Alternating host cell tropism shapes the persistence, evolution and coexistence of Epstein-Barr virus infections in human

Giao T. Huynh Frederick R. Adler

September 10, 2010

Abstract

Epstein-Barr virus (EBV) infects and can persist in a majority of people worldwide. Within an infected host, EBV targets two major cell types, B cells and epithelial cells, and viruses emerging from one cell type preferentially infect the other. We use mathematical models to understand why EBV infects epithelial cells when B cells serve as a stable refuge for the virus and how switching between infecting each cell type affects virus persistence and shedding. We propose a mathematical model to describe the regulation of EBV infection within a host. This model is used to study the effects of parameter values on optimal viral strategies for transmission, persistence, and intrahost competition. Most often, the optimal strategy to maximize transmission is for viruses to infect epithelial cells, but the optimal strategy for maximizing intrahost competition is for viruses to mainly infect B cells. Applying the results of the within-host model, we derive a model of EBV dynamics in a homogeneous population of hosts that includes superinfection. We use this model to study the conditions necessary for invasion and coexistence of various viral strategies at the population level. When the importance of intrahost competition is weak, we show that coexistence of different strategies is possible.

1 Introduction

Epstein-Barr virus (EBV) belongs to the herpesvirus family, infects over 90% of humans worldwide and persists for the lifetime of the person [18]. Most individuals infected with EBV are

asymptomatic, but the virus has been associated with many diseases and cancers including infectious mononucleosis (IM), Burkitt's lymphoma, Hodgkin's lymphoma, and nasopharyngeal carcinoma (NPC). EBV is transmitted by intimate contact, mainly through saliva and oropharyngeal secretion [2]. The virus primarily targets two cell types, B cells and epithelial cells. In B cells, EBV can establish a long-term infection. EBV can drive an infected B cell through different stages of latent infection where the viral genome remains inside the cell. The virus turns off most of its gene expression, stays quiescent, and remains invisible to the immune response within memory B cells. These memory B cells can be activated and become plasma-like B cells within which virions can replicate and burst out (lytic infection). In epithelial cells, an EBV infection often results in virus replication and producion. Latent infection of epithelial cells is rare and has been observed only in the case of NPC.

In vitro, viruses that emerge from one cell type preferentially infect the other [3]. The viral glycoprotein gp42 on EBV's envelope serves as the molecular switch that EBV uses to alternate infections between the two cell types. This viral protein is required for infection of B cells, but inhibits infection of epithelial cells. To enter a B cell, gp42 needs to bind to HLA class II molecules, antigen presenting proteins on the surface of antigen presenting cells, on the cell to trigger fusion. In B cells, gp42 interacts with HLA class II molecules, which are being synthesized before being presented on the cell surface, and becomes a target for degradation. Hence, viruses produced by B cells express low levels of gp42. Because epithelial cells do not express HLA-II, no such intracellular interactions occur and viruses produced by epithelial cells express higher levels of gp42 compared to viruses produced by B cells [3]. As a result, viruses emerging from B cells are approximately 5 times more efficient at infecting epithelial cells than the viruses emerging from epithelial cells, which are 100 times more efficient in infecting B cells than viruses derived from B cells [4, 11].

The viral infection of B cells and the maintenance of a persistent infection within the host have been intensively studied [19]. The dynamics of EBV infection of B cells and the T cell responses have been investigated using simulations of agent-based models, C-ImmSim and PathSim [6, 20]. The infection of epithelial cells, however, has not been included in these models. Simulations of these models reproduce qualitative features of real infections where the maximum number of

infected cells and viruses occur sometimes between one to a few weeks after initial infection, and a persistent infection is established within months. PathSim shows that EBV cannot result in a persistent infection unless it can maintain a latent infection within the memory B cell population. The simulation results of this agent-based model also highlight the sensitivity of the dynamics of infection to variation in the reactivation rate of lytic infection from infected memory B cells. A small increase in this rate causes the number of infected cells to expand quickly and remain at a high level [20]. However, the sensitivity may be due to the lack of T cell expansion and proliferation in this agent-based model.

A mathematical model describing the within-host dynamics of EBV infection has been constructed to study the T cell responses to persistent virus [7]. This model uses ordinary differential equations to track the number of latently and lytically infected cells, viruses, and T cells. However, it does not identify a specific class of target cells, such as B cells or epithelial cells. The model allows newly infected cells to produce viruses without going through latent stages of infection and allows lytically infected cells to become latently infected. The first of these two assumptions only applies for infection of epithelial cells. The second assumption does not reflect the biology of EBV infection of either cell type. Differential equation models have also been developed to study the dynamics of other herpesvirus infections like cytomegalovirus (CMV or HHV-5) [27] and HHV-6 [26]. Both CMV and HHV-6 can infect a wide range of cells in human and share the ability to establish persistent infection within the host cells with other herpesviruses [16, 28].

In this study, we develop and analyze mathematical models describing the within-host and between-host dynamics of infection to explore the effects of switching between host cell types on viral shedding, persistence, and evolution. Within a host, EBV must infect B cells to establish a long-term infection. However, infection of epithelial cells plays an important role in transmitting the infection. In fact, EBV is transmitted mainly through saliva and most viruses found in the saliva of infected hosts show characteristics of deriving from epithelial cells [13]. We propose a mathematical model to capture the within-host dynamics of virus and host-cell interaction, including the dynamics of epithelial cell infection. Assuming EBV can modify its ability to infect B cells and epithelial cells, we use this model to compute viral strategies that maximize transmission and the total virus

population being produced (intrahost competition). We then determine how changes in parameters affect the optimal viral strategy for transmission and for intrahost competition. While infection of epithelial cells plays a key role in transmission, intrahost competition emphasizes the role of infection of B cells. Finally, we apply the results of our within-host model to derive a model of EBV infection at the population level to study the conditions for invasion and coexistence of different viral strategies.

2 Model of the within-host dynamics of an EBV infection

2.1 Model

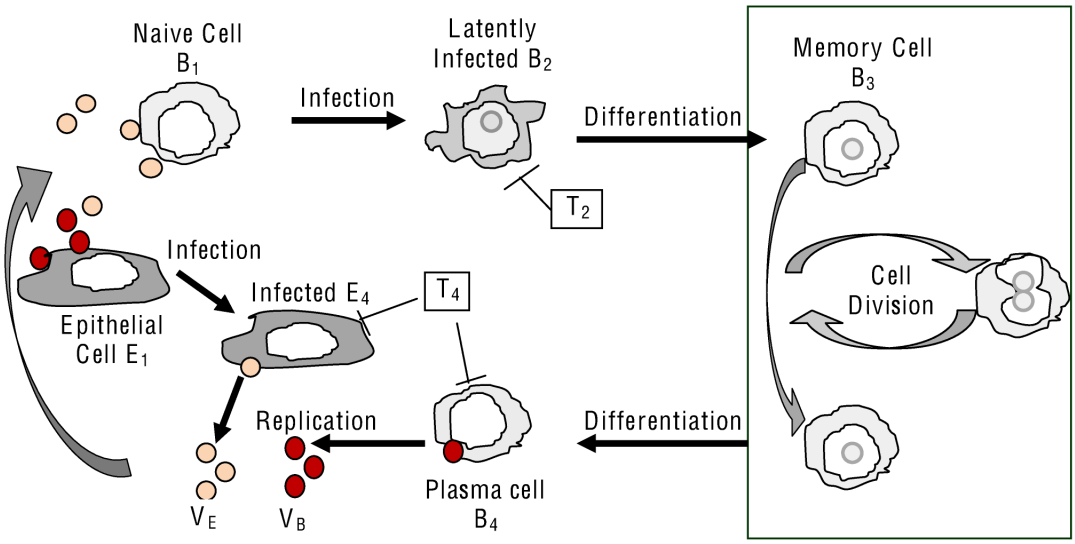

Figure 1: Model of EBV infection of B cells and epithelial cells. Our model adds the infection of epithelial cells to the model of EBV infection of B cells proposed by Thorley-Lawson [23].

