

Mathematical modelling the age dependence of Epstein–Barr virus associated infectious mononucleosis

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Most people get Epstein–Barr virus (EBV) infection at young age and are asymptomatic. Primary EBV infection in adolescents and young adults, however, often leads to infectious mononucleosis (IM) with symptoms including fever, fatigue and sore throat that can persist for months. Expansion in the number of CD8⁺ T cells, especially against EBV lytic proteins, are the main cause of these symptoms. We propose a mathematical model for the regulation of EBV infection within a host to address the dependence of IM on age. This model tracks the number of virus, infected B cell and epithelial cell and CD8⁺ T-cell responses to the infection. We use this model to investigate three hypotheses for the high incidence of IM in teenagers and young adults: saliva and antibody effects that increase with age, high cross-reactive T-cell responses and a high initial viral load. The model supports the first two of these hypotheses and suggests that variation in host antibody responses and the complexity of the pre-existing cross-reactive T-cell repertoire, both of which depend on age, may play important roles in the etiology of IM.

Keywords: infectious mononucleosis; mathematical model.

1. Introduction

Epstein–Barr virus (EBV) is a member of the herpesvirus family infects over 90% of humans worldwide and can persist for the lifetime of the person (Rickinson & Kieff, 2001). EBV is transmitted by intimate contact, mainly through saliva and oropharyngeal secretion (Andiman, 2006). Within a host, the virus primarily targets two cell types, B cells and epithelial cells. EBV enters B cells and epithelial cells through different routes using different glycoprotein complexes on its envelop (Hutt-Fletcher, 2007). Host saliva and antibodies, like IgA and IgG, to viral glycoproteins can decrease the infection of B cells but enhance the infection of epithelial cells (Sixbey & Yao, 1992; Turk *et al.*, 2006).

EBV can establish long-term infections in B cells, driving an infected B cell through stages of latent infection where the viral genome remains inside the cell. The virus stays quiescent and remains invisible to the immune response within memory B cells. These latently infected memory B cells can

be activated, becoming plasma-like B cells within which virions replicate and burst out (lytic infection). Infection of epithelial cells typically results in lytic replication with viruses bursting out and cell death (Hutt-Fletcher, 2005). Infections of both cell types are important, as *in vitro* experiment shows that virus produced from one cell type preferentially infects the other (Borza & Hutt-Fletcher, 2002).

Most people get EBV infection at young age and are asymptomatic. Adolescents and young adults infected with EBV develop infectious mononucleosis (IM) in up to 50% of cases, with symptoms including fever, fatigue and sore throat that can persist for months (Andiman, 2006; Cohen, 2005). These symptoms are caused mainly by expansion in the number of CD8⁺ T cells, especially against EBV lytic proteins expressed during lytic replication and production of virions (Hislop *et al.*, 2007).

Since its discovery more than 40 years ago, EBV has attracted many empirical studies of its ability to persist within one host and its association with cancers. However, EBV infects only humans and a limited range of host cells and lacks a good animal models to investigate EBV infection *in vivo* (Rickinson, 2005). Most hypotheses and conclusions about EBV infection are based on studies of cell cultures in epithelial and B cell lines. Viral loads and infected cell data must be obtained from saliva and blood collected from infected people. Many aspects of EBV infection still remain open questions including what factors affecting the dynamics of infection that may lead to IM.

In our previous work, we developed a mathematical model of the within-host dynamics to study EBV long-term infection and viral evolution (Huynh & Adler, 2010). In this study, we extend the within-host model to include features of immune system thought to be important in IM: the role of antibodies in shifting infections between the two cell types and the effect of specific and cross-reactive T-cell responses. The model tracks the number of viruses, infected B cells and epithelial cells, specific CD8⁺ T cells and cross-reactive CD8⁺ T cells responding to the infection.

We use this model to investigate the following three hypotheses:

- **Saliva and antibody effects**

Host saliva and antibodies to EBV proteins promote infection of epithelial cells which, in turn, can induce an elevated CD8⁺ T-cell response against lytic infection. This hypothesis comes from observations that some unknown factor in host saliva and antibodies to viral proteins have been observed to enhance epithelial cell infection and that salivary IgA level increases with age (Jafarzadeh *et al.*, 2008; Sixbey & Yao, 1992; Turk *et al.*, 2006; Weber-Mzell *et al.*, 2004).

- **Cross-reactive T-cell responses**

Continuous exposure to different pathogens as people age can increase the complexity of the pre-existing memory T-cell repertoire. Adolescents infected with EBV may recruit large numbers of cross-reactive memory T cells previously created in response to other viral infections. These cross-reactive memory T-cell responses may be easier to be activated than naive T cells but less efficient in controlling the infection than primary responses from naive T cells (Clute *et al.*, 2005; Rickinson & Kieff, 1996).

- **The initial viral load**

High viral challenges in adolescents, often acquired via kissing, may induce aggressive CD8⁺ T-cell response (Hislop *et al.*, 2007).

In one study using data collected from three IM and three asymptomatic donors, the levels of CD8⁺ T cells during the primary infection were observed to be between 4 and 26 folds higher in IM cases than in asymptomatic cases (Silins *et al.*, 2001). Our goal of applying the model in studying these hypotheses is not to predict the exact level of antibody effect, cross-reactive memory CD8⁺ T-cell response or

initial viral load that induces IM but to help in formulating questions and providing insights for specific biological study of IM in the laboratory.

2. Model

Addressing the three hypotheses for the causes of IM requires consideration of antibody effects and state variables representing cross-reactive T-cell responses to latent and lytic infection. Our mathematical model (Fig. 1 and (2.1)) tracks two types of target cells, B cells and epithelial cells, viruses, two types of specific cytotoxic T cells (CTLs) attacking latently infected B cells (T_2) and lytically infected cells (T_4), respectively, and two types of cross-reacting CTLs against latently (T_{2c}) and lytically (T_{4c})-infected cells. B cells is classified further 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 cells, expressing EBV latent genes (Thorley-Lawson, 2005) and thus can be recognized and killed by effector T cells. B_3 represents the next stage of latency with no expression of viral gene and hence no T cell response to these infected memory cells (Thorley-Lawson, 2005).

