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Cytotoxic Killing and Immune Evasion by Repair

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The interaction between the immune system and pathogens is a complex one, with pathogens constantly developing new ways of evading destruction by the immune system. The immune system's task is made even harder when the pathogen in question is an intra-cellular one (such as a virus or certain bacteria) and it is necessary to kill the infected host cell in order to eliminate the pathogen. This causes damage to the host, and such killing therefore needs to be carefully controlled, particularly in tissues with poor regenerative potential, or those involved in the immune response itself. Host cells therefore possess repair mechanisms which can counteract killing by immune cells. These in turn can be subverted by pathogens which up-regulate the resistance of infected cells to killing. In this paper, we explore the hypothesis that this repair process plays an important role in determining the efficacy of evasion and escape from immune control. We model a situation where cytotoxic T lymphocytes (CTL) and natural killer (NK) cells kill pathogen-infected and tumour cells by directed secretion of preformed granules containing perforin and granzymes. Resistance to such killing can be conferred by the expression of serine protease inhibitors (serpins). These are utilized by several virally infected and tumour cells, as well as playing a role in the protection of host bystander, immune and immune-privileged cells. We build a simple stochastic model of cytotoxic killing, where serpins can neutralize granzymes stoichiometrically by forming an irreversible complex, and the survival of the cell is determined by the balance between serpin depletion and replenishment, which in its simplest form is equivalent to the well known shot noise process. We use existing analytical results for this process, and additional simulations to analyse the effects of repair on cytotoxic killing. We then extend the model to the case of a replicating target cell population, which gives a branching process coupled to shot noise. We show how the process of

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repair can have a major impact on the dynamics of pathogen evasion and escape of tumour cells from immune surveillance.

KEY WORDS: Cytotoxic killing, granzyme, serpin, repair, immune evasion, shot noise, mean first passage time

1. INTRODUCTION

The primary function of the immune system is to detect and eliminate harmful pathogens. Conversely pathogens attempt to evade such destruction by a variety of means. The outcome of these competing mechanisms can be difficult to predict and there is therefore increasing interest in modelling the interaction of the immune system with pathogens within a host. Typically, such models have consisted of sets of coupled nonlinear ordinary differential equations (ODEs) that describe the time evolution of the numbers of various types of immune cells, pathogens, infected cells and so on.[23] The resulting models are closely related to those used in ecology to describe the population dynamics of interacting species, and are often analysed in very similar ways.

However, many aspects of the immune system are stochastic in nature. In the early stages of an immune response, the number of interacting cells is small, and stochastic fluctuations coupled with non-linear amplification in the subsequent expansion phase may render an ODE description inappropriate. Here we present a situation where killing of infected cells occurs in a stochastic manner due to the random interaction with immune effector cells. We present a simple model, which is equivalent to the well know phenomenon of shot noise in statistical physics, originally used to model the current through a vacuum tube due to the random arrival of electrons at the anode.[10] The key quantity of biological interest is the expected time to kill a cell, which is simply the Mean First Passage Time for a particular threshold. Expressions for this have been derived in the statistical physics literature[16,18,19] and we analyse their implication for the biological effects of the interaction of a killing mechanism with repair. We then incorporate pathogen proliferation which leads to an apparently novel type of stochastic system which as far as we are aware has not been previously studied. It has interesting statistical properties, and may therefore be worthy of further study.

Most of the research into how pathogens and tumours evade immune killing can be classified as evasion techniques, including dormancy, sequestration, failure of antigen display and antigenic variation.[24] However, in the past few years it has become increasingly evident that many host cells can acquire resistance to killing by the immune system by the over-expression of granzyme inhibitors, and that the same mechanism can be taken over by pathogens to help them evade the immune system. We build a simple stochastic model of cytotoxic killing, where serpins can neutralize granzymes stoichiometrically by forming an irreversible complex,

and the survival of the cell is determined by the balance between serpin depletion caused by repeated hits from cytotoxic cells of the immune system occurring at random times, and the replenishment of serpins by continuous synthesis. We show how the replenishment rate of serpins can have a major impact on the dynamics of pathogen evasion, escape of tumour cells from immune surveillance, and the regulation of the host immune response.

1.1. Cytotoxic Killing and Serpins

The killing of infected host cells by cytotoxic T lymphocytes (CTL) and natural killer (NK) cells, known as *cytolysis*, is the main immune mechanism for controlling intracellular pathogens and tumour cells.[2] It may also have an important role in immune regulation, for example in the killing of autologous immature dendritic cells by NK cells.[28] In the innate immune system, NK cells are important effectors of cytolysis, targeting cells which have down-regulated self Major Histocompatibility Complex (MHC) class I molecules ('missing self' hypothesis), or which express ligands indicating cellular stress like MICA and MICB ('altered self' hypothesis), as well as infected cells expressing ligands for the toll like receptors TLR3 and TLR9. In adaptive immunity, cytotoxic T lymphocytes are also effective at cytolysis, and recognise and lyse cells presenting either foreign or tumour peptides on MHC class I molecules.

