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Sharing the burden: antigen transport and firebreaks in immune responses

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Communication between cells is crucial for immune responses. An important means of communication during viral infections is the presentation of viral antigen on the surface of an infected cell. Recently, it has been shown that antigen can be shared between infected and uninfected cells through gap junctions, connexin-based channels, that allow the transport of small molecules. The uninfected cell receiving antigen can present it on its surface. Cells presenting viral antigen are detected and killed by cytotoxic T lymphocytes. The killing of uninfected cells can lead to increased immunopathology. However, the immune response might also profit from killing those uninfected bystander cells. One benefit might be the removal of future 'virus factories'. Another benefit might be through the creation of 'firebreaks', areas void of target cells, which increase the diffusion time of free virions, making their clearance more likely. Here, we use theoretical models and simulations to explore how the mechanism of gap junction-mediated antigen transport (GMAT) affects the dynamics of the virus and immune response. We show that under the assumption of a well-mixed system, GMAT leads to increased immunopathology, which always outweighs the benefit of reduced virus production due to the removal of future virus factories. By contrast, a spatially explicit model leads to quite different results. Here we find that the firebreak mechanism reduces both viral load and immunopathology. Our study thus shows the potential benefits of GMAT and illustrates how spatial effects may be crucial for the quantitative understanding of infection dynamics and immune responses.

Keywords: viral infection; immunology; T cells; spatial model; gap junctions

1. INTRODUCTION

To successfully fight off pathogens, the host immune response needs to rapidly sense the presence of an intruder. This is possible only with communication between cells. During viral infections, one important message that needs to be communicated is the presence of the virus inside a cell. One way cells can signal the presence of virus is through the display of viral antigen on MHC I. Recently, another mode of communication has been demonstrated, namely the transport of antigen from one cell to another through gap junctions (Neijssen *et al.* 2005).

Gap junctions are small channels that can form between two cells to allow the transfer of small molecules (Harris 2001; Evans *et al.* 2006). The building blocks used to form gap junctions are connexins, which are expressed by many different cell types (Saez *et al.* 2003). While a number of studies have provided evidence that gap junctions play an important part during immune responses (Hu & Cotgreave 1997; Krenacs & Rosendaal 1998; Alves *et al.* 2000; Saez *et al.* 2000; Oviedo-Orta *et al.* 2001; Eugenin *et al.* 2003; Oviedo-Orta & Evans 2004; Matsue *et al.* 2006; Zhao *et al.* 2006), a recent study by Neijssen *et al.* (2005) is the first to suggest a possible mechanism. In this study, it was shown that *in vitro*, antigen peptides can be transported through gap junctions and presented on MHC I of the receiving cell, with subsequent recognition of the peptide–MHC complex by cytotoxic T lymphocytes (CTL). It has been suggested that such gap junction-mediated antigen transport (GMAT) might be useful during *in vivo* viral infections (Griffiths 2005; Heath & Carbone 2005; Li & Herlyn 2005; Neijssen *et al.* 2005).

One way through which GMAT could be beneficial is through the transport of antigen from infected cells to professional antigen-presenting cells, such as dendritic cells (DC). Since DC need to present viral antigen to activate T cells, but are not always infected by the virus, they need to acquire antigen by different mechanisms, a process termed cross-presentation (Cresswell *et al.* 2005; Trombetta & Mellman 2005). Several pathways for cross-presentation are known (Heath *et al.* 2004; Yewdell & Haeryfar 2005); GMAT might be another such pathway (Neijssen *et al.* 2005; Handel *et al.* 2007*a*).

Another way in which GMAT might help the immune response is by sharing antigen between infected and uninfected target cells. This can lead to