Our mathematical model (Figure 1, and Equation 1) describing the dynamics of EBV infection within an infected host follows two types of target cells (B cells and epithelial cells), two types of viruses (B-cell-derived, V_B, and epithelial-cell-derived, V_E), and two types of cytotoxic T cells or CTLs (attacking latently infected B cells, T_2, and lytically infected cells, T_4, respectively). B cells break into four state variables: naive B cells (B_1), latently infected B cells (B_2), latently infected

memory B cells (B_3), and lytically infected B cells or plasma cells (B_4). B_2 and B_3 represent different stages of latency. B_2 are newly infected naive B cells and expressing EBV latent genes (up to 9 of them). Because of this latent gene expression, T cells can recognize and kill these B_2 cells. The latent gene expression helps driving a B_2 cell into a memory infected stage (B_3), in which no viral gene being expressed. There is no T-cell response to B_3 cells. Epithelial cells do not ordinarily harbor latent virus and require only two state variables: uninfected epithelial cells (E_1), and lytically infected epithelial cells (E_4). The model consists of a system of 10 ordinary differential equations:

$$\begin{aligned}
\dot{B_1} &= d_1(B_0 - B_1) - \mu_{Eb}V_E B_1 - \mu_{Bb}V_B B_1 \\
\dot{B_2} &= \rho(\mu_{Eb}V_E B_1 + \mu_{Bb}V_B B_1) - (d_2 + c)B_2 - k_2 B_2 T_2 \\
\dot{B_3} &= cB_2 + rB_3 - srB_3 \\
\dot{B_4} &= rB_3 - d_4 B_4 - k_4 B_4 T_4 \\
\dot{E_1} &= d_e(E_0 - E_1) - \mu_{Be}V_B E_1 - \mu_{Ee}V_E E_1 \\
\dot{E_4} &= \mu_{Be}V_B E_1 + \mu_{Ee}V_E E_1 - (d_e + \gamma)E_4 - k_4 E_4 T_4 \\
\dot{V_B} &= nd_4 B_4 - d_v V_B \\
\dot{V_E} &= n\gamma E_4 - d_v V_E \\
\dot{T_2} &= \phi_2 T_1 w(B_2) + \theta_2 T_2 w(B_2) - \delta T_2 \\
\dot{T_4} &= \phi_4 T_1[w(B_4 + E_4)] + \theta_4 T_4[w(B_4 + E_4)] - \delta T_4 \,.
\end{aligned} \tag{1}$$

The dynamics of B cells obey these assumptions:

- Naive B cells begin with an initial population of B_0 and turn over at rate d_1. They can encounter and be infected by the virus populations V_B and V_E with rates $\mu_{Bb}V_B$ and $\mu_{Eb}V_E$, respectively.
- An infection of a naive cell, B_1, gives rise latently infected cells, B_2, by proliferation of these newly infected cells, where ρ is the proliferation factor. These B_2 cells die at a rate d_2, can be recognized and killed by effector T cells at a rate $k_2 T_2$, and can enter the latently infected

memory state at rate c, which is driven by EBV gene expression switching off.

- Infected memory cells, B_3, obey homeostatic regulation similar to normal memory B cells. They are invisible to the T cell responses, undergo division with a rate r. On average, one cell becomes lytically infected and one remains in the memory state. The rate sr represents the death of B_3 due to homeostatic regulation of memory cells, where s is the regulation factor. For normal homeostasis, $s = 2$ balances the proliferation rate of $2r$ [15]. The frequent turnover of the memory B cell population helps maintain the supply of plasma B cells that produce antibodies against various types of antigens. EBV takes advantage of this mechanism to activate its lytic cycle.

- Lytically infected B cells, B_4, arise from lytic reactivation of infected memory B cells, B_3, at a rate r, die and release viruses at a rate d_4, and can be killed by effector T cells at a rate $k_4 T_4$.

The dynamics of epithelial cells assume the following:

- Uninfected epithelial cells start with initial population E_0, and turn over at a rate d_e. They can encounter and be infected by the virus populations V_B and V_E with rates $\mu_{Be} V_B$ and $\mu_{Ee} V_E$, respectively.

- Lytically infected epithelial cells, E_4, die naturally at a rate d_e, die from virus bursting out at a rate γ, and can be killed by effector T cells at a rate $k_4 T_4$.

Free viruses, V_B and V_E, are produced from B cells and epithelial cells at rates nd_4 and $n\gamma$ respectively, where n is the average burst size, and are cleared at a rate d_v.

To model the CTL response, we assume that the naive population, T_1, is regulated at a constant level and, upon stimulation by viral antigens, become effector cells against latent or lytic infection with a rate ϕ_2 or ϕ_4, respectively. The activated effector cells, T_2 and T_4, can proliferate further with rates θ_2 and θ_4, respectively, upon stimulation by viral antigens from infected cells. Each type of effector cell dies at a rate δ. Activation and proliferation of CTLs are saturating function of the

available infected cells,

$$w(B_j) \quad = \quad \frac{B_j}{K + B_j}\,, \tag{2}$$

where K is the number of infected cells at which activation or proliferation is half maximal. Table 1 presents the parameter values used for simulation and analysis of the model.

This system of Equation 1 has two equilibria. The infection-free equilibrium is given by

$$B_1^* = B_0\,, \qquad E_1^* = E_0\,,$$

and every other state variable equals zero. If virus replication is sufficiently efficient, there is also a persistent equilibrium, where all state variables take on positive values. The stability of the infection-free equilibrium is determined by the basic reproductive ratio,

$$\begin{aligned} R_0 \quad = \quad & \frac{n}{2d_v^2}\left(\frac{\rho\mu_{Bb}B_0c}{(s-1)(d_2+c)} + \frac{\mu_{Ee}E_0\gamma}{d_e+\gamma}\right) \\ & + \frac{n}{2d_v^2}\sqrt{\left(\frac{\rho\mu_{Bb}B_0c}{(s-1)(d_2+c)} - \frac{\mu_{Ee}E_0\gamma}{(d_e+\gamma)}\right)^2 + \frac{4\rho\mu_{Eb}B_0c\mu_{Be}E_0\gamma}{(s-1)(d_2+c)(d_e+\gamma)}}\,, \end{aligned} \tag{3}$$

of EBV in a naive host [9] that can be found by the next generation matrix [25]. Infections of both B cells and epithelial cells contribute to the basic reproductive ratio of EBV. If $R_0 < 1$, the infection-free equilibrium is stable and a long term infection cannot establish within a host. If $R_0 > 1$, the infection-free equilibrium is unstable and EBV can establish a persistent infection.

The dynamics of infected cells, viruses, and T cell responses for the case when $R_0 > 1$ are shown in Figure 2. The populations of infected cells and viruses peak during the second week of infection and then resolve down to low levels at equilibrium. Infection of epithelial cells plays a larger role during the primary infection than during the long-term infection. Simulation of EBV infection using the agent-based model, PathSim, produces clearance of virus and infected-cells in the case of a small viral burst size or the inability of EBV to enter latency within memory B cells [20]. Furthermore,

UU IR Author Manuscript

EBV infection also cannot be established within people with X-linked agammaglobulinemia because they do not have mature B cells. Our model produced similar results; small virus burst size (small value of n), no latency within memory B cells ($c = 0$), or no mature susceptible B cells ($B_0 = 0$) leads to $R_0 < 1$ and virus clearance.

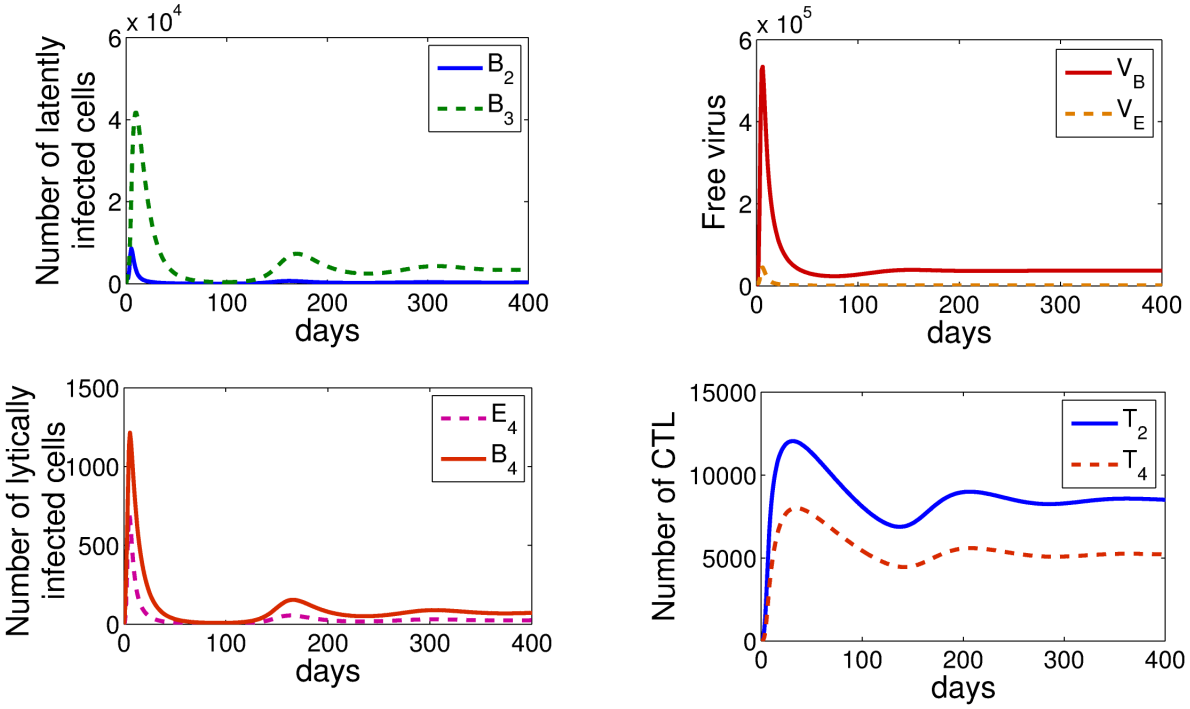

Figure 2: Dynamics of infected cells, viruses, and T cell responses using parameter values in Table 1

2.2 The effects of change in parameter values on transmission, persistent infection and the dynamics of EBV infection

Changes in the parameter values can alter the transmission efficiency, equilibrium viral loads, and short-term dynamics after initial infection. Transmission, as we have seen, depends on the amount of viruses produced by epithelial cells. Most EBV-infected individuals are healthy carriers and can transmit the virus throughout their lifetime. Although the amount of transmissible epithelial cell viruses varies during the course of the infection, over the long term, maximizing transmission is equivalent to maximizing the equilibrium value of epithelial cell viruses (V_E^*) produced in the long

run. A host can be infected by multiple strains of EBV, at least during primary infection [21]. In the case of a perfectly cross-reacting T cell response (i.e., one epitope of T cells responds to all strains of EBV) and a single cell type to infect, the principle of competition exclusion implies that multiple strains cannot coexist within a host [5]. In our model, there are two types of target cells and this principle does not necessarily apply. Numerical solutions of our model, however, do not display equilibrium coexistence in a host. Furthermore, the strain with higher total number of viruses being produced at equilibrium ($V_T^* = V_B^* + V_E^*$) wins out to establish a persistent infection within a host. Assuming EBV can modify its ability to infect B cells and epithelial cells, we investigate the optimal strategy the virus would used to maximize transmission or to maximize viral load in persistent infection.