Infection of epithelial cells often results in virus replication and production. Epithelial cells do not ordinarily harbour latent virus, which has been observed only in the cases of cancer like nasopharyngeal carcinoma. The model thus includes only two state variables: uninfected epithelial cells (E_1) and lytically infected epithelial cells (E_4). Viruses are classified into virus derived from B cells (V_B) and virus derived from epithelial cells (V_E) since virus produced from one cell type preferentially infects the other (Borza & Hutt-Fletcher, 2002). Cytotoxic T-cell responses against viral latent and lytic proteins have been detected in EBV positive individuals (Clute *et al.*, 2010). Expression of EBV proteins can also stimulate cross-reactive response from $CD8^+$ T cells specific to influenza virus (Clute *et al.*, 2005). Four state variables for T-cell responses are included to examine the effect of these responses

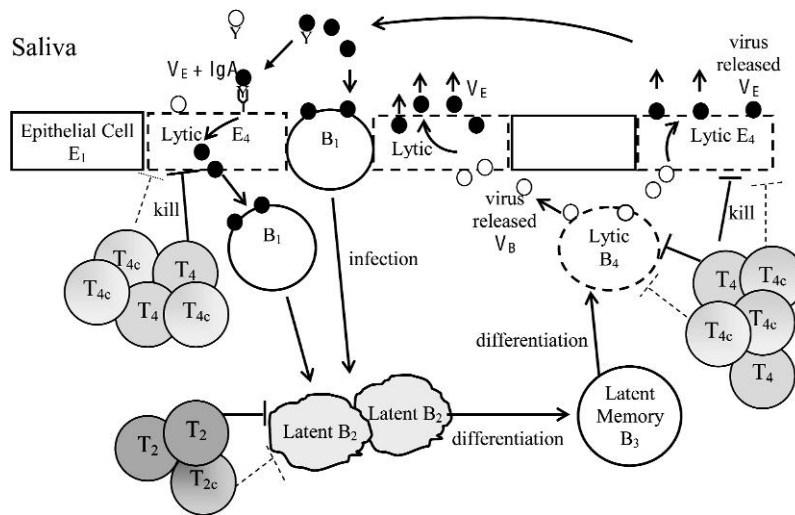


FIG. 1. Model of EBV infection of B cells and epithelial cells. Antibodies like IgA can shift the viral target from B cells to epithelial cells. Activation of cross-reactive memory T cells (T_{2c} , and T_{4c}) that are not efficient in killing infected cells may contribute to the pathology of IM.

on the dynamics of infection. The model consists of a system of twelve ordinary differential equations:

$$\begin{aligned}
\frac{dB_1}{dt} &= d_1(B_0 - B_1) - f(a)\mu_{Eb}V_E B_1 - f(a)\mu_{Bb}V_B B_1, \\
\frac{dB_2}{dt} &= \rho(f(a)\mu_{Eb}V_E B_1 + f(a)\mu_{Bb}V_B B_1) - (d_2 + c)B_2 - k_2 B_2 T_2 - \chi_2 k_2 B_2 T_{2c}, \\
\frac{dB_3}{dt} &= cB_2 + rB_3 - srB_3, \\
\frac{dB_4}{dt} &= rB_3 - d_4 B_4 - k_4 B_4 T_4 - \chi_4 k_4 B_4 T_{4c}, \\
\frac{dE_1}{dt} &= d_e(E_0 - E_1) - h(a)\mu_{Be}V_B E_1 - h(a)\mu_{Ee}V_E E_1, \\
\frac{dE_4}{dt} &= h(a)\mu_{Be}V_B E_1 + h(a)\mu_{Ee}V_E E_1 - (d_e + \gamma)E_4 - k_4 E_4 T_4 - \chi_4 k_4 E_4 T_{4c}, \quad (2.1) \\
\frac{dV_B}{dt} &= nd_4 B_4 - d_v V_B, \\
\frac{dV_E}{dt} &= n\gamma E_4 - d_v V_E, \\
\frac{dT_2}{dt} &= (1 - \sigma_2)\phi_2 T_N w(B_2) + \theta_2 T_2 w(B_2) - \delta T_2, \\
\frac{dT_{2c}}{dt} &= \sigma_2 m \phi_2 T_M w(B_2) + m\theta_2 T_{2c} w(B_2) - m\delta T_{2c}, \\
\frac{dT_4}{dt} &= (1 - \sigma_4)\phi_4 T_N [w(B_4 + E_4)] + \theta_4 T_4 [w(B_4 + E_4)] - \delta T_4, \\
\frac{dT_{4c}}{dt} &= \sigma_4 m \phi_4 T_M [w(B_4 + E_4)] + m\theta_4 T_{4c} [w(B_4 + E_4)] - m\delta T_{4c}.
\end{aligned}$$

The dynamics of B cells obey these assumptions:

- Naive B cells have an initial population size of B_0 and turnover rate d_1 . They encounter and are infected by V_B and V_E with rates $f(a)V_B\mu_{Bb}$ and $f(a)V_E\mu_{Eb}$, respectively, where $f(a)$ represents the inhibiting effect of host saliva and antibody responses on infection of B cells (2.3).
- An infection of a naive cell, B_1 , may give rise to one or more latently infected cells, B_2 , due to the limited proliferation of these newly infected cells, where ρ is the proliferation factor. These B_2 cells die at rate d_2 and are recognized and killed by specific or cross-reactive effector T cells at rate k_2 or $\chi_2 k_2$, respectively. They can also enter the latently infected memory state, driven by EBV turning off its gene expression, at rate c .
- Infected memory cells, B_3 , obey homeostatic regulation similar to normal memory B cells. They are invisible to the immune system and undergo cell division with rate r , where one cell goes into

lytic infection and one stays 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 a normal homeostasis, $s = 2$ balances the proliferation rate of $2r$ (Macallan *et al.*, 2005).

- Lytically infected B cells, B_4 , arise from lytic reactivation of memory infected B cells at rate r , die and release viruses at rate d_4 and can be killed by specific or cross-reactive effector T cells at rate k_4 or $\chi_4 k_4$, respectively.