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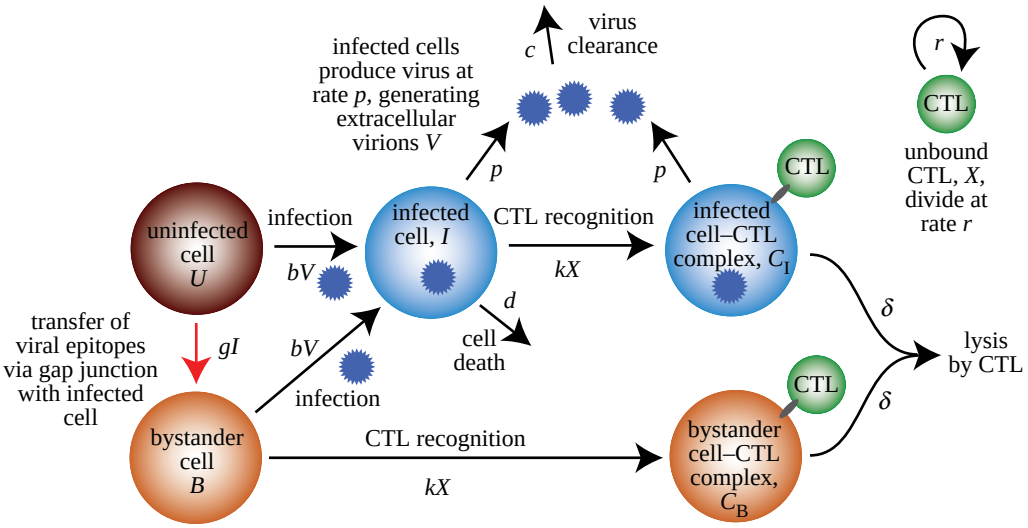

Figure 1. Schematic of GMAT between target cells. Uninfected cells become infected. The infected cells produce free virus, and they also transfer viral antigen via gap junctions to neighbouring uninfected cells, turning these into antigen-presenting, uninfected bystander cells. CTL can recognize antigen on both infected and bystander cells, form complexes with those cells and, after some time, kill the complexed cells. The specific meaning and values of the rates indicated by the symbols are described in §2.

cells that display antigen on MHC I without being infected. These uninfected but antigen-presenting bystander cells can be recognized and killed by CTL (figure 1).

The killing of target cells before they have become infected could lead to a reduction in viral load, due to the removal of potential 'virus factories'. The reduced viral load may in turn reduce immunopathology. However, the bystander killing by CTL could outweigh the virus-induced cell death, thereby increasing immunopathology. Additionally, if one takes into account that CTL diverted to kill bystander cells are not available to kill infected cells, increased GMAT might be even less beneficial (Handel *et al.* 2007*a*).

The killing of bystander cells adjacent to infected cells has also been suggested to be beneficial through the creation of something similar to a firebreak (FB), an area void of target cells, that requires virions to diffuse further to encounter potential targets and so making their clearance more likely (Griffiths 2005; Heath & Carbone 2005; Li & Herlyn 2005; Neijssen *et al.* 2005). We suggested previously that the effect of such a FB mechanism would be most marked for densely packed target cells and slowly diffusing virions (Handel *et al.* 2007*a*). Here, we use two models, one based on ordinary differential equations (ODE) and the other based on a spatially explicit, agent-based model (ABM), to further explore the potential impact of GMAT during immune responses. We find that under the assumption of a well-mixed system with no FB mechanism in place, the bystander killing by CTL always outweighs the virus-induced cell death, thereby increasing immunopathology. Additionally, CTL that kill bystander cells are not available to kill infected cells, which can make the effect of GMAT worse. However, once spatial effects are taken into account, the results change. Our spatially explicit model demonstrates that the proposed FB mechanism can indeed lead to a reduction in both viral load and immunopathology, outweighing the negative effects of increased CTL killing of bystander cells.

2. MODELS

We use two different models to study the impact of GMAT between infected and uninfected target cells. One model is based on ODE, and is implemented in MATLAB R2007a (The Mathworks). The other model is a spatially explicit ABM, and is implemented in the freely available NETLOGO platform, v. 4 (NETLOGO 2008, http://ccl.northwestern.edu/netlogo/). All programs and scripts are available from the authors. The models are described in §2.1 and 2.2.

