

Mathematical models of cancer dormancy

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Abstract

The objective of this paper is to present preliminary mathematical models of the interaction between tumor and antibody for the murine BCL1 lymphoma and illustrate how this interaction leads to dormancy of the tumor. We explicitly model the induction by the immune response of cell cycle arrest and apoptosis of the tumor cells. In the absence of large amounts of quantitative data and because the models are preliminary, they are deliberately simple. We neglect, for example, spatial effects on this lymphoid tumor and the synergistic effect of antigen-specific T cells. A comparison of alternative models shows that, although vaccination is necessary to stimulate a sufficient immune response to control tumor growth, boosting of the antibody response by the tumor itself is vital to the mechanisms that maintain dormancy. We determine parameters that control the size of the dormant tumors, and the fraction of proliferating cells. Finally, we discuss the implications for tumor immunotherapy.

Keywords: *Dormancy, mathematical models, tumor immunotherapy, BCL1 lymphoma*

Introduction

In cancer, the normal homeostatic processes which control the ratios of numbers of different cell types are upset. One clonal population grows and out-competes all others. However, it has been known for some time [1–4] that certain tumor cell populations can persist undetected for an extended period following removal of the primary tumor. This new equilibrium in which the cancer cells exist at presumably constant levels is referred to as dormancy. The tumor cells are undetected until they suddenly grow many years later. It is possible that during this period their numbers vary slightly.

Dormancy can also be induced in experimental animals, for example in murine B-cell lymphomas, such as the BCL1 lymphoma [5,6]. This tumor is a highly malignant, transplantable tumor, which grows initially in the spleen of the mouse, although the cells later circulate in the blood [7,8]. Such tumors arise from a single cell with a homogeneous antibody on its surface that contains a unique antigen-combining site, called an idiotypic [8]. An idiotypic can act as an antigen in the same animal because its concentration

is so low that the animal is not tolerant to it. Injection of an antibody to this idiotypic before and after injection of tumor cells can be sufficient to arrest growth of the tumor [9,10]. Active immunization with the cancer cell idiotypic (conjugated to an immunogenic carrier protein) or with irradiated tumor cells can also protect against unlimited tumor growth [11–13]. The tumors do not regress, but rather stay at a constant size (c. 1 million cells or 1% of the spleen) for an extended period (up to 2 years) (see Figure 1). Within the population of cancer cells, most are in cell cycle arrest, but some proliferate (see figure 1 in [6]). This proliferation is balanced by cell death. There is evidence that the antibodies signal directly to the cells to induce either cell cycle arrest or apoptosis [10,14]. Approximately 3–5 times as many cells are quiescent as in the absence of antibody (see figure 1 in [6]). The correlation between the development of dormancy and the serum level of antibody is significant (see figure 3 in [9]). When implanted into unimmunized mice, the tumor cells which lead to dormant tumors in the immunized mouse are capable of growing indefinitely and causing full-blown cancer (see figure 5 in [15]).

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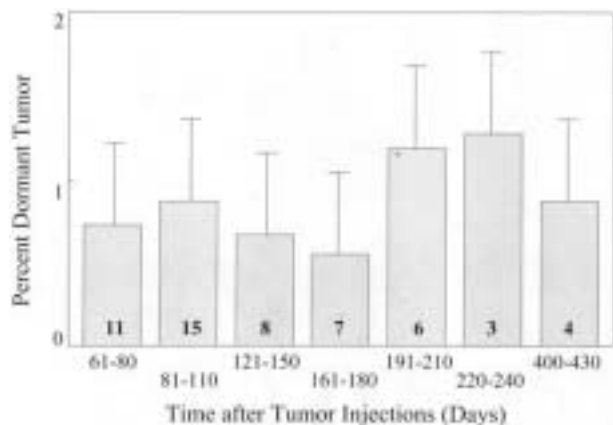


Figure 1. The population of dormant lymphoma cells is stable in size. The percentage of dormant tumor means the percentage of the total number of splenocytes that are dormant lymphoma cells. Id-immune mice carrying dormant lymphoma tumors were killed at various times. The number of large $\lambda +$ cells was determined by flow cytometric analysis. The number in each column indicates the number of mice analyzed for that timeframe with the standard deviation indicated by the error bars. The average number of $\lambda +$ cells in Id-immune animals not injected with BCL1 cells (normal B-cell blasts, 0.24 ± 0.09 ; $n = 6$) was subtracted from the value obtained for each animal before each group was averaged. The percentage of dormant tumor is the percentage of corrected $\lambda +$ cells in the total splenocyte population for each animal. For more details of the experimental protocols, see [6] and references therein.

