2} \right),
\]

(12)

\[
v_{ni} = \frac{b}{c} drug_{Pi} \left( \frac{y_n + y_{ni}}{2} \right),
\]

(13)

where \(i, j = 1, 2\), and \(i \neq j\).

Virions are produced from infected cells at a rate of \(b\), and have a
viral lifetime of \(1/c\). The proportion of infectious virions and non-
infectious virions, denoted \(v_i\) and \(v_{ni}\), are given by \(1 - drug_{Pi}\)
and \(drug_{Pi}\), respectively, where \(i = 1\) or 2 and \(drug_{Pi}\) denotes the effi-
cacy of protease inhibitor (PI) against virus 1 and 2, respectively.
Doubly infected cells were assumed to produce an equal number of
virions to that produced by singly infected cells, among which half
are virus 1 and half are virus 2.

2.6. T Cell activation and antigenic exposure

Following Fraser et al. [30] and their hypothesis that the occur-
rence of bursts of activation is predominantly localised, the rate of T cell
activation is limited by the availability of antigen presenting
sites and thus occurs at an approximately constant rate. The rates
of activation of CD4 and CD8 cells, denoted by \(a_k\) and \(a_k\), respec-
tively, were given as,

\[
a_i = a_0 k \frac{x_i}{x_0 + x_S}, \quad \text{where } i = 4 \text{ or } 8,
\]

(14)

and \(a_0\) is the average rate of T cell activation per antigenic exposure.
and \(k\) is the relative T cell pool size below which T cell activation
fails due to exhaustion of repertoire, and \(k\) (a non-negative integer)
is the number of concurrent antigenic exposures experienced by the
‘patient’ at a particular instance. The values of \(k\) vary randomly over
time, following Fraser et al. [30] with details given in Table 2. Note
the random nature of \(k\) results in model fluctuations about the
deterministic value, but that these are not the focus of this study.

3. Analytical results

Analytical solutions for the quasi-equilibrium states of the sys-
tem were obtained to identify major drivers of the level of each
state. The model can be solved to calculate a set of quasi-equilib-
rium values of immune cell levels and viral loads after primary
infection and before the onset of AIDS. This allows us to under-
stand the factors that influence the relationship between the immune cell levels and viral loads and the potential spread of virus within the host. Expressions for the basic reproductive ratios in the absence and presence of CTL response can also be derived which identify the factors that drive the initial growth of HIV infection in the host.

3.1. Equilibrium values

Following the example of James Griffin (Ph.D. Thesis, 2006), by setting the above equations to zero, equilibrium values of T cells and virions can be found. The only exception is the equation for quiescent CD4 cells that will not reach equilibrium within the time scale of the model representing a decade of infection. In the following equations, let $A$ denote the equilibrium value of any variable $A$ and let $\bar{Y}$ and $\bar{Y}_P$ be the equilibrium values of $(y_1 + y_2 + y_{12})$ and $(y_{p1} + y_{p2} + y_{p12})$, respectively.

For $dz_k/dt = 0$ and $dz_k/dt = 0$, we can solve the two simulations equations – Eqs. (10) and (11) – for $z_k$, and by assuming that $z_0 \leq z_k$, we get,

$$\bar{z}_A = \frac{dz_k}{P_x}.$$  

(15)

Substituting Eq. (11) with Eq. (15), gives,

$$\bar{Y}_P = \frac{dz}{\bar{a}_x}.$$  

(16)

Assuming that no antiretroviral therapy (ART) is taken, adding Eq. (12) for $i = 1$ and $i = 2$, gives,

$$v_1 + v_2 = \frac{b}{c} \bar{Y}_P.$$  

(17)

Substituting Eq. (16) into Eq. (17), we get the equilibrium viral load,

$$v_1 + v_2 = \frac{d_z}{\bar{a}_x} \frac{b}{c}.$$  

(18)

Eq. (18) implies that the equilibrium viral load is determined by (i) the CTL death rate, (ii) the CTL activation rate per productive infected cells, (iii) the viral production rate in productively infected cells, and (iv) the viral clearance rate.