2.1. The ODE model

For the first model, we use a set of coupled ODEs to describe the dynamics of the infection process as schematically shown in figure 1. Uninfected cells, U, become infected by virus, V, according to a mass–action interaction at rate b. Infected cells produce virus at rate p, die at rate d and transport antigen to uninfected cells at rate g, turning these cells into bystander cells. Bystander cells can also become infected. Both infected and bystander cells present viral epitopes on MHC I and can therefore be recognized by the virus-specific CTL. CTL form complexes, C_I and C_B, with infected and bystander cells, respectively, at rate k and eventually kill these cells at rate δ. We assume that CTL always kill upon complex formation. Inclusion of a term that describes complex dissociation without killing was found not to alter the results. While in a complex with CTL but not yet killed, infected cells continue to produce virus. As long as CTL are bound in complexes, they are not able to engage new cells, thereby reducing the number of free CTL. The total number of CTL increases exponentially (clonal expansion) at rate r. We only model the CTL expansion phase, since the peak and subsequent decline of the CTL response usually occur several days after virus has been cleared. Free virus is cleared at a constant rate c. Since the rate of clearance is usually much faster when compared with the rate of virus absorption by target cells,

Table 1. Model parameters. (Values are chosen in line with the reports for influenza infections and immunological studies of CTL kinetics.)

symbol	meaning	value	reference
d	death rate of infected cells	$1\ d^{-1}$	Price *et al.* (1997) and Brydon *et al.* (2003)
p	virus production rate	$200\ d^{-1}$	Cairns *et al.* (1952) and Stray & Air (2001)
c	free virus clearance rate	$2–10\ d^{-1}$	Baccam *et al.* (2006) and Handel *et al.* (2007*b*)
b	infection parameter	$10^{-5}\ d^{-1}$	Handel *et al.* (2007*b*)
r	CTL growth rate	$1\ d^{-1}$	Belz *et al.* (2001), Lee *et al.* (2005) and Legge & Braciale (2005)
k	complex formation term	$0.3\ d^{-1}$	Regoes *et al.* (2007) and Yates *et al.* (2007)
δ	killing rate	$48\ d^{-1}$	Mempel *et al.* (2006)
U_0	initial number of uninfected cells	10^4	our choice
I_0	initial number of infected cells	1	our choice
V_0	initial number of virions	0	our choice
T_0	initial number of activated CTL	1	our choice
g	GMAT parameter	varied	under investigation

we ignore the absorption term, as is customary for these models (Perelson & Nelson 1999; Perelson 2002). The equations for this model are given by

$$\frac{dU}{dt} = -bUV - gUI \qquad \text{uninfected cells},$$

$$\frac{dI}{dt} = bUV + bBV - dI - kXI \qquad \text{infected cells},$$

$$\frac{dB}{dt} = gUI - bBV - kXB \qquad \text{bystander cells},$$

$$\frac{dC_I}{dt} = kXI - \delta C_I \qquad \text{CTL–infected cell complex},$$

$$\frac{dC_B}{dt} = kXB - \delta C_B \qquad \text{CTL–bystander cell complex},$$

$$\frac{dV}{dt} = p(I + C_I) - cV \qquad \text{free virus},$$

$$\frac{dT}{dt} = rT \qquad \text{total CTL},$$

$$X = T - C_I - C_B \qquad \text{free CTL}.$$

In appendix A, we prove that this model describes an acute, transient infection, for instance an influenza infection. The parameters for the model are given in table 1. We use values for the virus dynamics that are in line with the reported ones from influenza infections. To facilitate comparison with the spatial model, we consider a local region containing 10^4 uninfected target cells.

2.2. The agent-based model

The ABM is spatially explicit. We study a localized infection of a patch of epithelial tissue containing 10 201 target cells on a grid of size 101×101. Initially, the target cell located at the central grid point starts out as infected and a CTL is randomly placed on the grid. The simulation runs in discrete time steps of 1 hour. In line with the values for the ODE model, an infected cell lives for 24 hours and produces eight virions per hour, for a total of approximately 200. Virions have a certain probability p_v of being cleared each hour. Since the ODE assumes a well-mixed system, the parameters describing mass–action rates for the ODE model have no direct equivalence in the spatially explicit model. For the ABM, we assume that both virions and CTL move randomly, with the virions diffusing at a slow speed, m_v, and the CTL at a higher speed, m_t, both given in units of grid points per hour. We further assume that a virion infects an uninfected or a bystander cell with a probability p_i upon landing on the same grid point. Equivalently, a CTL forms a complex with a bystander or an infected cell at probability p_c. Killing of infected or bystander cells by CTL after complex formation occurs with probability p_k. For $p_k = 1$, killing is instantaneous; lower values of p_k correspond to delayed killing. CTL increase in number according to a discrete version of the exponential growth used for the ODE model. Gap junctions between infected and uninfected cells are formed at every time step with probability G_p, the gap-junction parameter for the ABM model.