So how does the antibody signaling ensure that for each cell division there is exactly 1 death, so that the tumor remains at a fixed size, and what parameters determine the size of the tumor and the ratio of proliferating to quiescent cells? Here we present 3 (slightly different) models of tumor dormancy, in which the negative signaling by the antibody can prevent infinite growth of the tumors. We then present a more realistic model that illustrates the role of immunization in the induction of dormancy.

Tumor dormancy itself can arise through a variety of mechanisms and has been studied from numerous perspectives via mathematical models. Here we mention just a sample of these mechanisms and models. First, models of the cellular response to the BCL1 tumor have been presented by Kuznetsov and co-workers [16,17]. A variety of models of the interaction between cancer and the immune system have been studied, see for example [18–21]. Aside from immune mechanisms of limiting tumor growth, this growth may be hindered if the tumor cannot access adequate nutrients and, in particular, has insufficient blood supply. Sophisticated computational models of tumor dormancy in the context of generalized resource limitation have been developed [22]. Generally, tumors are limited to grow to a certain radius before they need to develop their own blood supplies (angiogenesis). Thus, the growth of avascular tumors involves a balance

between proliferation at the outside of the tumor and necrosis at the core, see for example [23]. Limiting angiogenesis can lead to small dormant tumors and is likely to be of particular importance therapeutically, as it targets non-neoplastic cells, which being genetically more stable are less likely to evolve resistance to therapy [24–27].

In the case of the BCL1 lymphoma, experiments show that anti-idiotypic antibody is the major anti-tumor factor by comparing the protection conferred by transfer of T cells with that conferred by transfer of antibody into SCID (immune-deficient) mice [9,28–30]. Here, for simplicity, we ignore cellular immunity, as, in the BCL1 system, antibody alone transferred to SCID mice can induce dormancy, while T cells alone cannot.

Idiotype vaccination for B-cell lymphoma in humans has led to long-term remissions [29]. Hence, immunization to a tumor-specific antigen and injection of tumor-specific antibodies are now in use to control several types of cancer [31–33], probably by inducing dormancy.

Tumor dormancy can have both positive and negative implications for cancer therapy. On the one hand, if tumor cells are not cycling then it can be difficult for many currently used chemotherapeutics to kill the cells. On the other hand, if the dormant state can be prolonged it can clearly contribute to the quality of life of those individuals for whom chemotherapy is not appropriate. The dormant state may last for the individual's lifetime and, hence, the cancer managed as a chronic condition. If we could learn more about how dormancy was induced and maintained it might be possible to substitute immunological therapies for high-dose chemotherapy with its major undesirable side-effects. A potential advantage of using the body's own cellular and biochemical defenses to attack tumors is that they are unlikely to have the same mutagenic effects as many chemotherapeutic agents. Thus, the problems with the evolution of resistance may be less severe. Immunotherapy such as this depends on the existence of an at least partially tumor-specific antigen. In the case of B-cell malignancies, this can be the immunoglobulin idiotype, but such tumor antigens are rarely present in other tumors. However, over-expression of a tumor-associated antigen can give a "window" in which injections of antibody can kill tumor cells with minimal effects on normal cells [34].

Here we present and analyze the mathematical models of tumor dormancy and then focus in the discussion on what these models suggest to be the most promising avenues for enhancing antibody immunotherapy: maximizing the chance of complete clearance and minimizing the size of the dormant tumor and the chance of escape from dormancy.

The models and results

Model a

We consider cancer cells that are either proliferating, with density denoted by y , or quiescent, with density x . There is antibody production at a rate proportional to the density of antigen-presenting (cancer) cells. Antibody concentration is represented by a variable z . The antibody signals to proliferating cells to induce either cell cycle arrest or apoptosis. It also signals apoptosis to quiescent cells. The model equations are as follows:

$$\begin{aligned} \dot{x} &= \alpha_1 zy - \alpha_3 zx - \lambda x + my \\ \dot{y} &= ry - \alpha_1 zy - \alpha_2 zy - my \\ \dot{z} &= \gamma(x + y) - bz \end{aligned} \tag{1}$$

The term $\alpha_1 zy$ corresponds to the initiation of cell cycle arrest and the terms $\alpha_2 zy$ and $\alpha_3 zx$ to apoptosis induced by the antibody. r is the replication rate minus the natural (antibody-independent) death rate of the proliferating cells, λ is the natural death rate of the quiescent cells, m is the rate of the initiation of cell cycle arrest in the absence of antibody¹, γ is the rate of production of antibody induced by the cancer cells and b is the decay rate of the antibody. For simplicity, we ignore spatial effects and assume that the cells and antibody are well mixed. Such spatial effects are probably of less significance in lymphoid tumors than in truly solid tumors in which access to

the blood supply and hence to antibody and lymphocytes is very heterogeneous. Processes such as apoptosis induced by interactions between antibody and tumor cells are here described by simple mass action kinetics. We represent this model in a simple schematic diagram in Figure 2.