Taking Eq. (14) and let $k_4$ be the mean number of CD4-specific antigens present, and let $\bar{x}_4$ be the number of quiescent CD4 cells in the absence of infection and $\bar{a}_4$ be the rate of activation of CD4 cells in the absence of infection, gives,

$$\bar{a}_4 = \frac{a_0 k_4 \bar{x}_4}{\bar{x}_4 + \bar{x}_5}.$$  

(19)

Provided that the CD4 count is not at a low level, $x_4 \gg x_5$, Eq. (19) can be simplified as,

$$\bar{a}_4 \approx \frac{a_0 k_4}{\bar{a}_x}.$$  

(20)

Assuming the rate of activation of CD4 at equilibrium is similar to that in the absence of infection (i.e. $\bar{a}_4 \approx a_4$), Eq. (2) gives the equilibrium of activated CD4 count as,

$$\bar{x}_{4A} \approx \frac{a_4}{\mu_A + \beta_1 v_1 + \beta_2 v_2}.$$  

(21)

Eq. (21) implies that the equilibrium activated CD4 count is determined by (i) pre-infection CD4 activation rate ($a_4$), (ii) activated T cell division rate ($\mu_A$), and (iii) average infection rate of an activated CD4 T cell (i.e. $\beta_1 v_1 + \beta_2 v_2$). However, $\bar{x}_{4A}$ will become low at low CD4 levels, i.e. $x_4 \sim x_5$, as Eq. (20) does not hold.

At equilibrium and in the absence of drugs, Eq. (5) gives,

$$\bar{y}_{ii} = \frac{f_i \beta_i \bar{a}_i \bar{x}_{4A}}{\bar{a}_i},$$  

(22)

where $i = 1$ or 2.

Substituting Eq. (22) into Eq. (4), gives,

$$\bar{y}_i = \frac{f_i \beta_i \bar{a}_i \bar{x}_{4A}}{a_i + x + \beta_4 \bar{a}_i \bar{y}_P}.$$  

(23)

where $i = 1$ or 2 and $i \neq j$.

The equilibrium value of target cells infected by one virus only is determined by (i) the infection event ($\beta_i \bar{a}_i \bar{x}_{4A}$), (ii) the activated infected cells becoming virally productive ($a_i$), (iii) the death rate of infected cells in the absence of CTL ($\gamma$), and (iv) the rate of second infection of infected cells by heterologous virus ($\omega_{hij} \beta_i$).

Let $\xi$ be 1 (i.e. CTL kills doubly infected cells at the same rate as singly infected cells). For $dy_{pi}/dt = 0$ and $dy_{p12}/dt = 0$, we can solve Eq. (6) (for $i = 1$ or 2) and Eq. (9) together and get,

$$\bar{z}_A = \frac{a_0 \bar{Y} - \gamma \bar{Y}_P}{\epsilon \sigma \bar{Y}_P}$$  

where $\epsilon = \bar{Y}_P / \bar{Y}_P + \bar{y}_P$.

(24)

If $\bar{y}_P \approx \bar{y}_T$, then $\epsilon = 1/2$, and as $\bar{Y} \approx \bar{Y}_P$, Eq. (24) becomes:

$$\bar{z}_A = \frac{2(\delta_R - \gamma)}{\sigma}.$$  

(25)

Eq. (25) implies that the equilibrium value of activated CTL is determined by (i) the rate of activated infected cells become virally productive ($a_i$), (ii) the death rate of infected cells in the absence of CTL ($\gamma$), and (iii) the maximum rate of CTL killing of HIV-infected cells ($\sigma$).