3. RESULTS

3.1. GMAT increases immunopathology in a well-mixed system

During viral infection, pathology is often caused by both virus-induced cell death and immune-mediated damage, such as killing of infected cells by CTL (Krakauer & Nowak 1999; Wodarz & Krakauer 2000; Ganusov & Antia 2005). The mechanism of GMAT could reduce viral load due to the removal of uninfected bystander cells by CTL before these cells become infected, reducing the number of potential future virus factories (infected, virus-producing cells). This in turn leads to reduced virus-induced cell death. However, increased GMAT also leads to increased killing of uninfected bystander cells and therefore could cause increased immunopathology. To achieve an overall reduction of dead cells, the reduction in viral load due to removal of virus factories must result in a decrease in virus-induced cell death that is large enough to offset the bystander killing caused by CTL. Otherwise, GMAT does more harm than good. We use the ODE model to study the impact of GMAT in a well-mixed system, which might for instance apply to the spleen. First, we assume that the killing of bystander or infected

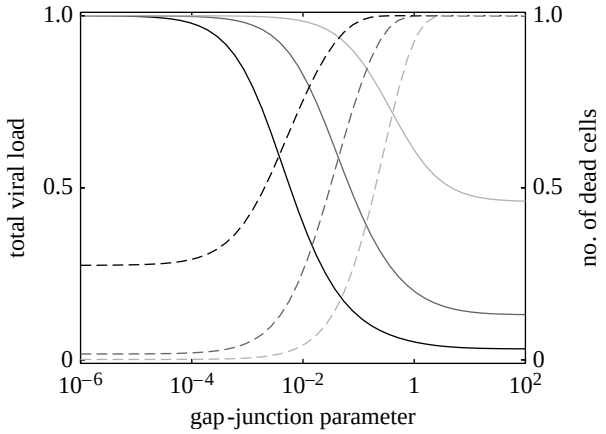

Figure 2. Viral load and dead cells for different values of the gap-junction parameter, immediate killing by CTL. Shown are total viral load, integrated over the whole infection (solid lines) and number of dead cells at the end of the infection (dashed lines) as a function of the gap-junction parameter. For better representation, each curve is scaled by its maximum value. The model used to obtain the results is the set of ODEs described in §2, with $\delta \to \infty$, corresponding to the immediate killing of target or bystander cells by CTL. The virion clearance rates corresponding to slow (black lines), medium (dark grey lines) and fast (light grey lines) virus clearances are $c = 2\,\mathrm{d}^{-1}$, $c = 4\,\mathrm{d}^{-1}$ and $c = 8\,\mathrm{d}^{-1}$, respectively.

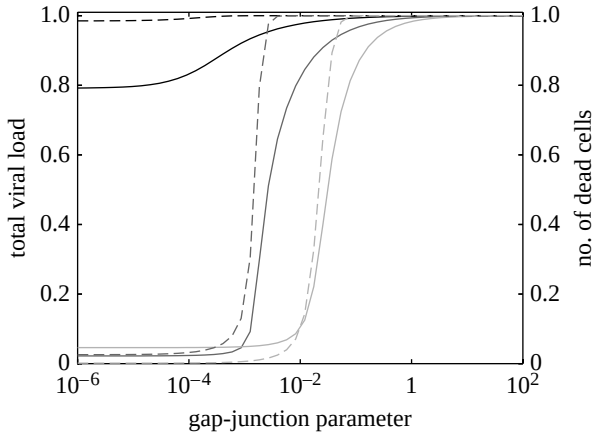

Figure 3. Viral load and dead cells for different values of the gap-junction parameter, delayed killing by CTL. Same scenario as shown in figure 2, but with $\delta = 48\,\mathrm{d}^{-1}$, corresponding to approximately 30 min between complex formation and killing by CTL.

cells by CTL is instantaneous (i.e. $\delta \to \infty$). Using the ODE model described in §2, we show in figure 2 that increased GMAT can reduce the total viral load (the integrated viral load over the course of an infection). However, the killing of bystander cells increases immunopathology (the number of dead target cells).