Here, χ_j ($j = 2$ or 4), with $0 \leq \chi_j \leq 1$, characterizes the efficiency of cross-reactive T cells in killing infected cells, compared to specific T cells. The smaller χ_j is the more inefficient cross-reactive T cells are in killing infected cells.

The dynamics of epithelial cells assume the following:

- Uninfected epithelial cells have initial population size of E_0 with turnover rate d_e . They encounter and are infected by V_B and V_E with rates $h(a)V_B\mu_{Be}$ and $h(a)V_E\mu_{Ee}$, respectively. Here, $h(a)$ represents the enhancement effect of host saliva and antibody responses on infection of epithelial cells (2.4).
- Lytically infected epithelial cells, E_4 , die at natural rate d_e , die due to virus bursting out at rate γ , and can be killed by specific or cross-reactive effector T cells at rate k_4 or $\chi_4 k_4$, respectively.

The effects of host saliva and antibody responses on the infection of the two cell types are represented by the functions f and h and included as parameters in the cell-specific infection terms. This is based on the observation that saliva from infected people and antibodies to viral glycoproteins interfere with infection of B cells and enhance infection of epithelial cells (Turk *et al.*, 2006). From limited data in this *in vitro* study, we obtain the linear relationship between f and h that can be described in the following equation:

$$h = 1 + \lambda - \lambda f, \tag{2.2}$$

where $\lambda \approx 32$. The functions f and h carry no units. Without the antibody effect, $f = 1$ and $h = 1$. With antibody effects, f decreases to represent decreased efficiency in infection of B cells and h increases to represent increased efficiency in infection of epithelial cells. To model the dependence of f and h on antibody response, we assume that the two functions take on the forms

$$f(a) = 1 - \frac{a^2}{A^2 + a^2}, \tag{2.3}$$

$$h(a) = 1 + \frac{\lambda a^2}{A^2 + a^2}, \tag{2.4}$$

where a represents the strength of saliva and antibody effects. We will refer to a as the antibody effect from now on because the factor(s) in saliva that can enhance infection of epithelial cells remain unknown. The functions $f(a)$ and $h(a)$ take the form of Hill functions, where λ is the maximum level of the antibody effect on the infection of epithelial cells and A is the level of a where the effect on the infection of B cells and epithelial cells is half maximal. As a increases, $f(a)$ decreases while $h(a)$ increases before saturating. This saturating form assumes that a certain level of antibody response is required to have strong effects on the infection of both cell types.

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. These viruses die at rate d_v . To model the CTL response, we

separate the specific responses against latent (T_2) and lytic (T_4) infection coming from naive T cells and the cross-reactive responses (T_{2c} and T_{4c}) coming from the memory T cells specific to other encountered pathogens.

We assume that the naive and memory populations, T_N and T_M , are fixed at constant levels due to homeostatic regulation of these two pools of T cells (Stockinger *et al.*, 2004) and also for the mathematical convenience of the model. Upon stimulation by viral antigens, T_N become effector cells against latent or lytic infection at rate $(1 - \sigma_2)\phi_2$ or $(1 - \sigma_4)\phi_4$, respectively, where σ_j is the fraction of cross-reactive T-cell response. With further stimulation by viral antigens from infected cells, the activated effector cells, T_2 and T_4 , can proliferate with rates θ_2 and θ_4 , respectively. Each type of effector cell dies at a similar rate δ . Activation and proliferation of CTLs saturate as a function of the available infected cells

$$w(B_j) = \frac{B_j}{K + B_j}, \quad (2.5)$$

where K is the number of infected cells at which activation or proliferation is half maximal and is assumed to be the same for both responses.

Cross-reactive responses, T_{2c} and T_{4c} , are activated from the memory population at rate $\sigma_j m \phi_j$, where $m \geq 1$ is a measurement of how much faster a response can be activated from memory T cells compared to activation from naive T cells. These cross-reactive memory cells are assumed to have faster dynamics than specific T cells. Although they may be activated quickly and proliferate rapidly, they die faster (by a factor m). This comes from observations that memory cells respond with fast kinetics (Kedl & Mescher, 1998) but are also more susceptible to death (Cerwenka *et al.*, 1999). Furthermore, T cells obtained from acute IM patients have been shown to have high expression of programmed-death-1 (Hislop *et al.*, 2007).

The system (2.1) has two equilibria: an infection-free equilibrium and a persistent equilibrium. The infection-free equilibrium is given by

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

with other state variables equal zero. The stability of the infection-free equilibrium is determined by the basic reproductive ratio, of EBV in a naive host (Heffernan *et al.*, 2005):

$$R_0 = \frac{n}{2d_v^2} \left(\frac{\rho f(a)\mu_{Bb}B_0c}{(s-1)(d_2+c)} + \frac{h(a)\mu_{Ee}E_0\gamma}{d_e+\gamma} \right) + \frac{n}{2d_v^2} \sqrt{\left(\frac{\rho f(a)\mu_{Bb}B_0c}{(s-1)(d_2+c)} - \frac{h(a)\mu_{Ee}E_0\gamma}{d_e+\gamma} \right)^2 + \frac{4\rho f(a)\mu_{Eb}B_0c h(a)\mu_{Be}E_0\gamma}{(s-1)(d_2+c)(d_e+\gamma)}}. \quad (2.6)$$

Infections of both B cells and epithelial cells contribute to the basic reproductive ratio of EBV. The antibody effects, $f(a)$ and $h(a)$, shift the weight of R_0 contribution from B cells to epithelial cells. If $R_0 < 1$, the infection-free equilibrium is stable and the infection cannot establish within a host. If $R_0 > 1$, the infection-free equilibrium is unstable and EBV can establish a persistent infection, where all state variables take on positive values. Tables 1 and 2 present the parameter values used for simulations and analysis of the model.