This system of equations has non-zero steady state,

$$\begin{aligned} z &= \frac{r - m}{\alpha_1 + \alpha_2} \\ x + y &= \frac{b r - m}{\gamma \alpha_1 + \alpha_2} \\ x &= \frac{\alpha_1 r + \alpha_2 m}{r(\alpha_1 + \alpha_3) + \lambda(\alpha_1 + \alpha_2) + m(\alpha_2 - \alpha_3)} \times \frac{b r - m}{\gamma \alpha_1 + \alpha_2} \end{aligned} \tag{2}$$

Analysis of the Jacobian matrices shows that the zero steady state ($x=y=z=0$) is always unstable (the dynamics move away from it) provided $r > m$. We will assume $r > m$ always holds, as it is the condition for tumor growth in the absence of an immune response. Such an analysis also shows that the non-zero steady state given by Equation (2) is typically stable (the dynamics move towards it) provided that b is sufficiently large or that α_3 is large. The precise conditions for stability are algebraically quite complex, so we do not give them here. We simply note that for certain values of the other parameters (for example, $\alpha_3 \gg \alpha_1 + \alpha_2$), this state is stable for all values of b , whereas for other values it is only stable if b is larger than a critical value. It seems somewhat

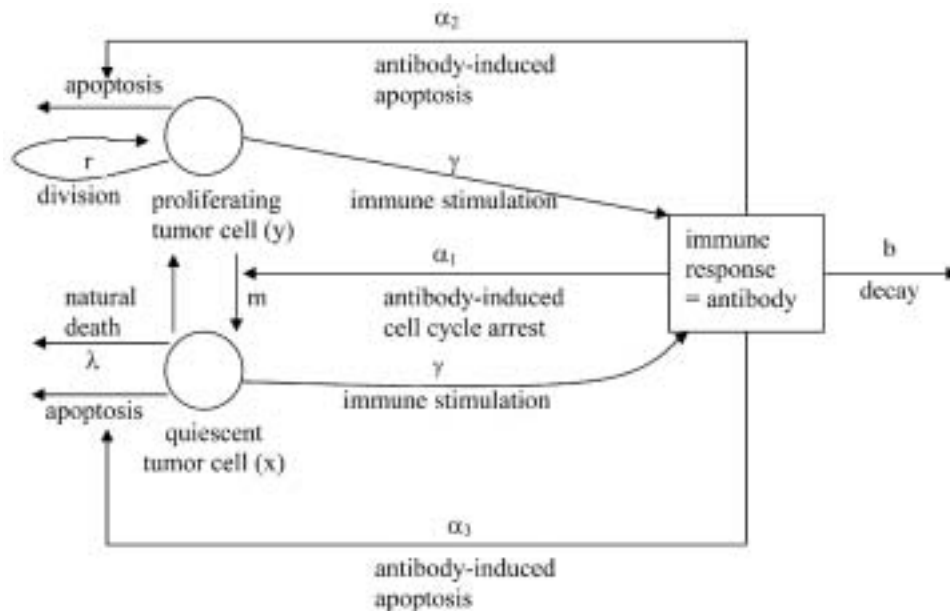


Figure 2. A schematic diagram representing models a and b. Proliferating tumor cells have a net growth rate of r , they naturally become quiescent at rate m . Antibody induces cell cycle arrest in these cells at rate α_1 and apoptosis at rate α_2 . Quiescent cells naturally die at rate λ and antibody induces apoptosis in these cells at rate α_3 . Antibody production is stimulated by the cancer cells at rate γ (in model b, this rate is also proportional to the amount of antibody already present) and antibody decays at rate b .

surprising that rapid degradation of the antibody should favor stability of a dormant tumor state.