Next, we consider how taking into account the time it takes CTL to bind to and kill infected or bystander cells influences the results (Pilyugin & Antia 2000; Mempel *et al.* 2006). We set $\delta = 48\,\mathrm{d}^{-1}$, which corresponds to an average of 30 min to kill, in agreement with a recent experimental study (Mempel *et al.* 2006). For this scenario, an increase in bystander cells leads to more CTL being diverted from the task of removing infected cells. Figure 3 shows that this leads to an increase not only in immunopathology with increasing GMAT, but also in virus production.

3.2. GMAT is beneficial in a spatially structured system

Section 3.1 showed that GMAT is not beneficial in a well-mixed situation. However, it has been proposed that GMAT and removal of bystander cells could work like a FB: by killing target cells that surround an infected cell, virions exiting this infected cell will have to diffuse further before they encounter the next uninfected target cell. This leads to an increase in time spent outside a cell and makes the virions more susceptible to being cleared by the immune system (Griffiths 2005; Heath & Carbone 2005; Li & Herlyn 2005). We previously suggested that such a FB mechanism might be most relevant in situations where target cells are tightly packed and stationary. If the distances between target cells are small when compared with their size, removal of uninfected cells around an infected cell can significantly increase the distance a virion has to diffuse before it finds the next cell it can infect. Since the diffusion time scales with the square of the distance, the increase in diffusion time can be large (Handel *et al.* 2007*a*).

The FB idea is a fundamentally spatial mechanism; therefore, a spatial model is required to properly study it. We implemented such a model using NetLogo (2008, http://ccl.northwestern.edu/netlogo/), as described in §2. Using this model, we simulated infections for different levels of GMAT. Figure 4 shows a snapshot for a typical simulation. One can see that for a low GMAT rate, there is no empty space between infected (red) and uninfected (green) cells. By contrast, for a high GMAT rate, CTL (white) kill bystander cells (yellow) surrounding an infected cell. This leads to 'empty' space (i.e. an area filled with dead cells, void of uninfected target cells) between virions (blue) and target cells (see figure 4, orange ellipse). Virions have to diffuse further before they reach a target cell, making it more likely that those virions are cleared.

First, we investigated the impact of GMAT for different parameter combinations. For each simulation, we recorded the total number of dead cells and the total viral load, in analogy to the quantities plotted above for the ODE model. Figure 5 shows the average of those quantities as a function of the GMAT rate, scaled by the maximum value as in the earlier figures. As the figure shows, the FB mechanism works: increased GMAT rates lead to a reduction in viral load and dead cells. This result is rather robust to changes in parameters. Conditions on the parameters are that the virus is able to spread and cause an infection, and further that the infection is controlled before it reaches the edges of the grid, otherwise spurious boundary effects would occur. A large number of parameter combinations satisfy these conditions. We tried many of those combinations and always find that the viral load and the number of dead cells are reduced with increasing GMAT.

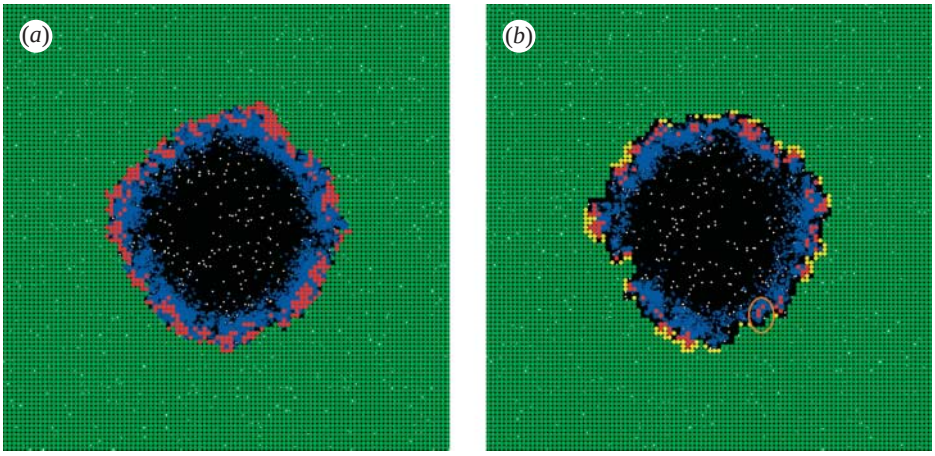

Figure 4. Snapshots of the infection simulation for (*a*) low and (*b*) high GMAT. Uninfected cells are green, infected cells red, bystander cells yellow, virus is blue and CTL are white. The black area represents tissue where epithelial cells have died. (New susceptible epithelial cells regenerate on a time scale that is slower than the time scale of acute viral infections such as influenza; this process is therefore neglected in the simulation.) In (*b*) FBs between the virions and uninfected cells are clearly visible (e.g. see the orange ellipse).