Assuming no cross-reactive responses ($\sigma_2, \sigma_4 = 0$), the dynamics of viruses and T cells for the cases without antibody effect ($a = 0$) and with antibody effect ($a = 10$) are shown in Fig. 2(i and ii),

TABLE 1 *Parameters for the dynamics of B cells and antibody effect used in the model simulations (2.1). We use many parameters from PathSim, where the rates are estimated and given in a unit of per 6 min (Shapiro et al., 2008) and convert them into the unit of per minute*

| Parameter | Description | Value | Value | Reference |
|------------|--|-----------------------|------------------------------------|-------------------------------------|
| d_1 | Turnover rate of naive B cells | 1/6000 | min^{-1} | (Shapiro et al., 2008) |
| μ_{Eb} | B cell infection rate per epithelial cell virus | 3.3×10^{-10} | $\text{min}^{-1}\text{virus}^{-1}$ | (Shapiro et al., 2008) [†] |
| μ_{Bb} | B cell infection rate per B-cell virus | $\mu_{Eb}/100$ | $\text{min}^{-1}\text{virus}^{-1}$ | (Hutt-Fletcher, 2005) |
| ρ | Proliferation factor | 2 | No unit | (Shapiro et al., 2008) |
| d_2 | Death rate of latently infected B cells | 1/11520 | min^{-1} | (Shapiro et al., 2008) |
| c | Rate of latently infected cells going into memory stage | 0.001 | min^{-1} | (Shapiro et al., 2008) [‡] |
| k_2 | Rate of latently infected B cells killed by T cells | 3.8×10^{-8} | $\text{min}^{-1}\text{cell}^{-1}$ | (Shapiro et al., 2008) [§] |
| r | Rate of reactivation of lytic infection from latent infection | 8.3×10^{-5} | min^{-1} | (Shapiro et al., 2008) |
| s | Regulation factor of memory B cells | 2 | No unit | (Macallan et al., 2005) |
| d_4 | Death rate of lytically infected cells due to viruses bursting out | 1/4320 | min^{-1} | (Shapiro et al., 2008) |
| k_4 | Rate of lytically infected B cells killed by T cells | 7.6×10^{-8} | $\text{min}^{-1}\text{cell}^{-1}$ | (Shapiro et al., 2008) [§] |
| a | The strength of antibody effect | Variable (0–40) | No unit | |
| A | Level of a where antibody effect is half maximal | 10 | No unit | |
| λ | Maximal level of antibody effect on epithelial cell infection | 32 | No unit | (Turk et al., 2006) [¶] |

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

[‡]We take this to be the same rate as the estimation of 0.1% of lymphocytes leaving the Waldeyer’s ring per minute.

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

[¶]Estimated from limited data given in an *in vitro* study (Borza & Hutt-Fletcher, 2002).

respectively. The antibody effect greatly increases the number of viruses being produced, with most of this increase coming from epithelial cell viruses. Elevated number of T cells against viral lytic proteins are induced during primary infection.

3. Application to IM

EBV infection in children of young age is usually asymptomatic. Adolescents and young adults infected with EBV may develop flu-like symptoms referred to as IM. These symptoms result from a massive T-cell response to EBV a few weeks after the initial viral infection that can last from a few weeks to several months (Cohen, 2005). The T-cell responses against viral latent proteins are generally smaller in

TABLE 2 Parameters for the dynamics of epithelial cells, virus and T-cell responses used in the model simulations (2.1)

| Parameter | Description | Value | Unit | Reference |
|------------|---|-----------------------|-------------------------------------|---|
| d_e | Turnover rate of epithelial cells | 1/6000 | min^{-1} | (Shapiro <i>et al.</i> , 2008) [†] |
| μ_{Be} | Epithelial cell infection rate per B-cell virus | 3×10^{-11} | $\text{min}^{-1}\text{virus}^{-1}$ | (Shapiro <i>et al.</i> , 2008) [‡] |
| μ_{Ee} | Epithelial cell infection rate per epithelial cell virus | $\mu_{Be}/5$ | $\text{min}^{-1}\text{virus}^{-1}$ | Hutt-Fletcher (2005) |
| γ | Death rate of infected epithelial cells due to viruses bursting out | 1/6000 | min^{-1} | (Shapiro <i>et al.</i> , 2008) [§] |
| n | Viral burst size | 1000 | $\text{virus}\cdot\text{cell}^{-1}$ | Shapiro <i>et al.</i> (2008) |
| d_v | Death rate of virus | 1/2160 | min^{-1} | Shapiro <i>et al.</i> (2008) |
| σ_j | Fraction of effector cells activated from cross-reactive memory T cells | Variable (0–1) | No unit | |
| m | Factor of faster response from memory T cells | 5 | No unit | Kedl & Mescher (1998) |
| ϕ_2 | Rate of T-cell activation against latent infection | 1.95×10^{-5} | min^{-1} | Shapiro <i>et al.</i> (2008) [¶] |
| ϕ_4 | Rate of T-cell activation against lytic infection | 4.48×10^{-5} | min^{-1} | Shapiro <i>et al.</i> (2008) [¶] |
| θ_2 | Rate of T-cell proliferation against latent infection | 3.25×10^{-5} | min^{-1} | Shapiro <i>et al.</i> (2008) |
| θ_4 | Rate of T-cell proliferation against lytic infection | 3.25×10^{-5} | min^{-1} | Shapiro <i>et al.</i> (2008) |
| K | Number of infected cells when T-cell activation is half maximal | 10^5 | Cell | Jones & Perelson (2005) |
| δ | Death rate of T cells | 1/156000 | min^{-1} | Shapiro <i>et al.</i> (2008) |

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

[‡]Estimated taken to be less than μ_{Eb} (Turk *et al.*, 2006).

[§]Estimated taken to be less than d_4 (Borza & Hutt-Fletcher, 2002).

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

^{||}Probability of lymphocyte encounter per minute multiplied by the frequency of cell division (every 8–12 h).

magnitude than the T-cell responses against viral lytic proteins during the acute phase of IM. The acute phase is followed by convalescence and eventually a virus carrier state where the CD8+ population resolves to a level comparable to that in asymptomatic carriers (Hislop *et al.*, 2007).