CTLs and NK cells kill most pathogen-infected and tumour cells by directed secretion of preformed granules containing perforin and granzymes. Recently, it has been discovered that serine protease inhibitors (serpins) can specifically inhibit the effect of granzymes. For instance, the orthopoxvirus serpins SPI-1 and SPI-2 are granzyme inhibitors and have been shown to be effective in decreasing CTL lysis as measured by *in vitro* cytolysis assays.[17] Similarly, the adenovirus protein L4-100K potently inhibits granzyme B-mediated cell death.[1] Some human cells also express the endogenous serpin PI-9 (also known as SPI-6 in mice), which is an irreversible stochiometric inhibitor of granzyme B. PI-9 is expressed in immune cells (CTLs, NK cells, mature DCs),[13] bystander cells (endothelial cells, mesothelial cells, renal cells undergoing rejection),[6,22] immune-privileged tissues (brain, testis, placenta)[3] and several different murine tumours,[20] and human malignancies including choriocarcinoma,[7] leukaemia[8] and melanoma.[27]

2. A SHOT NOISE MODEL OF CYTOTOXIC KILLING

2.1. The Basic Model

We assume that target cells are visited and hit by cytotoxic immune cells according to a Poisson process of rate λ. Each hit results in the delivery of some quantity d of granzyme. In the simplest case we assume that d is fixed, but more generally it can be a random variable d_i for the ith hit, assumed to occur at time t_i.

Inside the target cell, the granzyme can be neutralized by forming an irreversible complex with a protective serpin, in the ratio 1:1. This complex is then degraded and plays no further part in the process. We make the simplifying assumption that the affinity of serpin for granzyme is so high that no damage occurs as long as there is any protective serpin left in the cell. If the protective serpin is fully depleted, we assume that any residual granzyme present is sufficient to catalyse proteolytic reactions leading to the death of the target cell.

We assume that the amount of serpin in a cell is regulated so that in steady state the level of serpin is θ. Thus, if the size d of a hit is greater than θ then the target cell is immediately killed. Otherwise, the target cell is killed only if it is sequentially hit over a period of time such that the accumulated granzyme results in total depletion of serpins. We assume that following a hit, the amount of serpin recovers exponentially to the equilibrium θ at a rate r. Thus, if the cell is in equilibrium and is hit at time t_i then immediately after the hit, the level of serpin is $s(t_i) = \theta - d_i$ and at any later time $t > t_i$ it is $s(t) = \theta - d_i e^{-r(t-t_i)}$. Thus if a cell is hit by a sequence of hits of size d_i at time t_i, then its level of serpin at time t is given by

$$s(t) = \theta - \sum_{i=1}^{X(t)} d_i e^{-r(t-t_i)} \tag{1}$$

where $X(t)$ is the total number of hits in time $(0, t]$. The cell dies if following a hit, the serpin is completely depleted, that is if $s(t) < 0$. It is convenient to write this as

$$\sum_{i=1}^{X(t)} d_i e^{-r(t-t_i)} > \theta \tag{2}$$

where t_i is the time at which the ith hit occurs, d_i is the quantity of granzyme delivered by this hit, and $X(t)$ is the total number of hits in time $(0, t]$. It is convenient to refer to the sum on the left hand side above as the *accumulated damage* at time t (Fig. 1). This follows a process exactly equivalent to shot noise.[10] This has been widely analysed for either $d_i = d$ constant, or with d_i exponentially distributed with mean d, so that the probability of a hit of size h is de^{-dh}. This latter case is the most amenable to analysis, and thus is the case for which most rigorous results have been derived. It is, however, perhaps not particularly biologically realistic. We therefore present results both for this case, and for the biologically more plausible constant and normal distributions of hit size. In the last of these we are limited to numerical simulations since as far as we are aware rigorous derivations do not exist in the literature.

Observe that we model hits as a stochastic process, whilst repair is assumed to proceed deterministically at a constant rate. This is biologically plausible, since hits result from the random interaction with effector cells, whilst the repair machinery,

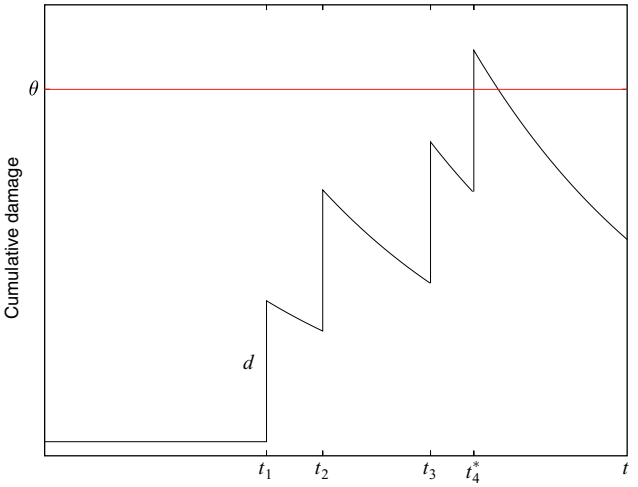

Fig. 1. Hits are received by the target cell at times t_1, t_2, t_3 and, t_4. Each hit causes an amount of damage d. However, this is attenuated with time due to cellular repair. At time t, the total damage done is found by summing $\sum_{i=1}^{3} de^{-r(t-t_i)}$. If at any time t this sum is greater than the cell threshold θ, the cell is killed. Death occurs at time t_4^* in the figure. Colour online.

once activated, can produce serpins at a continuous rate. There will of course be some random fluctuations in the production of such proteins, but this will be at a molecular scale, quite different to the cellular scale of the killing process.

Many aspects of this model have been analysed in detail in the case of the exponential and sometimes also the constant hit size distributions.[10,16,18,19] We begin by giving an elementary analysis of the evolution of the mean cumulative damage, which provides insight into the overall average behaviour of the model.