3.2. Basic reproductive ratio

The definition of the basic reproductive ratio for a virus within a host, $R_0$, is the number of virions produced by each virion at initial infection. This value determines whether, and how fast, virus will establish itself in the host. Its calculation below follows the examples of Griffin (Ph.D. Thesis, 2006). In the absence of infection, the equilibrium number of activated CD4 cells is $\bar{a}_x/\mu_A$ (see Eq. (21)). Virus 1 infects these cells at a per cell rate of $\beta_1 v_1$ and virus 2 infects cells at a per cell rate of $\beta_2 v_2$. Virions are produced at a per cell rate of $b$ by virally productively infected cells. At a rate of $c$ they are cleared from the blood. (In our model, $b/c$ is a composite parameter, see Table 1.) In other words, for a mean time of $1/c$ each virion persists in the blood. During this time period, the virion infects $\frac{b}{c} A$ or $\frac{b}{c} A_2$ cells (virus 1 or virus 2, respectively). These cells then become virally productive. If at the initial stage of infection, anti-HIV CTL response is not present and the virally productive cells die at a rate of $\gamma$. A number of $b/\gamma$ virions are produced by each virally productive cell. Therefore, the basic reproductive ratio of virus $i$, where $i = 1$ or 2, is calculated as,

$$R_0 = \frac{\beta_i \bar{a}_i}{\gamma} \frac{b}{c}$$  

(26)

where $i = 1$ or 2.

If at the initial infection there was a steady state CTL response, the virally productive cells will die at a rate of $(\gamma + \epsilon \sigma \bar{z}_A)$. Applying Eq. (24),

$$\gamma + \epsilon \sigma \bar{z}_A = \gamma + \frac{a_0 \bar{Y} - \gamma \bar{Y}_P}{\epsilon \sigma \bar{Y}_P} = \frac{\bar{Y}_P}{\bar{Y}_P}.$$  

(27)

Assuming that $\bar{Y}_P \approx \bar{Y}$, and applying Eq. (27), the basic reproductive ratio will become:

$$R_{0\text{CTL}} = \frac{\beta_1 \bar{a}_1}{\mu_A (\gamma + \epsilon \sigma \bar{z}_A)} \frac{b}{c} = \frac{\beta_1 \bar{a}_1 \bar{Y}_P}{\mu_A \bar{Y}}$$  

(28)

where $i = 1$ or 2. 

Eq. (28) implies that the initial growth of infection is determined by the following factors (i) viral fitness ($\beta$), (ii) the rate of activation of
CD4 cells in the absence of virus ($\bar{a}_4$), (iii) the rate of activated T cell division ($\mu_A$), (iv) the rate of activated infected cells becoming virally productive ($b_4$), (v) the viral production rate in productively infected cells ($b$), and (vi) the viral clearance rate ($c$). Factors (iii) to (vi) are fixed in the model, based on the assumption that these parameters do not vary according to the virus strain or the individual. This implies that it is the interplay between the given fitness of a viral strain and the activation of CD4 cells before infection (the latter being stochastic in the model) that determines the initial course of infection.

What difference does it make having two viruses? The calculation of the basic reproductive number for each virus is not influenced by the existence of the other when invading a treatment of the basic reproductive number for each virus is not influenced (fore, assuming activated cells will be reduced and hence the basic reproductive ratio lower, assuming a very low rate of co-infection of cells. Therefore, assuming $\omega_{het} \ll 1$ (where $i, j = 1$ or $2$, and $i, j$) and therefore $y_{12} \ll y_1$ or $y_2$, the basic reproductive ratio of virus 2 in the presence of virus at equilibrium will become:

$$R_{R2} = \frac{\beta_i}{\gamma} \frac{b}{c} \frac{a_4}{\mu_A + \beta_i V_1}$$  \hspace{1cm} (29)

The prevalence of one virus is influenced by the other through the infection rate of cells that are no longer susceptible to infection (depending on $\omega_{het}$). It seems that equilibrium number of activated CD4 cells is reduced by the presence of another virus from $\frac{\bar{a}_4}{\mu_A + \beta_i V_1}$ to $\frac{a_4}{\mu_A + \beta_i V_1}$. However, since there is a conservation of the viral population with an infection having one virus, $V_1 = \frac{\bar{a}_4}{\mu_A + \beta_i V_1}$ and two viruses, $V_1 + V_2 = \frac{a_4}{\mu_A + \beta_i V_1}$, the viral numbers stay constant and only if $\beta_i = \beta_j$ do activated cell numbers change.