We use numerical solutions of our model to investigate the three hypotheses for the high prevalence of IM in teenagers and young adults: saliva and antibody effects, cross-reactive T-cell responses and the initial viral load. The total number of T cells (both specific and cross-reactive ones) and the lytic T-cell ratio at the peak of infection are used as the two key measurements of IM. The lytic T-cell ratio is the ratio between effector T cells responding against lytic infection and effector T cells responding against latent infection, $(T_4 + T_{4C})/(T_2 + T_{2C})$. A wide range of values of these two measurements has been observed in IM patients. Individual epitope responses against latent and lytic infections can account for 0.1–5% and 1–40% of the total CD8+ T-cell population, respectively (Hislop *et al.*, 2007).

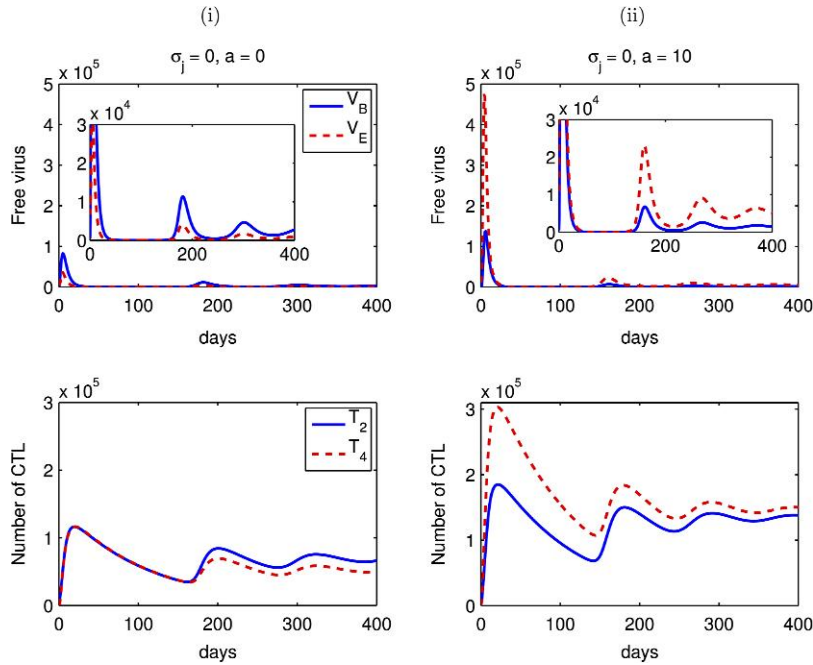


FIG. 2. Dynamics of viruses and T cells in the case of no cross-reactive T-cell responses ($\sigma_j = 0$). (i) Without antibody effect ($a = 0$). (ii) With antibody effect ($a = 10$). The insets show the level of persistent virus for the two cases. Parameter values used are shown in Tables 1 and 2.

3.1 Antibody effects

Race, sex and age are at least in part responsible for individual differences in antibody responses (Buckley & Dorsey, 1971; Childers *et al.*, 2003; Jafarzadeh *et al.*, 2008), which may influence the outcomes of EBV infection. Titers of antibody responses specific to EBV viral capsid antigen, IgA and IgG, have been observed to increase with age and IgA attains its highest level during the onset of disease within IM patients (Edwards & Woodroof, 1979; Oberender *et al.*, 1986). Furthermore, individuals are exposed to more pathogens as they age. EBV infection in young adults may activate antibody responses that are specific to other viruses but cross-reactive to EBV. As IgG and IgA responses to EBV glycoproteins can enhance the lytic infection of epithelial cells, the probability of getting IM may increase with age.

To examine this hypothesis with our model, we vary the strength of the antibody effect (a) and study its influence on the total number of T cells and the lytic T-cell ratio (Fig. 3) measured at the peak of infection. The total number of T cells increases with the level of a but then decreases when a is large. At high levels of antibody response, infection of B cells is strongly suppressed while the effect on enhancement of lytic infection of epithelial cells saturates, leading to a decreased total number of T cells (Fig. 3(i)) and increased lytic T-cell ratio (Fig. 3(ii)).

3.2 Cross-reactive T-cell responses

Massive expansion of CD8⁺ T cells responding to EBV causes the symptoms of IM (Silins *et al.*, 2001). It has been proposed that the high susceptibility of teenagers and young adults to IM may be

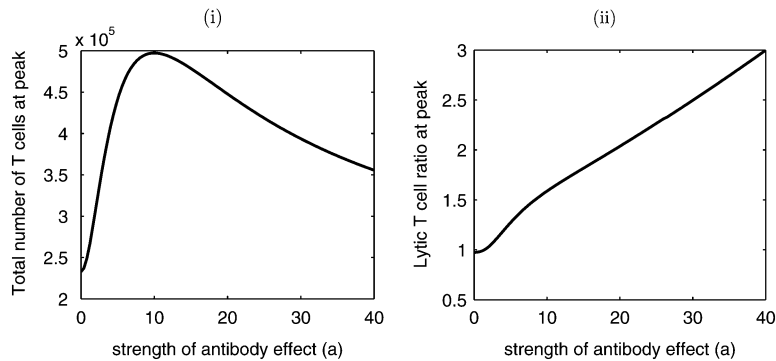


FIG. 3. Antibody effects on the total number of T cells ($T_2 + T_{2c} + T_4 + T_{4c}$) and the lytic T-cell ratio ($(T_4 + T_{4c})/(T_2 + T_{2c})$) in the absence of cross-reactive T cells ($\sigma_j = 0$). (i) Total number of $CD8^+$ T cells at the peak of infection. (ii) The lytic T-cell ratio at peak: ratio between the number of T cells against lytic infection (T_4) and the number of T cells against latent infection (T_2), evaluated at the peak of infection. Parameter values are shown in Tables 1 and 2.

due to a more complex memory CD8 repertoire than in young children. As individuals age, the memory CD8 repertoire gets more complex due to exposure to different pathogens. Adolescents infected with EBV may recruit a large number of cross-reactive memory T cells previously created in response to other viral infections (Rickinson & Kieff, 1996). In fact, it has been shown that memory $CD8^+$ T cells specific to influenza virus can be activated and respond to stimulation by EBV lytic proteins (Clute *et al.*, 2005). Both the magnitude and the efficiency of cross-reactive T cells in killing infected cells may contribute to the etiology of IM. The level of cross-reactive memory T cell can increase with age. These memory cells may be faster at activation and proliferation compared to naive T cells (Veiga-Fernandes *et al.*, 2000) but less efficient in controlling the infection (Thorley-Lawson, 2005).