2.2. Expected Cumulative Damage

As described above, the cumulative damage at time t is equivalent to the amount of serpin depletion and is thus given by the left hand side of Eq. (2). The expected value $M(t)$ of the accumulated damage at time t is therefore given by

$$M(t) = \mathbb{E}\left[\sum_{i=1}^{X(t)} d_i e^{-rW_i}\right]$$

where $W_i = t - t_i$. By conditioning on $X(t) = n$ we obtain

$$M(t) = \sum_{n=1}^{\infty} \mathbb{E}\left[\sum_{i=1}^{X(t)} d_i e^{-rW_i} | X(t) = n\right] \mathbb{P}[X(t) = n] \tag{3}$$

The locations of events in time of a Poisson process conditioned on a fixed total number of events are given by a uniform distribution.[26] Therefore, if $U_1, \ldots, U_n$ are independent random variables uniformly distributed in $(0, t]$, we have

$$\mathbb{E}\left[\sum_{i=1}^{X(t)} d_i e^{-rW_i} | X(t) = n\right] = \mathbb{E}\left[\sum_{i=1}^{n} d_i e^{-rU_i}\right] = n\mathbb{E}[d_1 e^{-rU_1}]$$

Since d_1 is independent of U_1, we have $\mathbb{E}[d_1 e^{-rU_1}] = \mathbb{E}[d_1]\mathbb{E}[e^{-rU_1}]$ and hence

$$\begin{aligned}\mathbb{E}\left[\sum_{i=1}^{X(t)} d_i e^{-rW_i} | X(t) = n\right] &= n\mathbb{E}[d_1]\mathbb{E}[e^{-rU_1}] \\ &= \frac{nd}{t}\int_0^t e^{-ru} du = \frac{nd}{rt}(1 - e^{-rt}) \qquad (4)\end{aligned}$$

Here d is either the magnitude of a fixed size hit, or the mean of the distribution of the independent random hits sizes d_i. Substituting back into Eq. (3)

$$\begin{aligned}M(t) &= \frac{d}{rt}(1 - e^{-rt}) \sum_{n=1}^{\infty} n\mathbb{P}[X(t) = n] \\ &= \frac{d}{rt}(1 - e^{-rt})\mathbb{E}[X(t)] = \frac{d\lambda}{r}(1 - e^{-rt}) \qquad (5)\end{aligned}$$

By using the series expansion for e^{-rt}, the accumulated damage at time t in the absence of repair is given by $d\lambda$ as expected, since we have assumed that hits are distributed according to a Poisson process of rate λ and each hit delivers d units of damage. Therefore, in the absence of repair, the average damage grows linearly with time, at rate $d\lambda t$, which is as expected. It is easy to see that the time taken to reach the critical threshold θ for cell death is inversely proportional to $d\lambda$.

In the presence of repair on the other hand, the average damage saturates to the equilibrium value $d\lambda/r$ as $t \to \infty$. More specifically, the average damage satisfies the ordinary differential equation

$$\frac{dM}{dt} = d\lambda - rM(t) \qquad (6)$$

However, this deterministic approximation gives a poor description of cytotoxic killing, since the latter is controlled by the peak values of the damage, rather than by the average. It is thus important to consider the variation around the mean value. In the limit $t \to \infty$, it is possible to calculate the asymptotic variance of the accumulated damage. In the case of fixed hit size, this is $d\lambda/2r$,[10] whilst for exponentially distributed hits with mean d it is $d\lambda/r$.[16] We see that not surprisingly, the variance for a random hit size distribution is larger than that for a constant hits size distribution.

2.3. Asymptotic Damage Distribution

We next examine the actual probability distribution of the accumulated damage since this determines the proportion of target cells killed for a given parameter set. We initially do this in the asymptotic limit $t \to \infty$, and restrict ourselves to the analysis of exponentially distributed hits with $\theta = \infty$, in other words the distribution of damage if the target cells are never actually killed. The asymptotic distribution in this case is simply a gamma distribution with parameters (d, λ/r).[16] Note that, as expected, it is only the ratio of λ to r that is important, rather than the absolute values of these parameters, which merely set the scale on which time is measured. From now on, we shall therefore take $\lambda = 1$ without loss of generality.

The qualitative behaviour of the asymptotic distribution then depends purely on the repair rate r. If the repair rate r is higher than the hit rate λ, then the distribution is concentrated near 0 (Fig. 2A) with an exponential decay. In such a case, the repair mechanism dominates and killing will be rare unless the threshold θ is