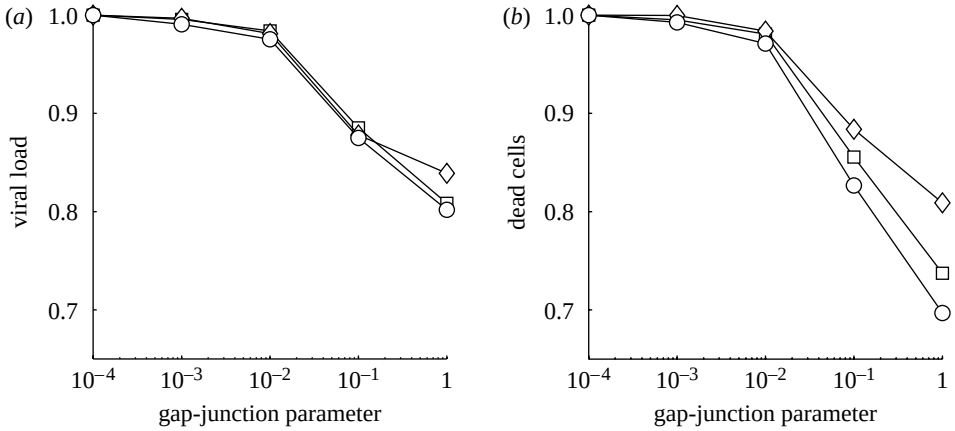

Figure 5. Normalized (*a*) total viral load and (*b*) number of dead cells obtained from the ABM for three different parameter combinations. Shown are averages of 200 simulations for different values of the gap-junction parameter. Parameters for scenario 1 (squares) are $p_v=0.28$, $p_k=1$, $p_i=1$, $p_c=0.5$, $m_v=0.25$ and $m_t=2$; parameters for scenario 2 (diamonds) are $p_v=0.34$, $p_k=1$, $p_i=0.3$, $p_c=1$, $m_v=0.5$ and $m_v=2$; and parameters for scenario 3 (circles) are $p_v=0.28$, $p_k=0.63$, $p_i=1$, $p_c=1$, $m_v=0.25$ and $m_t=2$.

Next, we investigated how virus diffusion speed, virus clearance rate and speed of bystander cell killing affect the impact of GMAT and the FB mechanism. To that end, we plot in figure 6 the normalized reduction in viral load and dead cells, divided by the number of FBs (bystander cells killed by CTL) for situations without GMAT and with high levels of GMAT. While significant variation between individual simulations exists, the trend as indicated by the mean values shows that increasing clearance rate or decreasing diffusion speed leads to a greater impact of GMAT. This is intuitively obvious since both fast clearance and slow diffusion increase the chances that during the time a virion traverses the empty space created by the killing of bystander cells, it is removed before it encounters the next target cell, thus increasing the impact of the FB. Figure 6 also shows that slower killing by CTL leads to a slightly higher impact of GMAT. This can be understood as follows: infected cells bound in complexes to CTL are able to produce virions until killing occurs. A delay in killing allows the complexed infected cells to produce more virions before CTL-induced death occurs. These virions diffuse to neighbouring uninfected cells and keep the infection alive. Therefore, the removal of neighbouring uninfected cells through GMAT becomes more important for the situation of delayed CTL killing.

4. DISCUSSION

The importance of gap junctions during immune responses seems to be clear (Oviedo-Orta & Evans 2004). Little is known about possible mechanisms although a number have been suggested (Griffiths 2005; Heath & Carbone 2005; Li & Herlyn 2005; Handel *et al.* 2007*a*). Here we used an *in silico* approach, based on mathematical models and simulations, to study the possible effects of one such recently discovered mechanism (Neijssen *et al.* 2005), GMAT.