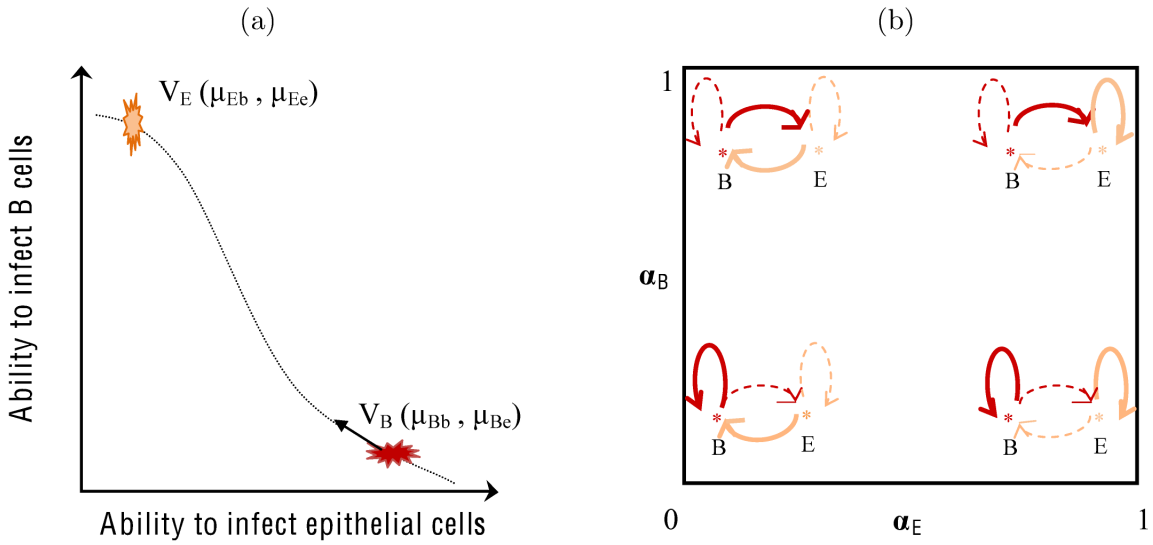

Figure 3: Trade-off in the ability to infect B cells and epithelial cells. (a) For a virus, increase in infection of B cells results in decrease in infection of epithelial cells and vice versa. (b) The strategies of V_B and V_E are defined as α_B and α_E, respectively; as these two parameters increase, both types of viruses prefer to infect epithelial cells.

We first develop a model to describe constraints in viral strategies within a host. We have assume that $\mu_{Eb} > \mu_{Bb}$ and $\mu_{Be} > \mu_{Ee}$ (Table 1) since V_E are more efficient at infecting B cells and V_B are more efficient at infecting epithelial cells [3]. This same study also found that infection efficiency varies among EBV strains. Assuming EBV can modify its ability to infect B cells and epithelial cells, it can only do so if there is a trade-off between the ability to infect B cells and

epithelial cells (Figure 3a). For example, V_B can improve its ability to infect B cells by increasing its expression of gp42. By doing so, however, it decreases its ability to infect epithelial cells.

To model this tradeoff, we constrain the infection rates to be weighted averages of the pure V_B and V_E strategies, which are represented by $\vec{\mu}_B$ and $\vec{\mu}_E$ respectively. Let $\vec{\eta}_B$ and $\vec{\eta}_E$ be the modified infection rates of V_B and V_E, respectively, given by

$$\begin{pmatrix} \eta_{Bb} \\ \eta_{Be} \end{pmatrix} = \alpha_B \begin{pmatrix} \mu_{Bb} \\ \mu_{Be} \end{pmatrix} + (1-\alpha_B) \begin{pmatrix} \mu_{Eb} \\ \mu_{Ee} \end{pmatrix}, \tag{4}$$

$$\begin{pmatrix} \eta_{Eb} \\ \eta_{Ee} \end{pmatrix} = \alpha_E \begin{pmatrix} \mu_{Bb} \\ \mu_{Be} \end{pmatrix} + (1-\alpha_E) \begin{pmatrix} \mu_{Eb} \\ \mu_{Ee} \end{pmatrix}. \tag{5}$$

The weighted parameters α_B and α_E define strategies of V_B and V_E, respectively. If $\alpha_B = 1$ and $\alpha_E = 0$ (Figure 3b, upper left), the η's take on the same values as the μ's. As α_B decreases, V_B switch from infecting epithelial cells to infecting B cells. As α_E increases, V_E switch from infecting B cells to infecting epithelial cells. When α_B and α_E are small, both V_B and V_E prefer to infect B cells (Figure 3b, bottom left). In contrast, when α_B and α_E are large, both V_B and V_E prefer to infect epithelial cells (Figure 3b, upper right). At the bottom right corner of Figure 3b, where α_B is small and α_E is large, V_B prefer to infect B cells and V_E prefer to infect epithelial cells. Replacing μ by η in the system of Equation 1, the equilibrium values $V_E^*(\vec{\alpha_i})$ and $V_T^*(\vec{\alpha_i})$ can be found numerically for any given strategy (α_B, α_E). Our next step is to analyze the effects of parameters on viral strategies that maximize V_E^* and V_T^*.

Natural variation between individuals in the expression of EBV receptors (HLA class II on B cells and CR2 or integrins on epithelial cells) can affect the susceptibility of these cells to EBV infection [8, 12]. The susceptibility of B cells and epithelial cells to virus classes V_B and V_E are described by the parameters μ_{Bb}, μ_{Be}, μ_{Eb}, and μ_{Ee}. We thus focus our sensitivity analysis on these four parameters and the other three parameters that are related to the production of viruses within a host: the rate of virus replication and production from B cells (d_4) and from epithelial cells (γ), and the viral burst size (n). We consider each of these parameters at three different values: the

value listed in Table 1 (middle), this value divided by two (small) and multiplied by two (large). For example, the three values for n are 500, 1000, and 2000. We thus have 3^7 (or 2187) parameter combinations to study. We ran the same sensitivity analysis on all other parameters in Table 1 but found minimal effects on the optimal strategy for transmission and establishment of a persistent infection.

2.2.1 Identifying strategies that maximize transmission

We assume that EBV maximizes transmission to new hosts by maximizing equilibrium amount of virus produced by epithelial cells (V_E^*). We divide the $\alpha_B\alpha_E$-plane in Figure 3b into a 21×21 grid where each of the 441 points on the grid represents a viral strategy. For every parameter set, we find V_E^* numerically for all strategies and locate the strategy where V_E^* is maximized. Figure 4 shows the influence of parameter values on the viral strategy that maximizes V_E^*. We focus our presentation of the results at the four corners where V_B and V_E prefer to switch infections between the two cell types (Region I), to primarily infect epithelial cells (Region II), to mainly target B cells (Region III), and to infect their own producing cells (Region IV).

- A minority of parameters (180 out of 2187 sets) favor a strategy similar to what has been observed in vitro where V_B preferentially infect epithelial cells and V_E preferentially infect B cells (Region I) [3]. The parameter sets that maximize V_E^* in this region tend to have μ_{Be}, n, and γ small and μ_{Ee} and d_4 large.

- A large number of parameter sets (1548 out of 2187) favor strategies where both V_B and V_E preferentially infect epithelial cells (Region II).

- The remaining parameter sets (459 out of 2187) favor strategies that lie in a range where α_B is large and α_E is at an intermediate value.

- There are no parameter sets favoring strategies in Region III and IV where α_B is small, implying that variation in parameter values has minimal effect on the strategy of B cell viruses in infecting epithelial cells to maximize transmission.