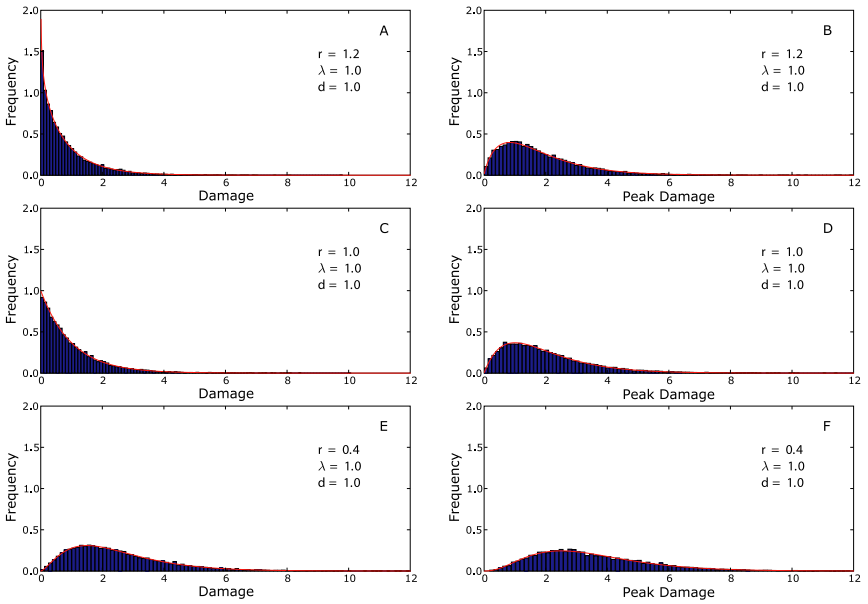

Fig. 2. Asymptotic damage (A, C, E) and peak damage distribution (B, D, F) for the case of exponentially distributed hit sizes. In all cases $\lambda = 1$ and $d = 1$; these parameters merely set the time and damage magnitude scales. Panels A, B $r = 1.2$; C, D $r = 1$ and E, F $r = 0.4$. For panels A, C, E the theoretical gamma distribution with parameters (d, λ/r) is shown in red and for panels B, D, F the distribution given by Eq. (7) is shown. In each case 10,000 encounters were sampled, after an initial transient of 1000 encounters which were ignored. Colour online.

low in relation to d. Conversely, if the repair rate r is lower than the hit rate λ, the peak of the distribution moves to the right, and killing becomes much more effective (Fig. 2E). At the critical case $r = \lambda$, the asymptotic distribution is precisely exponential (Fig. 2C; a well known special case for the gamma distribution).

Although the damage distribution is perhaps the most natural property to examine from the perspective of statistical physics, it is not the most relevant to the biological problem. This is because killing of the target cell is determined by the random variable representing instantaneous peak damage just after the time of each hit. By above, the damage immediately prior to the hit is a random variable with a gamma distribution with parameters $(d, \lambda/r)$. If we assume that the hit size is an exponentially distributed random variable with mean d then the damage immediately following the hit is simply the sum of the above two independent random variables. The mean of this sum is the sum $d\lambda/r + d$ of the two means and the full distribution is the convolution of a gamma $(d, \lambda/r)$ distribution and an exponential distribution with mean d. This evaluates to

$$P(u) = \int_0^u \frac{\left(\frac{v}{d}\right)^{\frac{\lambda}{r}-1} e^{-\frac{v}{d}}}{d\Gamma\left(\frac{\lambda}{r}\right)} \frac{e^{-\frac{u-v}{d}}}{d} dv = \frac{\left(\frac{v}{d}\right)^{\frac{\lambda}{r}} e^{-\frac{v}{d}}}{d\Gamma\left(\frac{\lambda}{r}+1\right)} \tag{7}$$

which we call the *asymptotic peak damage distribution*. This is plotted in Fig. 2B, D and F. As expected, the peak distribution is shifted to the right, and is broader than the underlying damage distribution. Numerical simulations suggest that the asymptotic distribution when the hit sizes are normally distributed is quite similar (Fig. 3). In Fig. 4, we show that the asymptotic distribution is reached rapidly for reasonable values of r, showing that Eq. (7) is a good approximation even for biologically relevant time scales.

We thus see that even when $r > \lambda$, a substantial number of cells will be subject to peaks of three to four times the average hit size d, and hence be susceptible to killing for thresholds θ well above d. However, the majority of cells will survive and hence this case does not represent effective elimination of a pathogen. Conversely, due to the large spread of the peak distribution, even at small repair rates, a significant proportion of cells will not exceed moderate θ for many hits. This suggests that it is important to analyse the time taken to kill a cell.

2.4. Expected Time to Killing

Observe that the probability of any cell eventually being killed is 1. However, this is of little biological relevance, since an infected cell needs to be killed relatively quickly before the pathogen is able to reproduce. In particular, if the killing time is long in comparison to the mean interval $1/\lambda$ between hits, then cytotoxic killing is ineffectual. We therefore wish to examine the effect of the various parameters on the average time taken to kill a cell. Note that the absolute

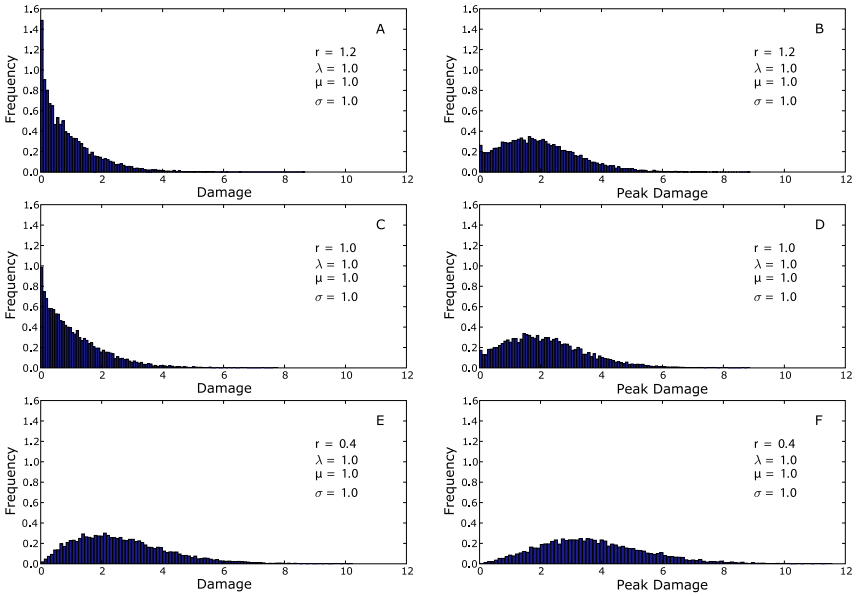

Fig. 3. Asymptotic damage (A, C, E) and peak damage distribution (B, D, F) for the case of normally distributed hit sizes with mean $\mu = 1$ and standard deviation $\sigma = 1$ ($d < 0$ are treated as $d = 0$). In all cases $\lambda = 1$. Panels A, B $r = 1.2$; C, D $r = 1$ and E, F $r = 0.4$. In each case 10,000 encounters were sampled, after an initial transient of 1000 encounters which were ignored. Colour online.

value of θ and d are not important, only their ratio θ/d. In effect, we measure the hit size in units of the threshold.