Our results suggest that in a situation where the infection dynamics can be described by a well-mixed model—which could for instance apply to organs such

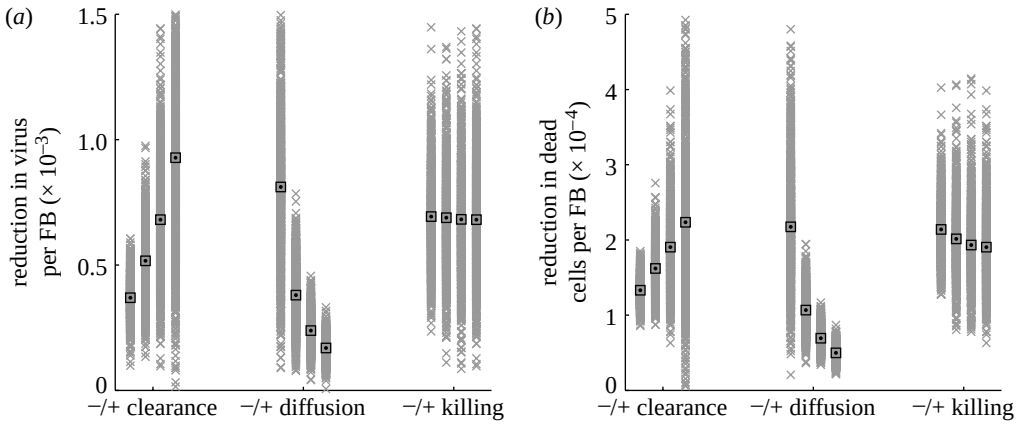

Figure 6. Reduction in (*a*) normalized viral load and (*b*) normalized number of dead cells per FB. A FB is defined as a bystander cell that gets killed by a CTL. The differences are between no GMAT and maximum GMAT. The notation (−/+) indicates an increase in that parameter. Clearance is increasing with values $p_v=0.15$, 0.22, 0.28 and 0.34, corresponding to $c=4$, 6, 8 and 10. Other parameters are $p_k=1$, $p_i=1$, $p_c=1$, $m_v=0.2$ and $m_t=2$. Values for the increasing diffusion rate are $m_v=0.25$, 0.3, 0.35 and 0.4. Other parameters for this scenario are $p_v=0.28$, $p_k=1$, $p_i=0.3$, $p_c=1$ and $m_t=2$. Values for complex killing are $p_k=0.39$, 0.63, 0.86 and 1, corresponding to $\delta=12$, 24, 48 and ∞. Other parameters are $p_v=0.28$, $p_i=1$, $p_c=1$, $m_v=0.2$ and $m_t=2$. The black squares show the mean and the grey crosses show individual results for 1000 simulations.

as the spleen—GMAT is not a good strategy for the immune response. However, in a situation where target cells are tightly packed and stationary—which applies for instance to epithelial cells (Sousa *et al.* 2005), a target of many viruses—GMAT might lead to a phenomenon similar to a FB, which hinders virus spread and can therefore constitute a useful immune response strategy. For such a situation, we showed that faster virion diffusion speed and higher virion clearance rate increase the impact of the FB mechanism. One additional interesting feature our model uncovered was the finding that delayed killing of complexes can lead to a slight increase in the GMAT impact. This can be attributed to the fact that without GMAT, infected cells that are tagged for killing by CTL still have enough time to produce a limited number of virions before they die. It is important to point out that while this is an interesting finding, it depends on the details of the model. For instance, we did not include an eclipse phase in our model, the delay time between infection of cell and release of new virions. Depending on the exact length of this phase compared with the delay in killing, this small effect could vanish.