A large fraction of $CD8^+$ T cells created during the course of IM respond to lytic infection (5–50% compared to 1–3% for T cells responding to latent infection) (Callan *et al.*, 1998; Hislop *et al.*, 2002). Since EBV has many more lytic genes than latent genes (Robertson, 2005), it is likely that there are more cross-reactive T cells to EBV lytic infection than to latent infection. We first assume cross-reaction of only T-cell responses against lytic infection. To address this assumption with our model, we set $\sigma_2 = 0$ and consider five different values of σ_4 , 0, 0.3, 0.6, 0.8 and 1. As σ_4 increases, the fraction of lytic T-cell response coming from cross-reactive memory T cells increases. At $\sigma_4 = 1$, there is no specific lytic T-cell response; all lytic T cells are cross-reactive.

To facilitate comparison with the antibody effect (Fig. 3), we present the effects of cross-reactive T cells on the development of IM using similar plots, with five curves in each representing different values of the level of cross-reactive lytic T cells (σ_4) (Fig. 4). This figure also illustrates the impact of χ_4 , the efficiency of cross-reactive T cells in killing lytically infected cells, on the two measurements of IM. Across all levels of antibody effects (a), the increase in σ_4 greatly elevates the total number of T cells and the lytic T-cell ratio. This effect, however, diminishes as χ_4 increases. At $\chi_4 = 1$, cross-reactive lytic T cells are as efficient as specific T cells in killing infected cells. In fact, due to their faster response, cross-reactive T cells reduce the overall T-cell responses and the probability of IM.

We now add the possibility of cross-reactive T-cell responses against latent infection. Figure 5 shows the effects of this addition on the two measurements of IM. For each level of σ_4 , we set $\sigma_2 = 0.2\sigma_4$ to assume lower levels of cross-reactive T cells against latent infection compared to lytic infection. We analysed and observed only minimal impacts of variation in the efficiency of cross-reactive T cells in killing latently infected cells (χ_2) on the results. We thus fix $\chi_2 = 0.5$ for this analysis. In comparison

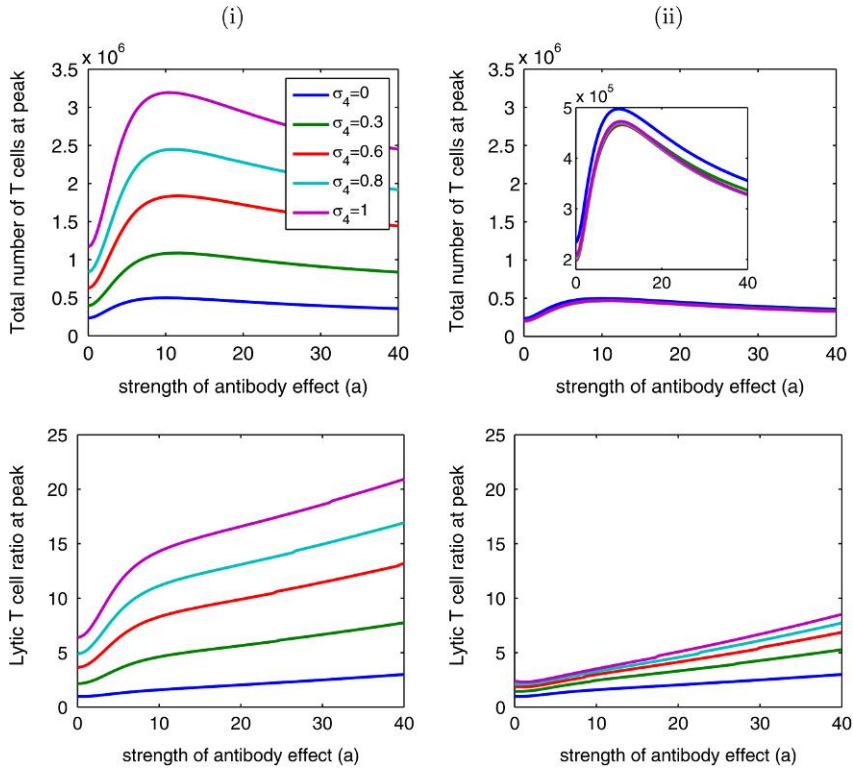


FIG. 4. The effects of cross-reactive T-cell responses to viral lytic proteins ($\sigma_4 > 0$) on the total number of T cells and the lytic T-cell ratio during primary infection as a function of the strength of the antibody effect (a). The five different degrees of cross-reactive responses are shown in each plot. (i) Left column: low efficiency of cross-reactive lytic T cells in killing infected cells ($\chi_4 = 0.1$). (ii) Right column: cross-reactive T cells are as efficient as specific T cells in killing infected cells ($\chi_4 = 1$). Other parameter values are shown in Tables 1 and 2.

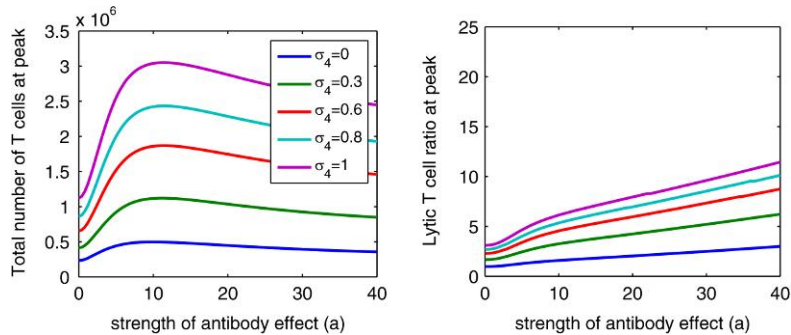


FIG. 5. The effects of both latent and lytic cross reactive T cells ($\sigma_j > 0$) on the total number of T cells and the lytic T-cell ratio during primary infection as a function of the strength of the antibody effect (a). The five different degrees of cross-reactive responses are shown in the plots. For each level of σ_4 , $\sigma_2 = 0.2\sigma_4$. We set $\chi_2 = 0.5$ and $\chi_4 = 0.1$. Other parameter values are shown in Tables 1 and 2.

to the results presented in Fig. 4(i), addition of cross-reactive T cells to latent infection does not induce visible effect on the total number of T cells while the lytic T-cell ratios are significantly reduced. This implies that cross-reactive T-cell responses to latent infection do not induce the high lytic ratio observed in IM patients.