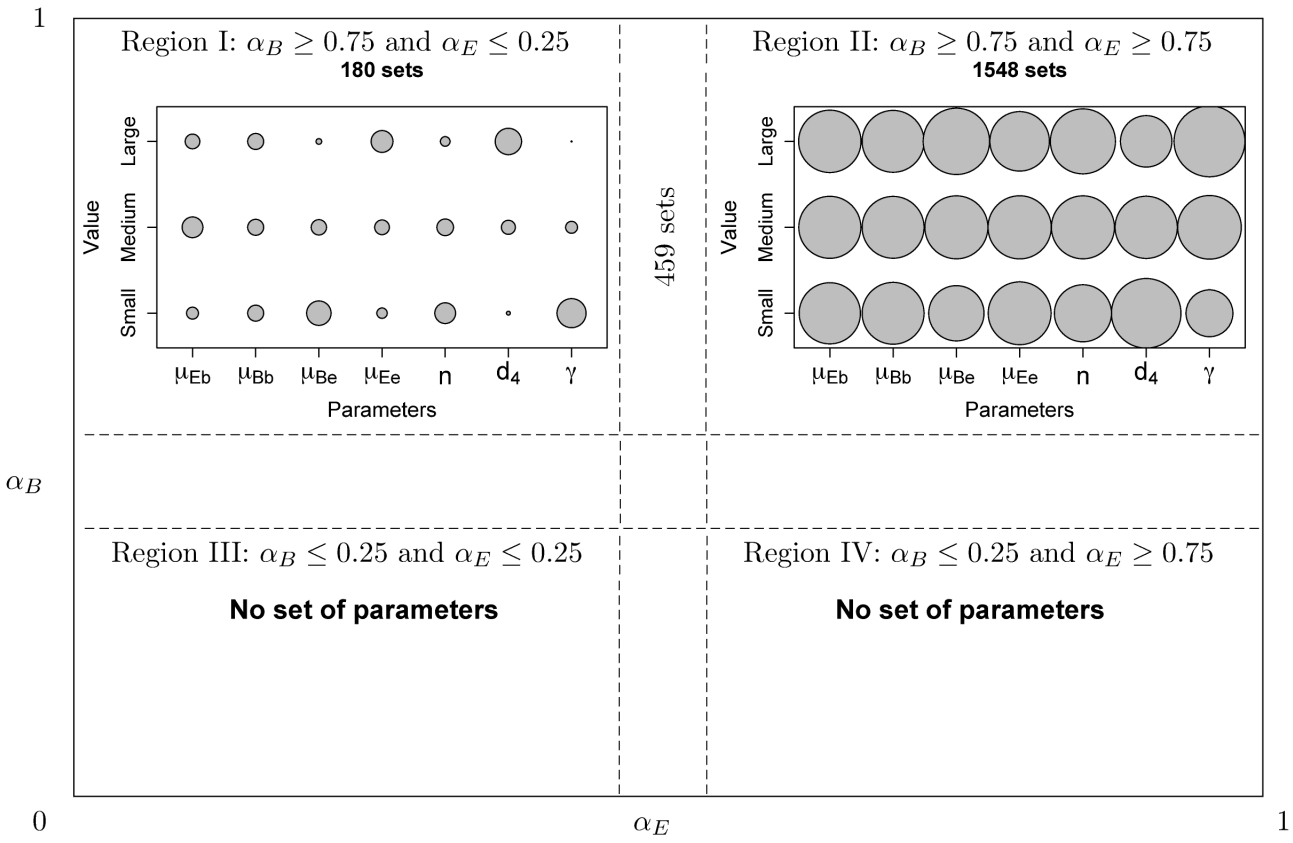

Figure 4: Effects of parameter values on optimal transmission (maximizing V_E^*). Each parameter takes on three different values: small, medium, and large. The four panels summarize parameter sets that give optimal V_E^* at the four corners in Figure (3b). Region I: $\alpha_B \geq 0.75$ and $\alpha_E \leq 0.25$; Region II: $\alpha_B \geq 0.75$ and $\alpha_E \geq 0.75$; Region III: $\alpha_B \leq 0.25$ and $\alpha_E \leq 0.25$; Region IV: $\alpha_B \leq 0.25$ and $\alpha_E \geq 0.75$. The dot size represents the number of times the parameters take on particular values.

Figure 5 shows the time-course dynamics of free viruses and the T cell responses for sample sets of parameters from Region I and Region II in Figure 4. The viral load initially elevates, peaks during the second week of infection, and then resolves down to a low equilibrium level. Strategies in Region II of the $\alpha_B\alpha_E$-plane give a higher V_E^* at the expense of a lower V_B^*.

2.2.2 Identifying strategies that maximize total viral load

We now make the assumption that a host gets infected by two or more EBV strains that differ only in their strategies on the $\alpha_B\alpha_E$-plane. In the case of a perfectly cross-reacting T cell response, our numerical results show that a virus with a higher V_T^* would win out to establish a persistent infection with no stable coexistence of multiple strains within a host. We thus assume that the

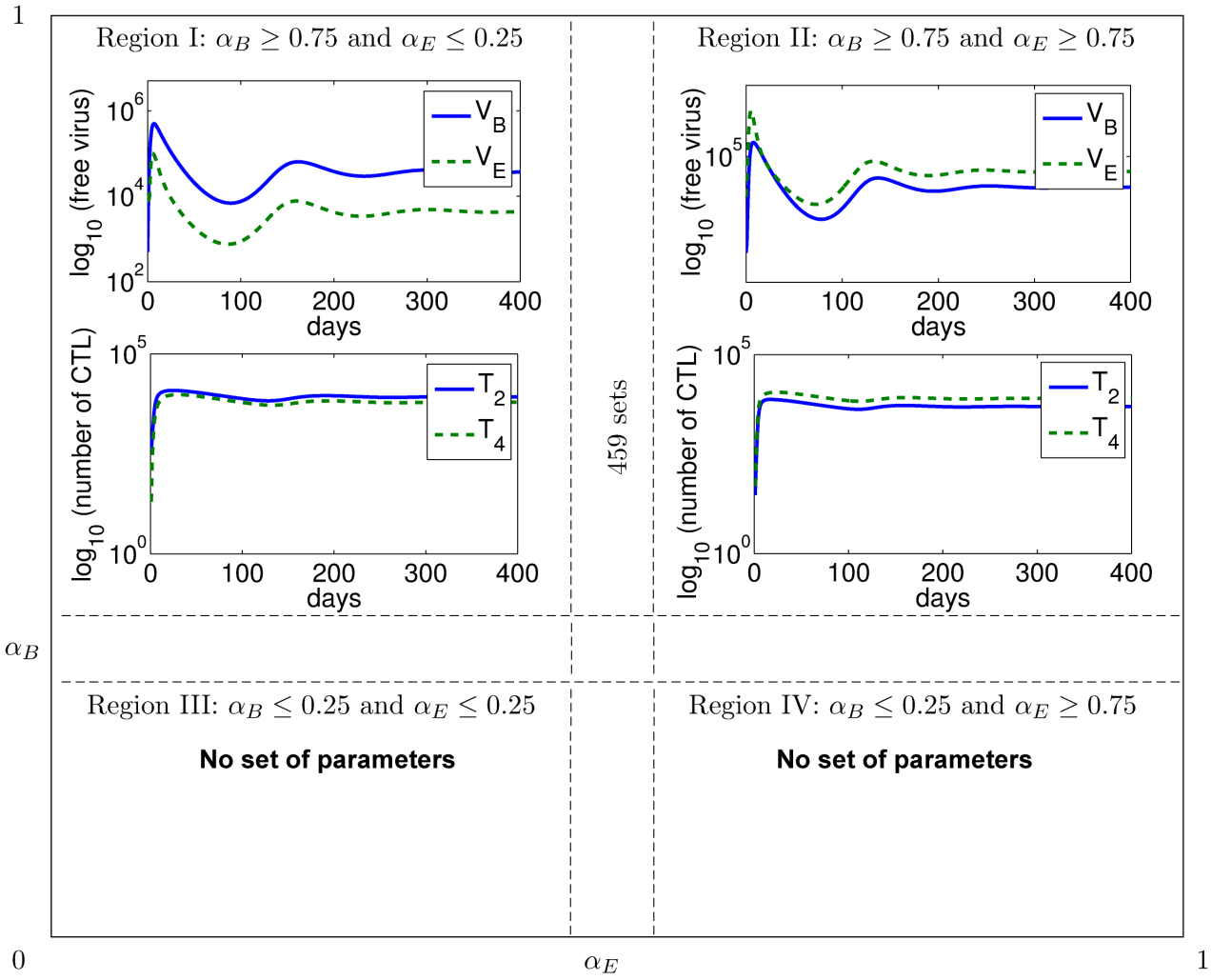

Figure 5: Effects of parameter values on dynamics of the model for sample sets of parameters from the two upper corners in Figure 4.

intrahost competition will select the virus that maximizes the total number of virions produced at equilibrium. For each of the 2187 sets of parameters, we found V_T^* numerically and located the strategy where V_T^* is maximized.

Figure 6 shows the influence of parameter values on viral strategy that maximizes V_T^*. The optimal strategies lie predominantly in Region III (1449 out of 2187 sets of parameters), 294 sets in Region II, with only a few conditions favoring a strategy in Region I (12 sets) or Region IV (33 sets). The remaining parameter sets (389) favor strategies in the middle and between the four regions.