While we use parameter estimates that are in line with the experimental data, some values, such as the size of the different cells and virions, were chosen rather arbitrarily. As such, our study should be interpreted qualitatively. Details will differ between our models and reality. Nevertheless, we found the same qualitative results for a wide range of different parameter choices, suggesting that GMAT and the FB mechanism might be a robust feature that could play a role in viral infections. We therefore conclude that, if the biological system resembles a well-mixed situtation, GMAT would be a poor strategy. If, on the other hand, the system has the spatial structure of (for instance) a layer of epithelial cells, GMAT could be a good strategy. Of course, suggesting that GMAT could be beneficial for the immune response does not establish its occurrence *in vivo*. This will need to be checked with experimental studies. The contribution of our study is to provide theoretical, *in silico* evidence that GMAT could be beneficial and that it might well be worth looking for this mechanism *in vivo*. Overall, we find that taking into account spatial structure can lead to important differences in results. This agrees well with other recent studies showing that details of infection dynamics depend on the assumption of spatial structure (Beauchemin *et al.* 2005; Funk *et al.* 2005; Beauchemin 2006; Howat *et al.* 2006) and illustrates that the choice of modelling framework needs to be targeted to the problem at hand.

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APPENDIX A. SOME MATHEMATICAL PROPERTIES OF THE SYSTEM

The main property of our ODE model is that for all initial conditions and parameter values (which are all non-negative, on biological grounds), the infection is always transient. In the long run, both the number of infected cells $I(t)$ and the viral load $V(t)$ vanish. Indeed, by adding appropriate equations, we find that

$$\dot{U}+\dot{I}+\dot{B}+\dot{C}_I+\dot{C}_B=-dI-\delta(C_I+C_B)\le 0,$$

which implies that the non-negative sum

$$U(t)+I(t)+B(t)+C_I(t)+C_B(t)$$

is non-increasing in time, and hence bounded at all times. Moreover, we find that the sum of the integrals on the right-hand side is bounded above,

$$d\int_0^t I(s)\mathrm{d}s+\delta\int_0^t(C_I(s)+C_B(s))\mathrm{d}s \le U(0)+I(0)+B(0)+C_I(0)+C_B(0),$$

for all times. Even in the absence of the immune response ($\delta = 0$), we have that

$$\int_0^t I(s)\mathrm{d}s \leq \frac{1}{\delta}(U(0) + I(0) + B(0) + C_{\mathrm{I}}(0) + C_{\mathrm{B}}(0)).$$

Since $I(t) \geq 0$, the integral

$$\int_0^\infty I(s)\mathrm{d}s$$

converges, and in the limit we have $\lim_{t\to\infty} I(t) = I_\infty = 0$. In other words, in the long run, the number of infected cells always vanishes.

The viral load $V(t)$ has a similar behaviour. Indeed, we have that

$$V(t) - V_0 = p\int_0^t (I(s) + C_I(s))\mathrm{d}s - c\int_0^t V(s)\mathrm{d}s,$$

or equivalently,

$$c\int_0^t V(s)\mathrm{d}s = p\int_0^t (I(s) + C_{\mathrm{I}}(s))\mathrm{d}s + V_0 - V(t)$$
$$\leq V_0 + p\int_0^\infty (I(s) + C_{\mathrm{I}}(s))\mathrm{d}s.$$

Hence, the integral

$$\int_0^\infty V(s)\mathrm{d}s = \frac{1}{c}\left(V_0 + p\int_0^\infty (I(s) + C_{\mathrm{I}}(s))\mathrm{d}s\right)$$

also converges. This implies that $\lim_{t\to\infty} V(t) = V_\infty = 0$, i.e. in the long run, the viral load also vanishes. All infections are therefore transient, with or without the immune response. Additionally, we have shown that the total viral load is always finite.

The second observation is that the infection always dies out before it entirely exhausts the available pool of uninfected cells. Again, this holds with or without the immune response. To show this, we observe that

$$\dot{U} = -bUV - gUI \leq 0,$$

hence the uninfected pool is constantly declining (a rather trivial observation) and the limit $U_\infty = \lim_{t\to\infty} U(t)$ exists. Dividing out U and integrating, we find that

$$\ln U_\infty - \ln U_0 = -b\int_0^\infty V(s)\mathrm{d}s - g\int_0^\infty I(s)\mathrm{d}s.$$

Substituting from above, we find that

$$\ln U_\infty = \ln U_0 - \frac{bV_0}{c} - \left(\frac{b}{c} + g\right)\int_0^\infty I(s)\mathrm{d}s > -\infty.$$

Hence the residual pool of uninfected cells is always positive, i.e. $U_\infty > 0$.

This analysis shows that the model has the right properties needed to describe an acute, transient infection. Since analytical expressions for the total viral load and the total number of dead target cells are not available, we use numerical simulations of the system to determine those values.

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