3.3 High initial viral load

A third hypothesis suggests that transmission often occurs through kissing in adolescents which may transmit a large number of viruses and hence lead to aggressive CD8+ T-cell responses. To analyse this hypothesis, we numerically solve (2.1) with five different levels of the initial viral load, V_0 . In comparison to antibody and cross-reactive T-cell effects, the initial viral load has very little effect on either the total number of T cells or the lytic T-cell ratio (Fig. 6).

3.4 Combined effects of antibody and cross-reactive T-cell responses

So far, our model supports the roles of antibody effects and the cross-reactive T cells in the development of IM. To summarize our analysis of the two hypotheses, we define two new ratios. The relative lytic T-cell ratio gives the lytic T-cell ratio for given value of σ_j and a compared with a baseline at $\sigma_j = 0$ and $a = 0$,

$$\left(\frac{T_4 + T_{4C}}{T_2 + T_{2C}} \right) / \left(\frac{T_4 + T_{4C}}{T_2 + T_{2C}} \right)_{\sigma_j=0, a=0}$$

The relative total T-cell number gives the ratio between the total number of T cells given values of σ_j and a and the one with a baseline $\sigma_j = 0$ and $a = 0$,

$$\frac{(T_2 + T_4 + T_{2C} + T_{4C})}{(T_2 + T_4 + T_{2C} + T_{4C})_{\sigma_j=0, a=0}}$$

We examine five different levels of cross-reactive T cells to lytic infection (σ_4), four different levels of cross-reactive T cell against latent infection (σ_2), five different levels of the efficiency of lytic T cells in killing infected cells (χ_4) and fix $\chi_2 = 0.5$ (Fig. 7).

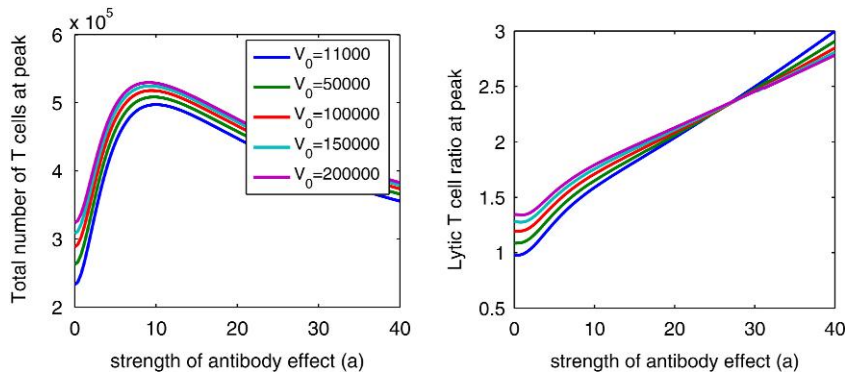


FIG. 6. The effect of initial viral load (V_0) on the total number of T cells and the lytic T-cell ratio during primary infection as a function of the strength of the antibody effect (a). We set $\sigma_j = 0$ which represents no cross-reactive T-cell response. Other parameter values are shown in Tables 1 and 2.

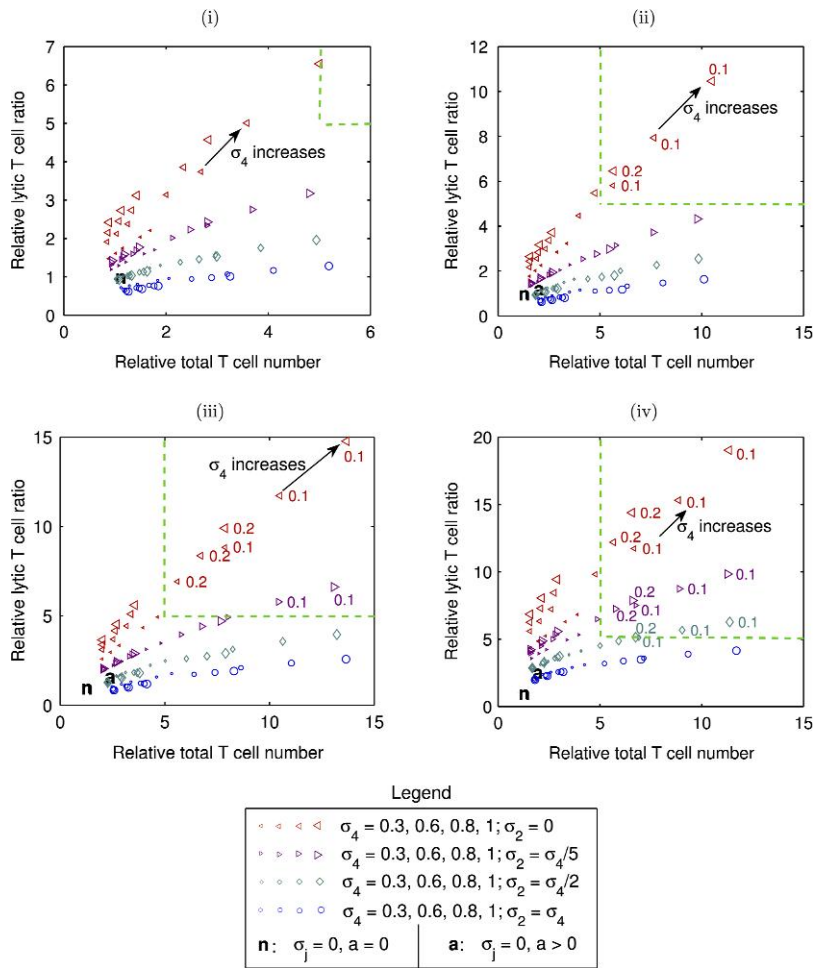


FIG. 7. Combined effects of antibodies with cross-reactive T cells on the relative T cell number and the relative lytic T cell ratio. (i) $a = 0$. (ii) $a = 4$. (iii) $a = 10$. (iv) $a = 30$. The green lines show the area of possible IM cases with high levels of the relative total T cell number and the relative lytic T cell ratio (≥ 5). Four different colors (symbols) represents different levels of cross-reactive latent T cells (σ_2). Symbol size represents different levels of cross-reactive lytic T cells ($\sigma_4 = 0.3, 0.6, 0.8, 1$). The label numbers next to the symbol represent the efficiency of lytic T cells in killing infected cells (χ_4). We examine five different levels of χ_4 (0.1, 0.2, 0.5, 0.7, 1) and only label the points of possible IM cases. The characters **n** and **a** represent the normal condition ($\sigma_j = 0, a = 0$) and the conditions with only antibody effects ($\sigma_j = 0, a > 0$), respectively.