4. Numerical results

Numerical solutions to the model were obtained through computer simulations to elucidate the impact of viral fitness and co-infection of cells upon the infection outcome and the impact of superinfection upon progression to AIDS.

The model was written in C++ programming language and was compiled using Microsoft Visual Studio.NET 2003 programming environment. Outputs of simulation were generated by the programme in comma separated files and were then analysed with Microsoft Office Excel 2003 and SAS 9 for Windows (SAS Institute Inc., Cary, NC, USA, 2002–03).

All simulations start on day –730. Infection by virus 1 takes place on day 0. Infection by virus 2 (superinfection event), if there is any, takes place on day 365 (Figs. 2 and 3). Simulations end on day 3650.

4.1. Viral fitness and co-infection of cells determine the outcome

It was found that the susceptibility of cells to superinfection by a heterologous HIV virion is crucial to the outcome of HIV superinfection of the host with respect to the relative fitness of the two strains involved. Fig. 2 shows that if target CD4 cells that are already infected with one strain of HIV are less susceptible to the infection of a heterologous strain than their uninfected counterparts, competitive exclusion between the two strains will arise.

In this scenario, the relative fitness of the two strains determined which strain will prevail in the host after the superinfection event. However, if cells are equally (or more) susceptible to the virus infection, regardless of whether they are infected by another strain, then co-infection may arise given that the superinfecting strain is sufficiently fit with respect to the established strain.

![Fig. 2. Competitive exclusion or co-existence of two strains of HIV. The vertical axis is the log ratio of virus 2 and virus 1 (median of 10 runs per set of parameters) and it is presented against a parameter space of the log rate of infection of infected cells by a heterologous virus relative to that of healthy cells (x-axis), and the log ratio of $\beta_2$ and $\beta_1$ (y-axis). Latin hypercube sampling [59] was used to draw numbers without replacement from the parameter space of $\beta_2/\beta_1$ (within a range of –2 and 2, by varying $\beta_2$ while keeping $\beta_1$ constant at 754) and the infection rate ratio (by varying the rate of superinfection of infected cells, $\alpha_i$ from 0.001 to 1000, assuming $\alpha_{het} = 0.001$). For each combination of parameters, 10 simulations were performed. The median of log ($\log_{10}(\text{ratio})$) between the final viral load of virus 2 and that of virus 1, i.e. log$(V_2/V_1)$, of the 10 simulations were obtained and using the software SAS, performing procedures g3grid and g3d, a three-dimensional interpolated smooth surface was created as shown. It is evident that when an infected cell is very unlikely to be re-infected by a second strain, a scenario of competitive exclusion (either virus 1 or virus 2 persists) occurs with varying degree of exclusiveness depending on the ratio between $\beta_2$ and $\beta_1$. Take for example, when $\log_{10}($infection rate ratio$) = –3$, with log $(\beta_2/\beta_1)$ between 0 and 0.5, log$(V_2/V_1)$ falls between $–7.1$ and $11.4$. Given that the simulation viral load is of the order $10^6$, beyond this range, one is certain that only one strain prevails. If an infected cell is equally or more likely to be infected by a second strain, a scenario of co-existence of two strains can occur, depending on the fitness of the second strain. For example, when $\log_{10}($infection rate ratio$) = 0$, log$(V_2/V_1)$ varies between $–24.1$ and $3.4$; and when $\log_{10}($infection rate ratio$) = 3$, log$(V_2/V_1)$ varies only between $–1.7$ and $0.4$.