As far as we are aware, it is not possible to derive the distribution of such times analytically. However, the mean time to kill a cell is simply the mean first passage time to θ for the process starting at 0. This has been derived for both the case of fixed size hits,[18] and for exponentially distributed hits.[16,18]

In the case of fixed size hits, it is easy to see that if the hit size d is larger than the threshold θ, i.e. $\theta/d < 1$, then a single hit will kill the cell, irrespective of the repair rate. Hence, the expected time to kill a cell is simply $1/\lambda$. For $\theta/d > 1$ multiple hits are required to kill a cell, and the expressions for the average kill time become somewhat involved, with a different expression each time θ/d passes an integer,[18] Given that in a biological context neither the threshold, nor the hit size are likely to be precise, we do not expect such fine detail to be relevant, and instead focus on the exponentially distributed hit situation, which is somewhat simpler.

For exponentially distributed hits, both Refs. 16 and 18 provide expressions for the mean time $T_{\lambda,r,d}(\theta, s)$ to reach the threshold θ, starting at a damage level s.

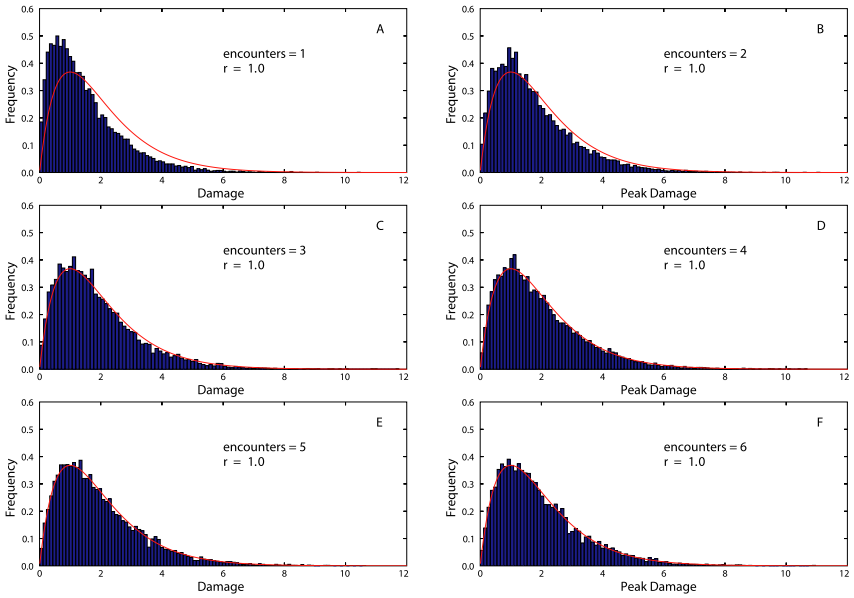

Fig. 4. The transient converges to the asymptotic distribution for peak damage rapidly. Shown here is the convergence when $\lambda = 1$, $d = 1$ and $r = 1$. Each histogram shows the peak damage distribution for 10000 cells for the first k encounters, with the value of k indicated in each subplot. The asymptotic distribution is shown as the solid line. Colour online.

The one given in the latter reference is computationally more convenient

$$T_{\lambda,r,d}(\theta, s) = \frac{1}{\lambda}\,{}_1F_1\left(1, 1+\frac{\lambda}{r}, \frac{s}{d}\right) + \frac{\theta}{d\lambda}\,{}_2F_2\left(1, 1; 2, 1+\frac{\lambda}{r}; \frac{\theta}{d}\right) - \frac{s}{d\lambda}\,{}_2F_2\left(1, 1; 2, 1+\frac{\lambda}{r}; \frac{s}{d}\right) \tag{8}$$

Here ${}_1F_1$ is the confluent hypergeometric (or Kummer) function of the first kind and ${}_2F_2$ is a generalized hypergeometric function. Observe that if the starting damage is $s = 0$, then the third term vanishes, and ${}_1F_1\left(1, 1+\frac{\lambda}{r}, 0\right) = 1$, so the first term simplifies to $1/\lambda$. Other than this, this formula provides little insight into the qualitative behaviour of the expected time to kill a cell. Using Maple, we obtain the first few terms of the expansion of the second term as

$$\frac{\theta}{d\lambda}\,{}_2F_2\left(1, 1; 2, 1+\frac{\lambda}{r}; \frac{\theta}{d}\right) = \frac{1}{\lambda}\left(\frac{\theta}{d}\right) + \frac{1}{2}\frac{r}{(r+\lambda)\lambda}\left(\frac{\theta}{d}\right)^2 + O\left(\left(\frac{\theta}{d}\right)^3\right) \tag{9}$$