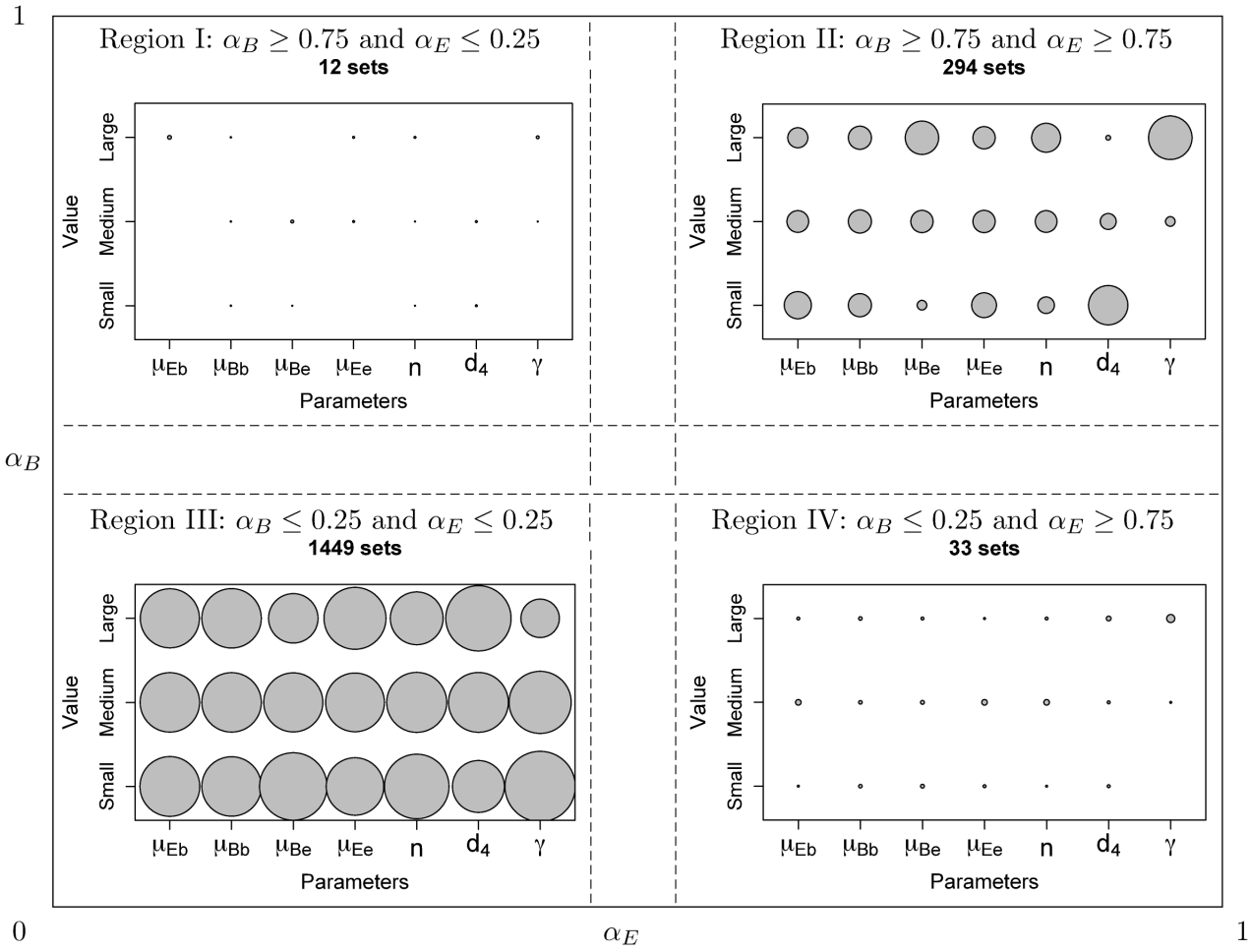

Figure 6: Effects of parameter values on a persistent infection (maximizing V_T^*) with similar notation as in Figure 4.

- In Region II, both V_B and V_E prefer to infect epithelial cells. The parameters values for which V_T^* is maximized in this region are characterized by a high rate of virus production in epithelial cells (γ) and a low rate of virus production in B cells (d_4).

- The majority of parameter values favor strategies in Region III where both V_B and V_E prefer to infect B cells. This implies that the infection of B cells plays a key role in the intrahost competition and the establishment of a long term infection.

Time-course dynamics of free viruses and the T cell responses for the four sample sets of parameters from the four Regions I-IV are shown in Figure 7. Higher levels of V_E^* often come at the expense of a lower V_B^*.

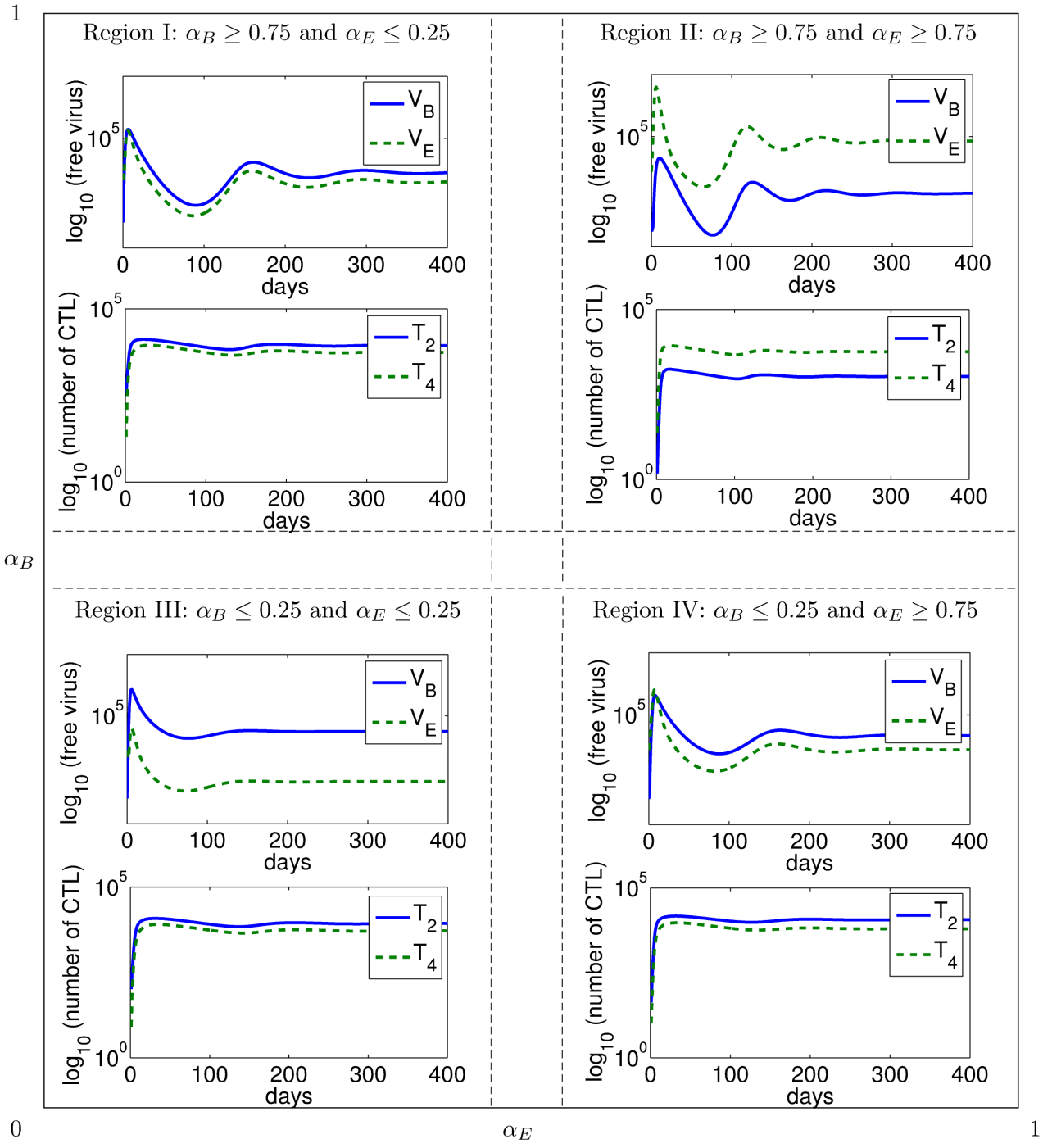

Figure 7: Effects of parameter values on dynamics of the model for sample sets of parameters from the four Regions I-IV in Figure 6.

3 A model of between-host dynamics

For most sets of parameters, maximizing V_E^* for transmission and maximizing V_T^* for intrahost competition favor different strategies. To predict the outcome of this conflict, we develop an implicit model of EBV dynamics in a homogeneous population of hosts that includes both transmission and superinfection. This model tracks susceptible hosts and hosts infected by one of the m strains of viruses. We make the assumption that virus strains do not coexist within a single host. A more competitive virus, with a higher value of V_T^*, can take over a host from the one with a lower value of V_T^* although the takeover requires time [1, 17]. We first analyze the model for the case where $m = 2$ and find the conditions for coexistence of different viral strategies within a population. We study the case where $m > 2$ primarily through simulation.