IM is assumed to be possible when both ratios, the relative total T-cell number and the relative lytic T-cell ratio are large (≥ 5). Studies give a wide range for these ratios (Callan *et al.*, 1998; Hislop *et al.*, 2002; Silins *et al.*, 2001), so these threshold levels of ≥ 5 are not to be conclusive. In the absence of antibody effects ($a = 0$), IM can only be explained with very high levels of cross-reactive lytic T cells together with a low efficiency of these cells in killing infected cells. In the absence of cross-reactive T cells ($\sigma_j = 0, a > 0$), antibody effects induce increases in the total number of T cells and the lytic T-cell ratio. However, these increases are not as significant as those induced by the combined effects of antibodies with cross-reactive T cells. Thus, IM is characterized by high level of antibody

effects, high level of cross-reactive T cells to lytic infection and low efficiency of cross-reactive T cell in killing infected cells. As individuals age, the levels of antibody effects and the cross-reactive T cells increase; hence, the probability of IM increases if the cross-reactive T cells do not efficiently kill infected cells.

4. Discussion

IM is characterized by a large T-cell response, primarily to the lytic phase of the infection and thus can result from two broad changes in the course of acute infection. First, the virus could be biased towards creating a large fraction of lytically infected cells. EBV alternates between infecting B cells (its primary target), with either latent or lytic infection, and epithelial cells (important in viral persistence and shedding), as lytic infection only. Any factor that biases infection towards epithelial cells can increase the importance of lytic infection and potentially increase the probability of IM. Switching between B cell and epithelial cell virus is modulated by antibody responses and unknown constituents in the saliva (Turk *et al.*, 2006). Hosts with increased IgA antibodies may be prone to large expansions of T cell against viral lytic proteins. Second, a host could have a less efficient T-cell response against the virus. EBV infection can activate cross-reactive memory T cells that are specific to other pathogens (Clute *et al.*, 2005). If these cells are activated in large number but recognize and kill target cells inefficiently, IM may result. The high initial viral load hypothesis cannot produce large expansions of T cells and thus cannot be used to explain the age dependence of IM.

In economically developed countries, IM has highest incidence in the 15- to 25-year-old age group. In developing countries like Brazil, the age distribution of IM is shifted downwards with mean age of IM around 13 years (Niederman & Evans, 1997). If people in developing countries are exposed to more diseases at an earlier age, they could have both higher antibody level and a larger pre-existing memory CD8 repertoire compared to age matched counterparts from developed countries. Together, these effects may explain the difference in age distribution of IM.

We built the component of antibody effects in our model based on an *in vitro* study of the host saliva and antibody effects on the infections of B cells and epithelial cells with limited data from saliva samples of infected and uninfected individuals (Turk *et al.*, 2006). The goal of our study was not to predict the exact level of antibodies that induces large expansion of T cells and symptoms of IM but to identify the potential risks in their effects. Our model highlights a need for further studies on the constituents of the saliva influencing infection of the two cell types, and studies to compare the levels of antibodies, especially IgA, to EBV viral capsid antigens and glycoproteins during the acute phase of infection between asymptomatic and symptomatic patients. These studies would help to identify the existence of thresholds of antibody levels or other factors in the host saliva that direct the course of infection.

We have used our model to show that both the magnitude and the quality of T cells in killing the infected cells are critical determinants of the outcomes of the infection. Indeed, our result suggests that large expansion of CD8⁺ T cells occur only when they are inefficient at killing. A study on mice has shown that infection with lymphocytic choriomeningitis virus, Pichinde virus or vaccinia virus can activate cross-reactive T cells that are specific to one of these viruses (Selin *et al.*, 1998). These cross-reactive responses are fast, functionally efficient, and hence help to clear the secondary virus infection. Study of T-cell responses to dengue virus has shown that different cross-reactive T-cell clones can have very different efficiencies in recognizing and killing the infected cells (Imrie *et al.*, 2007). *In vitro* study has shown that EBV antigen can activate cross-reactive T cells that are specific to influenza-A

virus, but the killing efficiency of these cells has not yet been determined (Clute *et al.*, 2005). As the pre-existing memory CD8 repertoire evolves with age, we do not know how the functional efficiency of these memory cells changes. Further studies to compare the recognizing and killing efficiency of effector T cells during primary infection of EBV between different age groups and between healthy and IM patients are needed to address this question and to validate the results of our model.

Studies have also suggested that genetic factors can contribute to differences in efficiency of T-cell responses to EBV, which implies difference in susceptibility to IM between individuals (McAulay *et al.*, 2007). Individuals with certain human leukocyte antigen (HLA) class I alleles are linked to higher risk of IM. HLA class I plays a key role in the process of antigen presentation by infected cells to T cells (Farrell, 2007). Hence, a difference in HLA alleles can induce different rates at which T cells can be activated, proliferate, recognize and kill infected cells. Similar to the way we model the cross-reactive T-cell responses, we can utilize our model to address this hypothesis on the genetic predisposition to IM.

Even though IM is rarely lethal, it may induce long-term effects on the population of T cells (Sauce *et al.*, 2006; Hislop *et al.*, 2007). IM is strongly correlated with increased risk of EBV-positive Hodgkin's lymphoma in the years after infection (Hjalgrim *et al.*, 2003). Understanding risk factors for IM may help to investigate the long-term effects of the disease and its association with more serious disease like cancers.

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