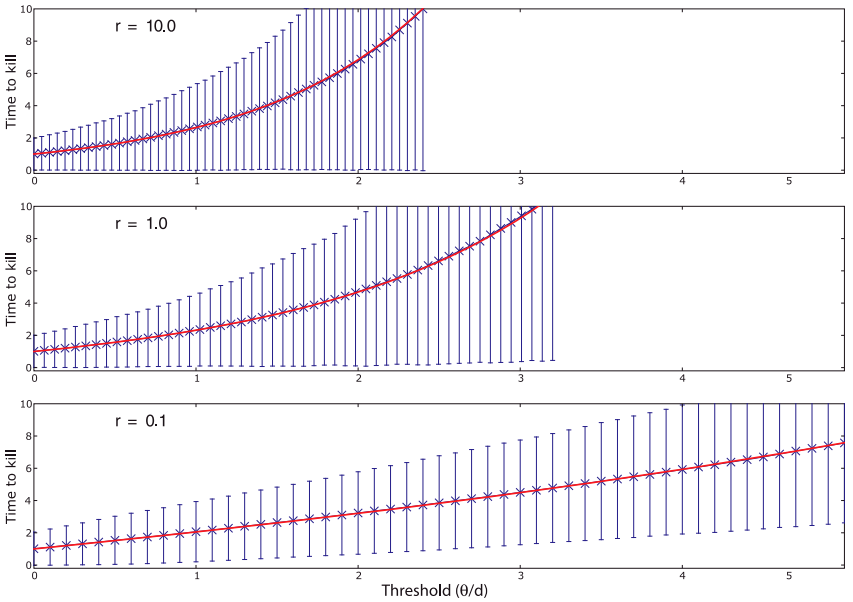

Fig. 5. (Left) Mean time to kill a cell, for three different repair rates and a hit rate of $\lambda = 1$. Analytical results using Eq. (8) is shown by the red curve, while the mean of 10000 simulation runs for the same three repair rates are indicated by blue circles. Error bars indicate ± 1 standard deviation for the simulation results. Colour online.

We thus see that for small repair rates, the time to kill a cell rises linearly with the normalized threshold θ/d but for r of the same magnitude as λ and larger, the dependence on θ/d increases rapidly. This is illustrated in Fig. 5. We see that for even moderate repair rates, the time to kill a cell becomes very sensitive to the threshold θ.

Once θ/d is significantly above 1, Eq. (9) suggests that either increasing the repair rate r or decreasing the hit rate λ rapidly allows target cells to escape immune killing altogether. Of the two, the quadratic term in Eq. (9) suggests that increasing the repair rate is slightly better than attempting to evade cytotoxic cells by decreasing λ but given the simplicity of the model, this difference is unlikely to be of biological significance.

Finally, note that from the point of view of the cytotoxic cell, the series expansion above in Eq. (9) suggests that it is more effective to increase the size d of individual hits, rather than the hit rate λ.

We have also carried out simulations to determine the variability around the mean of the killing time (Fig. 5). The interesting aspect of these is how the lower tail of the distribution rises relatively slowly with θ/d. Hence, even if on average it may take so long to kill a cell as to render cytotoxic killing ineffectual, a significant

proportion of cells will always be killed quickly. This may be useful to control a slowly replicating pathogen or slowly growing tumour. We explore this in Sec. 3 below where we extend the model to allow for such proliferation.

3. CYTOLYTIC CONTROL OF PROLIFERATING CELLS

So far, we have simply considered one infected cell being targeted by cytotoxic immune cells. However, both for virally infected cells and tumour cells, there will be proliferation (either by cell division or by infection) of the target cells which counters the killing of such cells by the immune system. The outcome will depend on which process can dominate in the long term. We shall model proliferation as a simple branching process. The combination of this with cytotoxic killing and repair leads to a class of stochastic models which as far as we are aware has not been previously considered. We give a preliminary heuristic analysis here, which attempts to give qualitative insight into the behaviour of the system, and is sufficient to draw some biological conclusions. However, we believe that this class of process would be worthy of more rigorous analysis using the tools of statistical physics.

3.1. A Branching Process Model of Proliferating Target Cells

Consider a pathogen infected cell that infects k other cells in each generation. If we let $k = 2$, this description also describes a tumour cell that either divides once or dies in each generation. The standard description of such a situation is a branching process[15] where a cell gives rise to k daughter cells with a given probability, which we denote a, or dies with probability $1 - a$. We could also consider a random distribution of the number of offspring but restrict to the above case for simplicity. We scale time so that one generation of the target cell represents unit time. As is well known, and intuitively obvious, the cell population becomes extinct with probability 1 if $a < 1/k$, and has a finite probability of survival if $a > 1/k$.[15] For simplicity, we assume that the process of cell division resets the damage in each daughter cell to 0.

3.2. The Critical Hit Rate in the Absence of Repair

We first consider the case $r = 0$ where there is no repair. To eliminate the pathogen we thus need to ensure that it is hit with at least θ/d hits with probability $(k - 1)/k$ or greater in unit time. Since the rate of hits is modelled by a Poisson process with rate λ, the probability of getting j hits per generation is given by

$$\mathbb{P}[j] = \frac{\lambda^j e^{-\lambda}}{j!}$$

and hence the probability of at least N hits is

$$1 - \sum_{j=0}^{N-1} \mathbb{P}[j] = 1 - \sum_{j=0}^{N-1} \frac{\lambda^j e^{-\lambda}}{j!} \tag{10}$$

which is simply the death probability $1 - a$ described above.