A host population infected with two strains of virus described by $\vec{\alpha}_1$ and $\vec{\alpha}_2$, where $\vec{\alpha}_i = (\alpha_B^i, \alpha_E^i)$, is captured by the following system of equations with three state variables: susceptible hosts (S), hosts infected by strain 1 (I_1), and hosts infected by strain 2 (I_2).

$$\begin{aligned}
\dot{S} &= \sigma N - \mu(\vec{\alpha}_1)\frac{I_1}{N}S - \mu(\vec{\alpha}_2)\frac{I_2}{N}S - \sigma S\,, \\
\dot{I}_1 &= \mu(\vec{\alpha}_1)\frac{I_1}{N}S - \mu(\vec{\alpha}_2)\nu(\vec{\alpha}_2,\vec{\alpha}_1)\frac{I_2}{N}I_1 + \mu(\vec{\alpha}_1)\nu(\vec{\alpha}_1,\vec{\alpha}_2)\frac{I_1}{N}I_2 - \sigma I_1\,, \\
\dot{I}_2 &= \mu(\vec{\alpha}_2)\frac{I_2}{N}S + \mu(\vec{\alpha}_2)\nu(\vec{\alpha}_2,\vec{\alpha}_1)I_1\frac{I_2}{N} - \mu(\vec{\alpha}_1)\nu(\vec{\alpha}_1,\vec{\alpha}_2)\frac{I_1}{N}I_2 - \sigma I_2\,.
\end{aligned} \tag{6}$$

This model assumes that

- There is no infection-induced death because mortality due to EBV infection is very rare even in the case of severe infectious mononucleosis. The total population, $N = S + I_1 + I_2$, is conserved by setting the per capita birth rate equal to the death rate, σ.
- Susceptible individuals are infected by strain i at a rate $\mu(\vec{\alpha}_i)I_i/N$, where $\mu(\vec{\alpha}_i) = aV_E^*(\vec{\alpha}_i)$. This implies that the infection rate of strain i is proportional to the number of epithelial cell viruses of that strain being produced within an infected host.
- Hosts infected by strain i can be superinfected, or taken over, by virus strain j at a rate

$\mu(\vec{\alpha}_j)\nu(\vec{\alpha}_j,\vec{\alpha}_i)I_j/N$. Because intrahost competition is determined by V_T^*, we assume that

$$\nu(\vec{\alpha}_j,\vec{\alpha}_i) = g(V_T^*(\vec{\alpha}_j) - V_T^*(\vec{\alpha}_i))$$

where g is an increasing function with $g(0) = 0$ and $g(-x) = -g(x)$. We set

$$g(x) = \frac{2}{\pi}\arctan(x/A), \tag{7}$$

which is scaled so that $g'(0) = 1/A$ and $\lim_{x\to\infty} g(x) = 1$.

- For all i, the transmission rate is larger than the birth and death rates ($\mu_i > \sigma$).

Equation 6 can be rewritten as

$$\begin{aligned} \dot{I}_1 &= \mu(\vec{\alpha}_1)\frac{I_1}{N}(N - I_1 - I_2) - f_{21}\frac{I_2}{N}I_1 - \sigma I_1\,, \\ \dot{I}_2 &= \mu(\vec{\alpha}_2)\frac{I_2}{N}(N - I_1 - I_2) + f_{21}\frac{I_1}{N}I_2 - \sigma I_2\,, \end{aligned} \tag{8}$$

where

$$f_{21} = \mu(\vec{\alpha}_2)g(V_T^*(\vec{\alpha}_2) - V_T^*(\vec{\alpha}_1)) - \mu(\vec{\alpha}_1)g(V_T^*(\vec{\alpha}_1) - V_T^*(\vec{\alpha}_2))$$

summarizes the competitive ability of strain 2 relative to strain 1.

If $V_E^*(\vec{\alpha}_1) > V_E^*(\vec{\alpha}_2)$ and $V_T^*(\vec{\alpha}_1) > V_T^*(\vec{\alpha}_2)$, strain 1 is better at both transmission and intrahost competition and will thus always win. If $V_E^*(\vec{\alpha}_1) > V_E^*(\vec{\alpha}_2)$ and $V_T^*(\vec{\alpha}_1) < V_T^*(\vec{\alpha}_2)$, strain 1 is better at transmission while strain 2 is better at intrahost competition. Then $\mu_1 = \mu(\vec{\alpha}_1) > \mu_2 = \mu(\vec{\alpha}_2)$ and $f_{21} > 0$.

UU IR Author Manuscript

Equation 8 has four equilibria:

$$\begin{aligned} P_0 &= (0,0), \\ P_1 &= \left(N(1-\frac{\sigma}{\mu_1}),0\right), \\ P_2 &= \left(0, N(1-\frac{\sigma}{\mu_2})\right), \text{ and} \\ P_{12} &= \left(\frac{(\mu_1-\sigma)N-(\mu_1+f_{21})I_2^*}{\mu_1}, I_2^* = \frac{\mu_1 N}{\mu_1-\mu_2+f_{21}} - \frac{\sigma N}{f_{21}}\right). \end{aligned} \tag{9}$$

Linear stability analysis implies that:

- The disease-free equilibrium, P_0, is unstable if $\mu_i > \sigma$.
- The strain 1 equilibrium, P_1, is positive and stable if $f_{21} < \frac{\sigma(\mu_1-\mu_2)}{(\mu_1-\sigma)}$.
- The coexistence equilibrium, P_{12}, is positive and stable if $\frac{\sigma(\mu_1-\mu_2)}{(\mu_1-\sigma)} < f_{21} < \frac{\sigma(\mu_1-\mu_2)}{(\mu_2-\sigma)}$.
- The strain 2 equilibrium, P_2, is positive and stable if $f_{21} > \frac{\sigma(\mu_1-\mu_2)}{(\mu_2-\sigma)}$.

If f_{21} is small, the force of superinfection is weak and strain 1 wins since it has a higher transmission rate. As f_{21} increases, strain 2 can coexist, and will eventually outcompete strain 1 for larger values of f_{21}.

The model with $m > 2$ strains of viruses can be written as

$$\begin{aligned} \dot{S} &= \sigma N - \sum_{i=1}^{m} \mu_i \frac{I_i}{N} S - \sigma S, \\ \dot{I}_i &= \mu_i \frac{I_i}{N} S + \sum_{j=1}^{m} f_{ij} \frac{I_i}{N} I_j - \sigma I_i. \end{aligned} \tag{10}$$

Let

$$F_i = \sum_{j=1}^{m} f_{ij} \frac{I_j}{N},$$

where $f_{ij} = 0$ when $i = j$. Using the assumption of constant population size, Equation 10 can be

reduced to

$$\dot{I}_i \quad = \quad \mu_i \frac{I_i}{N} \left(N - \sum_{j=1}^{m} I_j \right) + F_i I_i - \sigma I_i \,. \tag{11}$$

We can think of F_i as the total force of strain i taking over other strains. If $V_T^*(\vec{\alpha}_i)$ is small, f_{ij} can be negative and, hence, F_i can have a negative value. If $V_T^*(\vec{\alpha}_i)$ is large, f_{ij} is more likely to be positive and so is F_i. At equilibrium, we have

$$I_i^* = 0 \qquad \text{or} \qquad I_i^* = \frac{N \left(\mu_i \left(1 - \sum_{j \neq i}^{m} I_j^*/N \right) + F_i^* - \sigma \right)}{\mu_i} \,.$$

Strain i thus can exist at equilibrium if

$$F_i^* + \mu_i \left(1 - \sum_{j \neq i}^{m} I_j^*/N \right) \quad > \quad \sigma \,. \tag{12}$$

The condition for invasion can be expressed in terms of Equation 12. Let G be a set of strains (G $\subset \{1, 2, ..., k\}$) that can exist in the equilibrium state. Strain i, which is not in G, can invade the equilibrium if Equation 12 is satisfied. This implies that if strain i is not intrahost competitive (low $V_T^*(\vec{\alpha}_i)$ and hence F_i^* is negative), then it can only invade the equilibrium if it has a high transmission rate (high $V_E^*(\vec{\alpha}_i)$ and hence μ_i).

The force of infection, F_i^*, depends on the scaling factor A (Equation 7). When A is small, $|f_{ij}|$ is large and the force of superinfection is strong making it easier for a strain with a higher V_T^* to win via intrahost competition (Figure 8: row 1, col. 1). When A is large, the force of superinfection is weaker. It is more difficult for a strain with a higher V_T^* to take over a host from a strain with a lower V_T^*. Instead, strains with a lower V_T^* but a higher V_E^* (higher transmission) can invade and exist at equilibrium (Figure 8).