![Fig. 3. Kaplan–Meier curve showing the time (months) to achieve CD4 count <200 cells/ml among three categories of simulated patients. Each category consists of 500 simulations. Black: no superinfection; red: $\beta_2 \leq \beta_1$; green: $\beta_2 > \beta_1$. Only patients superinfected with a fitter strain (green: $\beta_2 > \beta_1$) experienced a faster progression to AIDS. The data for both categories with superinfection (red and green) are the same as that used for the analysis in Fig. 2, with a range of $\beta_2$ from 0.01 to 100 times of $\beta_1$. For each combination of $\beta_2$ and $\beta_1$, 10 simulations were performed. Because Latin hypercube sampling was used to draw number across the parameter space, the uncertainty of the rate of superinfection of infected cells is averaged out between the two categories (red and green) and its influence upon the aggregated outcomes therefore is minimised.
4.2. Superinfection and progression to AIDS

Fig. 3 shows the Kaplan–Meier curves for 1500 simulated HIV positive individuals leading to CD4 count < 200 cells/μl in the absence or presence of superinfection of a heterologous HIV strain, respectively. It was found that only superinfection with a fitter strain (green line; $\beta_2 > \beta_1$) leads to a faster progression to a status of low CD4 count when compared with simulations without superinfection (black line). Superinfection with a weaker strain (red line; $\beta_2 < \beta_1$) does not lead to a faster progression, assuming that no recombination takes place or such recombination events do not lead to a mutant strain with a higher $\beta$.

5. Discussion

Through the analysis of a mathematical model incorporating superinfection into previously published within-host models of HIV [27,30–32], we have identified the variables that influence the quasi-equilibrium levels of CD4 cells and viral loads and the criteria for the establishment of infection. The results confirm that susceptibility of cells to HIV superinfection is of paramount importance to the outcome of superinfection, as it determines whether competitive exclusion or co-existence prevails. The relative viral fitness between the two strains determines the final outcome, with the path to achieve it determined by the cellular susceptibility to superinfection. Additionally only if the superinfecting strain is fitter will superinfection result in a higher viral load that in turn leads to a faster progression to AIDS. This result is derived numerically but is consistent with our analytical findings, where we found the total virus population to be the same with one and two virus types, leading to similar activated and infected CD4 cell numbers and progression to disease. Even in the case of fitter superinfecting virus, the difference in the pace of progression to AIDS is small. This explains the observation that not all known cases of superinfection are associated with a faster progression to AIDS.

There are examples of co-existence of the initial and superinfecting strains [34–41], and also examples of competitive exclusion between two strains in the event of superinfection. Most of the latter were displacement of the first strain by the second strain [6,11,12,36,40–46], but there were also two cases in which a transient viraemia of the second strain was observed and then disappeared soon afterwards [44,47]. These different reports lead to uncertainty in our parameter estimation for the susceptibility of cells to co-infection. However, in two of the displacement cases, the replicative capacity as conferred by pol of the second strain was lower than that of the first strain [12,45], suggesting a possible difference in immune containment between the two viruses or the genetic basis of HIV viral fitness lying somewhere other than the pol coding region.