Heuristically, therefore, to eliminate the pathogen, we need to set $a < 1/k$, giving

$$1 - \sum_{j=0}^{N-1} \frac{\lambda^j e^{-\lambda}}{j!} > \frac{k-1}{k} \tag{11}$$

The critical hit rate λ required to achieve this is the implicit solution of

$$1 - \sum_{j=0}^{N-1} \frac{\lambda^j e^{-\lambda}}{j!} = \frac{k-1}{k} \tag{12}$$

This is shown in Fig. 6 for the cases $\theta/d = 1, 2, 3, 4$. We can obtain some insight into the qualitative behaviour by considering the case $\theta \leq d$, so that one hit is sufficient to kill the cell. The probability of at least 1 hit per generation is $1 - \mathbb{P}[0] = 1 - e^{-\lambda}$. For elimination of target cells, we therefore require that

$$1 - e^{-\lambda} > \frac{k-1}{k} \tag{13}$$

which gives

$$\lambda > \log k \tag{14}$$

Surprisingly, the rate λ need only increase as the logarithm of the number of offspring per generation k. Qualitatively, this also holds for higher θ/d.

It is instructive to substitute some illustrative values for the parameters into these formulae. Thus, when $\theta/d = 1$, a tumour dividing mitotically at a rate of once per day requires $\lambda > 0.69$ day^{-1} for effective control, while a virus that results in infection of 1000 other cells a day requires $\lambda > 6.9$ day^{-1} for effective control. A 500-fold difference in the rate of production of target cells requires approximately only a 10-fold increase in the number of immune effector cells for effective control. Even for $\theta/d = 4$, $\lambda > 13.06$ day^{-1} will control a proliferation rate of 1000 cells per day. It is therefore plausible that such pathogenic cells without the ability to replenish depleted granzyme inhibitors can usually be controlled with immune surveillance by the immune system.

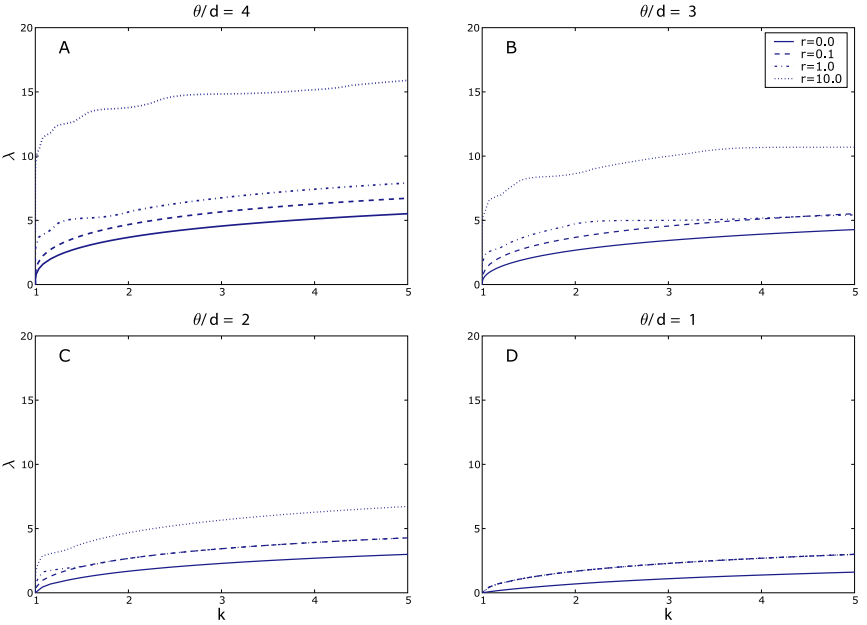

Fig. 6. Critical hit rates required to eliminate a proliferating cell population. In the absence of repair, the mean number of hits required to kill a cell is just θ/d, and this is shown by the lines labelled $r = 0$ for each subplot, corresponding to Eq. (11). With repair, the mean number of hits required to kill a cell is the MFPT multiplied by λ and is thus given by Eq. (15). The critical curves are calculated for repair rates $r = 0.1$, 1 and 10, as labelled. It is clear that a high repair rate is most effective in the presence of a high θ/d in preventing cell killing. The value of θ/d for plots A, B, C and D is 4, 3, 2 and 1 respectively. Colour online.

3.3. The Critical Hit Rate in the Presence of Repair

In the presence of repair it is difficult to determine the rate λ required to eliminate the pathogen, since in general it will require more than θ/d hits to kill the target cell. However, we can argue heuristically as follows: the average time taken to kill a cell is $T_{\lambda,r,d}(\theta, 0)$ and the average number of hits in this time is just $\lambda T_{\lambda,r,d}(\theta, 0)$. Hence, from Eq. (8), the average number of hits required to kill a cell is

$$N_{\lambda,r,d,\theta} = 1 + \frac{\theta}{d}\,{}_2F_2\left(1, 1; 2, 1\frac{\lambda}{r}; \frac{\theta}{d}\right) \tag{15}$$

We can substitute the largest integer N less than or equal to $N_{\lambda,r,d,\theta}$ into Eq. (12) and then solve for the critical hit rate λ required to eliminate the pathogen. This is shown in Fig. 6 for a selection of repair rates. We see that a high repair rate is most effective in the presence of a high θ/d in preventing cell killing. At high values of

λ however, there is little time for repair between hits, and so the curves plateau. Again, it appears as if the hit rate λ required to eliminate the pathogen behaves asymptotically as $\log k$, as in Eq. (14) for the no repair case.

3.4. Multiple Hits, Sensitivity and Specificity

Possibly in response to the evolution of immune evasion tactics, CTLs are highly sensitive to the presence of foreign-peptide MHC molecules on the target cell. It has been reported that activated CTLs can respond to the presence of as few as 1–10 copies of their cognate peptide-MHC ligand.[14,25] However, such high sensitivity poses a problem since it must come at a cost of increased false positives and hence lower specificity. This is because T cells in general appear to distinguish between peptide-MHC ligands mainly on their binding time to the TCR, and this is a random function of the ligand dissociation rate. Since self-peptide MHC complexes greatly outnumber foreign-peptide MHC complexes even on an infected cell, there is obviously a risk that an uninfected cell can activate a highly sensitive CTL.