The pressure of intrahost competition thus favors strains with small α_B and small α_E to maximize V_T^*. The pressure of transmission, however, supports strains with large α_B and large α_E such

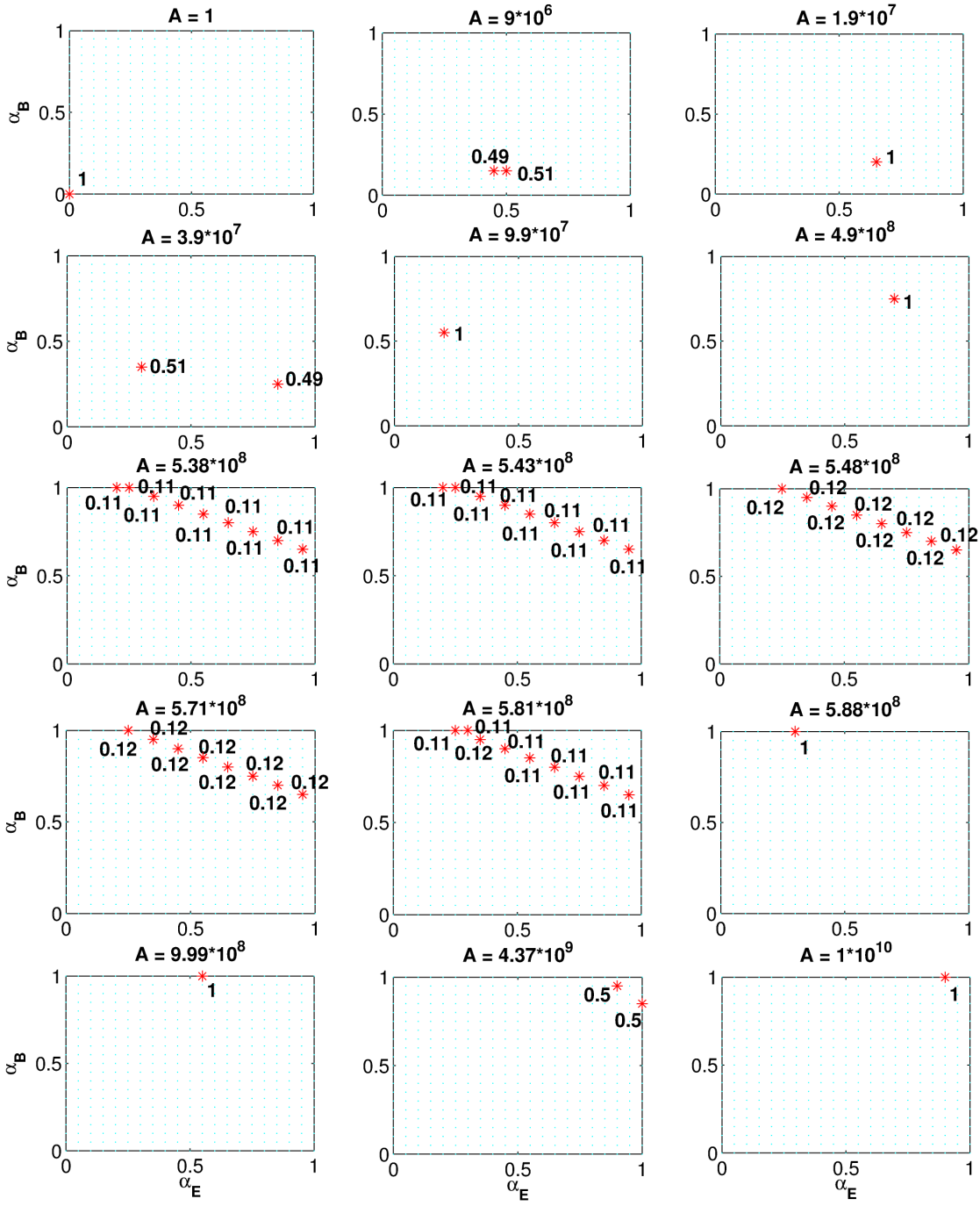

Figure 8: Snapshots of the winning and coexisting strains with the corresponding density of infected hosts at equilibrium (red '*') as A increases ($|f_{ij}|$ decreases) for a sample set of parameters from Region III in Figure 6. We consider 441 strains (blue '*') representing different viral strategies on the $\alpha_B\alpha_E$ plane.

that V_E^* is maximized. When the importance of intrahost competition is weak, strategies of higher transmission become dominant and coexistence of multiple strains is possible at the population level.

4 Discussion

We have developed models of the within-host and the between-host dynamics of EBV infection to study the effects of switching host cell tropism on transmission, persistence, and viral evolution. The model of the within-host dynamics tracks B cells, epithelial cells, T cells, and viruses. Although our model does not explicitly include spatial dynamics, the numerical solution of the dynamics of B cell infection (Figure 2) are consistent with simulations of the agent-based model, PathSim, such that small virus burst size or exclusion of a memory stage produces clearance [20]. This result supports the key role of virus latency within memory B cells in the establishment of a persistent EBV infection within a host. The agent-based models, however, have not yet address the importance of epithelial cell infection.

Viruses derived from B cells (V_B) preferentially infect epithelial cells and viruses derived from epithelial cells (V_E) preferentially infect B cells [3]. Viruses derived from epithelial cells are the main type that is shed in saliva, and play a key role in transmission [13]. Assuming EBV can modify its ability to infect B cells and epithelial cells, we compute viral strategies that maximize transmission and persistent infection. Maximizing transmission is assumed to be equivalent to maximizing the equilibrium amount of virus produced from epithelial cells. Maximizing intrahost competitive ability, in contrast, favors strains that produce the highest total number of viruses at equilibrium. Under most conditions, the optimal strategy for transmission is having both types of viruses (V_B and V_E) to preferentially infect epithelial cells. Under conditions when the rate of virus replication and production from B cells is high and production from epithelial cells is low, maximizing transmission favors viral strains that have viruses produced by one cell type infecting the other, in accord with observations of viral behavior in vitro [3]. In contrast, the optimal strategy for establishing and maintaining a persistent infection (intrahost competition) is for both types of

viruses to mainly infect B cells, an observation consistent with reports that, at least in healthy carriers, EBV primarily targets and maintains its persistent infection within the population of B cells [10].

For the majority of parameter combinations we studied, maximizing V_E^* for transmission and maximizing V_T^* for intrahost competition favor different strategies. No stable coexistence of multiple strategies is observed within a host. This may be due to the assumption of perfect cross-reactivity of T cells in our model. To further understand the conflict of strategies, we combine these results of the within-host model with a model at the population level and show that coexistence is possible. This between-host model includes both transmission and superinfection. In this case, when the force of superinfection is strong, viruses that preferentially infect B cells survive within and between hosts. When the importance of intrahost competition is weak, strategies of higher transmission become dominant and coexistence of multiple strains is possible at the population level.

Multiple strains of EBV, indeed, have been detected within healthy carriers for periods of more than 300 days [21]. There are at least two possible explanations for this. If multiple strains coexist at the host population level, as in our model, but within-host takeovers are slow, individuals could have transient multiple infections. Alternatively, coexistence of multiple strains within a host could result from either partial or no cross-reactivity of T cell responses. Our model can be modified to allow for either of these complications.

The existence of multiple strains, T cell responses, and the switching of infection between cell types may not only play important roles in the evolution of EBV but also in the pathology of EBV-associated diseases like infectious mononucleosis and nasopharyngeal carcinoma. Infectious mononucleosis (IM) is thought to be caused by primary infection of EBV in teenagers and young adults with symptoms including fever, fatigue, and sore throat. The overwhelming number of T cells, especially against viral lytic proteins cause these symptoms [10]. With the existence of multiple strains, it is possible that IM may be caused by a secondary infection with a strain that is particularly efficient at lytic replication and production of new viruses. For example, an infection with a strain that is highly efficient at infecting epithelial cells may generate an extreme T cell response against EBV lytic proteins and cause symptoms of IM.

Nasopharyngeal carcinoma is a cancer of epithelial cells in the nose and pharynx. EBV infection of epithelial cells normally results in lytic infection. In NPC patients, however, EBV can maintain latency within epithelial cells. Expression of virus latent proteins within these cells contributes to cell proliferation, cell survival, and inefficient T cell responses, all of which can accelerate tumor development. The shift of tropism from B cell to epithelial cell disease may be induced by immunoglobulin A (IgA) [22], which can enhance the viral entry into epithelial cells while interfering with the infection of B cells. Ongoing research extends the basic model of within-host dynamics (Equation 1) to study the association of EBV infection with these two important pathologies, IM and NPC.

Acknowledgements

We would like to thank Dr. Thorley-Lawson and other members of his laboratory for the opportunity to visit their lab and study the biology of EBV infection of B cells, and Dr. Hutt-Fletcher for insightful discussions on EBV infection of epithelial cells. Funding for this work was provided by the National Science Foundation (NSF) Research Training Group (RTG) (Award Number DMS0354259) and a 21st Century Science Initiative Grant from the James S. McDonnell Foundation.

References

[1] F.R. Adler and J. Mosquera. Is space necessary? Interference competition and limits to biodiversity. *Ecology*, 81(11):3226–3232, 2000.

[2] W.A. Andiman. Epidemiology of primary Epstein-Barr virus infection and infectious mononucleosis. In A. Tselis and H.B. Jenson, editors, *Epstein-Barr Virus*, volume 1, pages 39–57. Taylor & Francis Group, New York, NY, 1st edition, 2006.