Mathematical models of multiple infections of target cells have been proposed [48–50]. Dixit and Perelson's model [48] captured two mechanisms of multiple infections of cells, one being a series of sequential infectious contacts of a target cell with infected cells and free virions, and one being a single contact between a target cell and an infected cell leading to transfer of multiple viral genomes. With an extended model [49], they found that they could replicate the scaling law as observed in experiments by Levy et al. [20], and generalise it so that number of cells infected by three viruses and the cube of the number of infected cells are proportional to each other. They also found that if the rate of production of virus was independent of the number of times the cells are infected, viral dynamics is not influenced by multiple infections of target cells. This model was further extended to study the emergence of recombinant HIV [50]. In essence what these three models have in common is an exponential decay of susceptibility of cells. However, all three models were based on the hypothesis that CD4 down-modulation was the underlying mechanisms of 'Superinfection resistance', which was unlikely to be case as argued by Nethe et al. [22]. Given that Levy et al. [20] observed that little inhibition to multiple infection events was there during HIV-1 replication, this feature was not incorporated into our model in order to keep the model simple. However, there was a recent report that most of the CD4 cells in the blood of HIV-positive patients are infected with one copy of viral DNA (Sarah Palmer et al., 'Single-cell Analysis of HIV DNA from Infected Patients', Abstract 442, 16th Conference on Retroviruses and Opportunistic Infections 2009, Montreal, Canada, http://www.retroconference.org/2009/Abstracts/35935.htm). This may help parameterise the model in the future.

It has also been suggested that superinfection is associated with an accelerated progression to AIDS. Temporal association between high viral load and superinfection event has been observed in a number of cases [4,6,7,11,12,34,36–47,51,52]. While as it was noted before [1] that some cases might be associated with recent treatment interruptions [6,34,47,51], a majority of reported superinfection events took place in the absence of ART [4,7,11,12,36–46,52]. The increase in viral load associated with superinfection event may be a consequence of viral burst as seen in primary infection. Given that a high viral load is an indicator for a faster progression to AIDS, these patients were predicted to experience an accelerated disease progression. However, this is not always the case. There have also been cases where the superinfection event took place without any significant changes in viral load [8,35,36,39,41].

In addition to increased viral load, decreased CD4 count has also been reported to be associated with superinfection events. Superinfection may be associated with an abrupt decline in CD4 cell count observed in an untreated long-term HIV survivor [53]. An individual experienced superinfection around one year after seroconversion and his CD4 cell count dropped below 200 cells/μl 2.4 years after seroconversion, and his first clinical AIDS-defining illness 3.4 years after seroconversion [7,43]. However, this individual's rapid CD4 decline was not associated with the superinfection event in a regression analysis [43]. In addition, there have been reports of superinfection identified retrospectively among individuals with long-term non-progressive HIV infection [8]. Therefore, there is evidence that HIV superinfection does not necessarily lead to high viral load or accelerated decline in CD4 count.

This model is limited in a number of ways: for the sake of simplicity, humoral responses are not incorporated though neutralising antibodies are known to play a role against HIV superinfection [4]. Within-host evolution and recombination of viral strains were not captured by this model either. Models with alternative formulations of these dynamics may lead to different interpretations [54]. Therefore, we should not exclude the possibility that superinfection of a less fit virus can also lead to the emergence of a mutant strain through recombination leading a faster progression to AIDS, but if and only if such a recombined strain has a higher fitness. As the possibilities of recombination and mutations are not included in the model, the model outputs should be carefully interpreted in the light of these simplifications.

HIV superinfection is an important issue in relation to public health intervention and vaccine development. This mathematical model shows that HIV superinfection in and of itself is not a problem, but a fitter virus could lead to faster progression. Acquisition of resistant virus, and the possibility of cellular superinfection and therefore recombination leading to emergence of mutant strains are two other possibilities that needed to be taken into account. A less fit superinfecting strain will not establish itself if cellular superinfection is not possible. Superinfection can only go one way – either making things worse or generating no difference. Such model findings are consistent with observations of viral
competition and co-existence in cases studies. It warrants future studies including fitting the model outputs to empirical data from observational cohort where superinfection is carefully monitored should such become available.

Author contributions

I.C.H.F., F.d.W. and G.P.G. designed the experiments. I.C.H.F. wrote the mathematical models and the computer codes, and conducted the experiments and the data analysis. M.G. and A.v.s. contributed ideas on mathematical modelling and data analysis. I.C.H.F. drafted the manuscript. All of the authors contributed to the manuscript preparation. F.d.W. and G.P.G. supervised the project.

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Competing interests

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