During a viral infection, the number of activated CTLs undergoes a several orders of magnitude expansion, probably including CTLs which do not recognise the viral antigen but are stimulated to proliferate because of the large amounts of cytokines released by other activated cells ('bystander CTLs').[5,21] It is clear that with the massive numbers of activated CTLs found during a viral infection, even such a low false positive rate can lead to significant self-killing if a single hit is sufficient to kill a normal cell. While this may not matter much in tissues with high replicative potential, it may be problematic for tissues with no or low replicative potential.

However, PI-9 has been found to be expressed in endothelial and mesothelial cells, as well as in immune-privileged organs including the eye, ovary, testis and placenta.[3,6] If this results in $\theta > d$, then it is possible to have both high specificity and sensitivity in these tissues. Suppose that a CTL has a sensitivity of 100% for infected cells, but also makes a mistake once for every 1,000 normal cells scanned. Other things being equal, this implies that the hit rate λ_{Normal} for normal cells will be only 1/1000th the hit rate $\lambda_{\text{Infected}}$. If this results in the ratio r/λ being of the order of 1 or greater for normal cells and substantially less than 1 for infected cells, there will be minimal damage caused to non-infected tissue by occasional false positives since normal cells will very rarely accumulate sufficient damage to be killed. However, infected cells will still be efficiently eliminated.

4. DISCUSSION

In this paper, we have suggested that some cells may be resistant to a single hit by a cytotoxic immune effector cell, due for example, to the expression of PI-9,

a serpin that specifically inhibits granzyme B. This raises the possibility that a single CTL hit is insufficient to kill the target cell and that a stochastic succession of hits coupled to a repair or replenishment mechanism is a more appropriate model, with a threshold for cytotoxic killing determined by the steady state level of serpins. It is also possible that once the levels of serpin are above a threshold (so that $\theta > d$) cells could increase their repair rate without altering their steady state levels of serpins, by increasing both production and degradation of the molecule. This might have advantages for the target cell in settings where high concentrations of the serpin are deleterious to other cellular functions.

For pathogen infected and tumour cells, there is obvious survival advantage in evolving mechanisms to exceed this threshold. For slowly replicating or immune privileged tissue, this threshold provides a means for the immune system to kill infected cells effectively, while avoiding damage to normal cells, thus improving specificity and sensitivity.

Therapeutically, the multiple hits hypothesis suggests that immunotherapy against resistant pathogen infected cells or tumours may be more effective if 'repair' is prevented, for example, with a protein synthesis inhibitor. In the absence of 'repair,' the threshold vanishes, and target cell killing will vary linearly with immune effector density, assuming that the hit rate λ is directly proportional to effector cell density. Another prediction is that in settings where the perforin or granule exocytosis pathway is deficient, there would be an increased incidence of tumours (due to reduced immunosurveillance) and that these tumours would have a lower frequency of PI-9 up-regulation than those arising in immunocompetent individuals.

We suggest that resistance to cytotoxicity and cytolysis is a major mechanism for escaping immune control for both pathogens and tumour cells, as evidenced by the expression of granzyme inhibitors in adenoviruses, orthopoxviruses and multiple tumours. However, this seems to have generated relatively little attention relative to the mechanisms of evasion. The reason for this may simply be that resistance and repair are difficult to detect with the standard chromium release assay for cytotoxicity.[4] The induced proximity of highly activated effector and target cell in this assay for a prolonged period will mask all but the highest levels of resistance. Furthermore, this assay does not reflect the *in vivo* situation where CTLs crawl over potential targets and deliver a hit when activated, then rapidly move on to the next cell to be scanned. It may be more physiological to do the CTL assay in 3D collagen gels, where T cells appear to engage in 'hit-and-run' behaviour rather than maintain prolonged contact.[9,11,12] In such a scenario, we predict that resistance to cytolysis will be found to be a common mechanism for immune escape.

While we believe that this model is a plausible and useful abstraction of a highly complex biological process, it is necessarily based on simplifying assumptions. For instance, we assume that no damage is done to the cell while serpins

are present, i.e. that serpin neutralization is fully effective. If this is not so, and some residual damage (in terms of proteolysis, DNA damage etc.) is inflicted with each hit even in the presence of excess serpins, then a complete model would also need to include a separate irreversible damage threshold and repair process that might determine the actual time to kill the target cell (since it is now possible to accumulate irreversible damage before serpin exhaustion). If the damage process dominates, we can ignore the role of serpin depletion and replenishment and would end up with a qualitatively similar model. If neither process dominates and they also interact (e.g. the rate of serpin production is lower when there is DNA damage), then we would have a rather complicated model to analyse, with a corresponding trade-off in our ability to gain insight into the role of resistance of cytolysis. Therefore, we have chosen to use the simplifying assumptions described in the manuscript, supplemented with numerical simulations of less analytically tractable models where appropriate.

In conclusion, our simple mathematical model provides insight into the dynamics underlying immune resistance and escape from cytotoxic killing. In the experimental literature, it is usually assumed that target cells are either susceptible to a single hit kill or else are resistant to cytotoxic attack. Our model suggests that it is useful to consider resistance to cytotoxic killing as a continuum from single hit susceptibility to complete resistance, where the level of resistance varies with the parameters d, r, θ, and λ as described in this manuscript. If resistance is relative, it follows that 'resistant' cells can be rendered susceptible to immune killing by the therapeutic manipulation of the various parameters, thereby extending the potential scope of immunotherapy.

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