[3] C.M. Borza and L.M. Hutt-Fletcher. Alternate replication in B cells and epithelial cells switches tropism of Epstein-Barr virus. *Nat. Med.*, 8(6):594–599, June 2002.

UU IR Author Manuscript

[4] C.M. Borza, A.J. Morgan, S.M. Turk, and L.M. Hutt-Fletcher. Use of gHgL for attachment of Epstein-Barr virus to epithelial cells compromises infection. *J. Virol.*, 7(10):5007–5014, May 2004.

[5] H.J. Bremermann and H.R. Thieme. A competitive exclusion principle for pathogen virulence. *J. Math. Biol.*, 27(2):179–190, 1989.

[6] F. Castiglione, K. Duca, A. Jarrah, R. Laubenbacher, D. Hochberg, and D.A. Thorley-Lawson. Simulating Epstein-Barr virus infection with C-ImmSim. *Bioinformatics*, 23(11):1371–1377, Mar. 2007.

[7] M. Davenport, C. Fazou, A.J. McMichael, and M.F.C. Callan. Clonal selection, clonal senescence, and clonal succession: the evolution of the T cell response to infection with a persistent virus. *J. Immunol.*, 168(7):3309–3317, 2002.

[8] K.M. Haan, W.W. Kwok, R. Longnecker, and P. Speck. Epstein-Barr virus entry utilizing HLA-DP or HLA-DQ as a coreceptor. *J. Virol.*, 74(5):2451–2454, Mar. 2000.

[9] J.M. Heffernan, R.J. Smith, and L.M. Wahl. Perspectives on the basic reproductive ratio. *J.R. Soc. Interface*, 2(4):281–293, 2005.

[10] A.D. Hislop, G.S. Taylor, D. Sauce, and A.B. Rickinson. Cellular responses to viral infection in humans: lessons from Epstein-Barr virus. *Annu. Rev. Immunol.*, 25(1):587–617, 2007.

[11] L.M. Hutt-Fletcher. EBV entry and epithelial infection. In Robertson ES, editor, *Epstein-Barr Virus*, volume 1, pages 359–378. Caister Academic Press, Norfolk, England, 1st edition, 2005.

[12] L.M. Hutt-Fletcher. Epstein-Barr virus entry. *J. Virol.*, 81(15):7825–7832, Aug. 2007.

[13] R. Jiang, R.S. Scott, and L.M. Hutt-Fletcher. Epstein-Barr virus shed in saliva is high in B-cell-tropic gp42. *J. Virol.*, 80(14):7281–7283, 2006.

[14] L. Jones and A. Perelson. Opportunistic infection as a cause of transient viremia in chronically infected HIV patients under treatment with HAART. *B. Math. Biol.*, 67(6):1227–1251, 2005.

[15] D.C. Macallan, D.L. Wallace, Y. Zhang, H. Ghattas, B. Asquith, C. Lara, A. Worth, G. Panayiotakopoulos, G.E. Griffin, D.F. Tough, and P.C. Beverley. B-cell kinetics in humans: rapid turnover of peripheral blood memory cells. *Blood*, 105(9):3633–3640, 2005.

[16] E.S. Mocarski, T. Shenk, and R.F. Pass. Cytomegaloviruses. In D.M. Knipe and Howley P.M, editors, *Field's Virol*, volume 2, pages 2701–2772. Lippincott Williams and Wilkins, Philadelphia, PA, 5th edition, 2007.

[17] J. Mosquera and F.R. Adler. Evolution of virulence: a unified framework for coinfection and superinfection. *J.Theor.Biol*, 195(3):293–313, 1998.

[18] A. Rickinson and E. Kieff. Epstein-Barr Virus. In D.M. Knipe and Howley P.M, editors, *Field's Virol*, volume 2, pages 2575–2627. Lippincott Williams and Wilkins, Philadelphia, PA, 4th edition, 2001.

[19] E.S. Robertson, editor. *Epstein-Barr virus*, volume 1. Caister Academic Press, Norfolk, England, 1st edition, 2005.

[20] M. Shapiro, K.A. Duca, K. Lee, E. Delgado-Eckert, J. Hawlins, A.S. Jarrah, R. Laubenbacher, R. Laubenbacher, N.F. Polys, V. Hadinoto, and D.A. Thorley-Lawson. A virtual look at Epstein-Barr virus infection: Simulation mechanism. *J. Theor. Biol.*, 252(4):633–648, Feb. 2008.

[21] D. Sitki-Green, M. Covington, and N. Raab-Traubb. Compartmentalization and transmission of multiple Epstein-Barr virus strains in asymptomatic carriers . *J. Virol.*, 77(3):1840–1847, Feb. 2003.

[22] J.W. Sixbey and Q.Y. Yao. Immunoglobulin A-induced shift of Epstein-Barr virus tissue tropism. *Science*, 255(5051):1578–1580, 1992.

[23] D.A. Thorley-Lawson. EBV persistence and latent infection in vivo. In Robertson ES, editor, *Epstein-Barr Virus*, volume 1, pages 309–349. Caister Academic Press, Norfolk, England, 1st edition, 2005.

[24] S.M. Turk, R. Jiang, L.S. Chesnokova, and L.M. Hutt-Fletcher. Antibodies to gp350/220 enhance the ability of Epstein-Barr virus to infect epithelial cells. *J. Virol.*, 80(19):9628–9633, Oct. 2006.

[25] P. van den Driessche and J. Watmough. Reproduction numbers and sub-threshold endemic equilibria for compartmental models of disease transmission. *Math. Biosci.*, 180(1-2):29–48, 2002.

[26] G. Wang, G.R.F. Krueger, and L.M. Buje. Mathematical model to simulate the cellular dynamics of infection with human herpesvirus-6 in EBV-negative infectious mononucleosis. *J. Med. Virol.*, 71(4):569–577, 2003.

[27] D. Wodarz, S. Sierro, and P. Klenerman. Dynamics of killer T cell inflation in viral infections. *J.R.S. Interface*, 4(14):533–543, 2007.

[28] K. Yamanishi, Y. Mori, and P.E. Pellett. Human herpesviruses 6 and 7. In D.M. Knipe and Howley P.M, editors, *Field's Virol*, volume 2, pages 2701–2772. Lippincott Williams and Wilkins, Philadelphia, PA, 5th edition, 2007.

Table 1: Parameters used in simulations of the within-host model (Equation 1). The rates are given in a unit of per minute. We use many parameters from PathSim where the rates are estimated and given in a unit of per 6 minutes [20]. We convert them into the unit of per minute.

Parameter	Description	Value	Reference
d_1	Turnover rate of naive B cells	1/6000	[20]
μ_{Eb}	B cell infection rate per epithelial cell virus	3.3×10^{-10}	[20] [a]
μ_{Bb}	B cell infection rate per B cell virus	$\mu_{Eb}/100$	[11]
ρ	Proliferation factor	2	[20]
d_2	Death rate of latently infected B cell	1/11520	[20]
c	Rate of latently infected cell going into memory stage	0.001	[20] [b]
k_2	Rate of latently infected B cells killed by activated T cell	3.8×10^{-8}	[20] [c]
r	Rate of reactivation of lytic infection from latent infection	8.3×10^{-5}	[20]
s	Regulation factor of memory B cells	2	[15]
d_4	Death rate of lytically infected cell due to viruses bursting out	1/4320	[20]
k_4	Rate of lytically infected B cell killed by activated T cell	7.6×10^{-8}	[20][c]
d_e	Turn-over rate of epithelial cell	1/6000	[d]
μ_{Be}	Epithelial cell infection rate per B cell virus	3×10^{-11}	[e]
μ_{Ee}	Epithelial cell infection rate per epithelial cell virus	$\mu_{Be}/5$	[11]
γ	Death rate of infected epithelial cell due to viruses bursting out	1/6000	[f]
n	Viral burst size	1000	[20]
d_v	Death rate of virus	1/2160	[20]
ϕ_2	Rate of CTL activation against latent infection	1.95×10^{-5}	[20] [g]
ϕ_4	Rate of CTL activation against lytic infection	4.48×10^{-5}	[20][g]
θ_2	Rate of effector CTL proliferation against latent infection	3.25×10^{-5}	[20] [h]
θ_4	Rate of effector CTL proliferation against lytic infection	3.25×10^{-5}	[20][h]
K	Number of infected cells when T cell activation is half maximal	10^5	[14]
δ	Death rate of T cells	1/156000	[20]

[a] Probability of virus and cell encounter per minute multiplied by the probability of infection and divided by the number of viruses ($\approx 10^7$).

[b] We take this to be the same rate as the estimation of .1% of lymphocytes leaving the Waldeyer's ring per minute.

[c] Probability of lymphocytes encounter per minute multiplied by the probability that T_i kills its target and divided by the number of $T_i (\approx 10^4)$.

[d] Estimated, taken to be the same as d_1.

[e] Estimated, taken to be less than μ_{Eb} [24].

[f] Estimated, taken to be less than d_4 [3].

[g] Probability of lymphocyte encounter per minute multiplied by the probability of T_i activation by B_i, where $i = 2$ or 4.

[h] Probability of lymphocyte encounter given per minute multiplied by the frequency of cell division (every 8-12 hours).