When the parameters are such that the non-zero steady state (2) is stable, tumors will grow to the size $x + y = \frac{b}{\gamma} \frac{r-m}{\alpha_1 + \alpha_2}$. If the antibody production is fast and its decay slow, then the tumor will be small. If the production is slow or its decay fast then the tumor will be large. The tumor size also depends on the replication rate and rate of induction of cell cycle arrest and apoptosis of the proliferating cells. Thus, a tumor whose cells replicate fast and die or become quiescent at a low rate will be large, otherwise it will be small. The ratio of proliferating to quiescent cells in the dormant tumor is given by $\frac{y}{x} = \frac{\lambda(\alpha_1 + \alpha_2) + \alpha_3(r-m)}{\alpha_1 r + \alpha_2 m}$. In general, rapid death of quiescent cells and a high rate of antibody-induced apoptosis of quiescent cells will lead to a largely proliferating tumor. In the absence of apoptosis, $y/x = \lambda/r$ and so slow proliferation and rapid death of quiescent cells (which will lead to small tumors) favor a high proportion of proliferating cells within the tumor.

It is clear from Equation (1) that x , y and z must remain bounded (finite).² When the non-zero steady state (2) is unstable, because the zero steady state is always unstable, the system must converge to some attractor. From simulations we find that the system converges to a limit cycle and thus oscillations in the size of the tumor and the concentration of antibody ensue.

Figure 3a shows the size of the tumor plotted against time for various values of the parameters. It is possible to see that decreasing α_1 increases the size of the tumor proportionally, as does decreasing γ . Figures 3b and c show the tumor burden and the concentration of antibody for 2 different parameter sets. In Figure 3b, the amounts of proliferating and quiescent cells are shown. In Figure 3c, only the total tumor burden, $x + y$, is shown. Whereas in Figure 3a, b the system undergoes decaying oscillations and ultimately converges to the dormant state, for the parameter values in Figure 3c, the oscillations persist. For yet smaller values of b (other parameters, for example, as in Figure 3c), the oscillations are sharper and cell density levels drop so low that in reality the tumor would probably be eliminated. Provided elimination does not occur, the system displays marked oscillations. In experiments, approximately 8% of animals with dormant tumors show oscillations in the sizes of the tumors, causing oscillations in spleen size [5]. Such oscillations have also been observed in non-Hodgkins lymphoma in humans [35].

What we are really looking at here is the number density of cells not the absolute numbers. This is because, at this stage, we are ignoring spatial effects

and so essentially are considering the density of cells in a fixed volume. The population is assumed to be well mixed. In this context, number density and absolute number are equivalent. Clearly this assumption of a fixed volume is not realistic, as the tumor may be detected via measurement of enlargement of the spleen. However, in experiments, dormant tumors are typically around 1% of the size of the spleen, so this enlargement may not be too significant. The assumption of equivalence of cell number and cell density is a first approximation and we intend to account more explicitly for spatial effects in future models.

Model b

If normal antibody-producing B cells interact with antigen-presenting cancer cells to induce the secretion of more antibody or the proliferation of the antibody-producing B cells, then the rate of production of antibody will be proportional to the product of the density of cancer cells and the amount of antibody (or equivalently antibody-producing B cells) already present. Thus, the third equation becomes:

$$\dot{z} = \gamma z(x + y) - bz \quad (3)$$

The other equations are the same as for model a.

The steady state is then given by:

$$\begin{aligned} z &= \frac{r - m}{\alpha_1 + \alpha_2} \\ x + y &= \frac{b}{\gamma} \\ x &= \frac{\alpha_1 r + \alpha_2 m}{r(\alpha_1 + \alpha_3) + \lambda(\alpha_1 + \alpha_2) + m(\alpha_2 - \alpha_3)} \times \frac{b}{\gamma} \end{aligned} \quad (4)$$

For this model, the criterion for stability of the non-zero steady state (4) takes a simple form. The state is stable if and only if $\alpha_3 > \alpha_2 + \frac{(r+\lambda)}{m} \alpha_1$ (provided $m, \lambda, \gamma, b, \alpha_1 + \alpha_2, r - m > 0$). Typically this is unlikely to be the case, as it implies that the antibody is more efficient at inducing apoptosis in quiescent cells than in proliferating cells.

The other steady state is at $x = y = z = 0$. It is stable only if $r < m$, which we assume will not be the case (otherwise the tumor would not grow even in the absence of antibody). Thus, it seems that, in this model, generically the tumor burden and antibody concentration will undergo sustained oscillations. (This is because we do not see in numerical simulations x , y or z become unbounded or converge to an attractor stranger than a limit cycle.) If the unlikely condition $\alpha_3 > \alpha_2 + \frac{(r+\lambda)}{m} \alpha_1$ is satisfied, the tumor will grow to a size $x + y = \frac{b}{\gamma}$. In

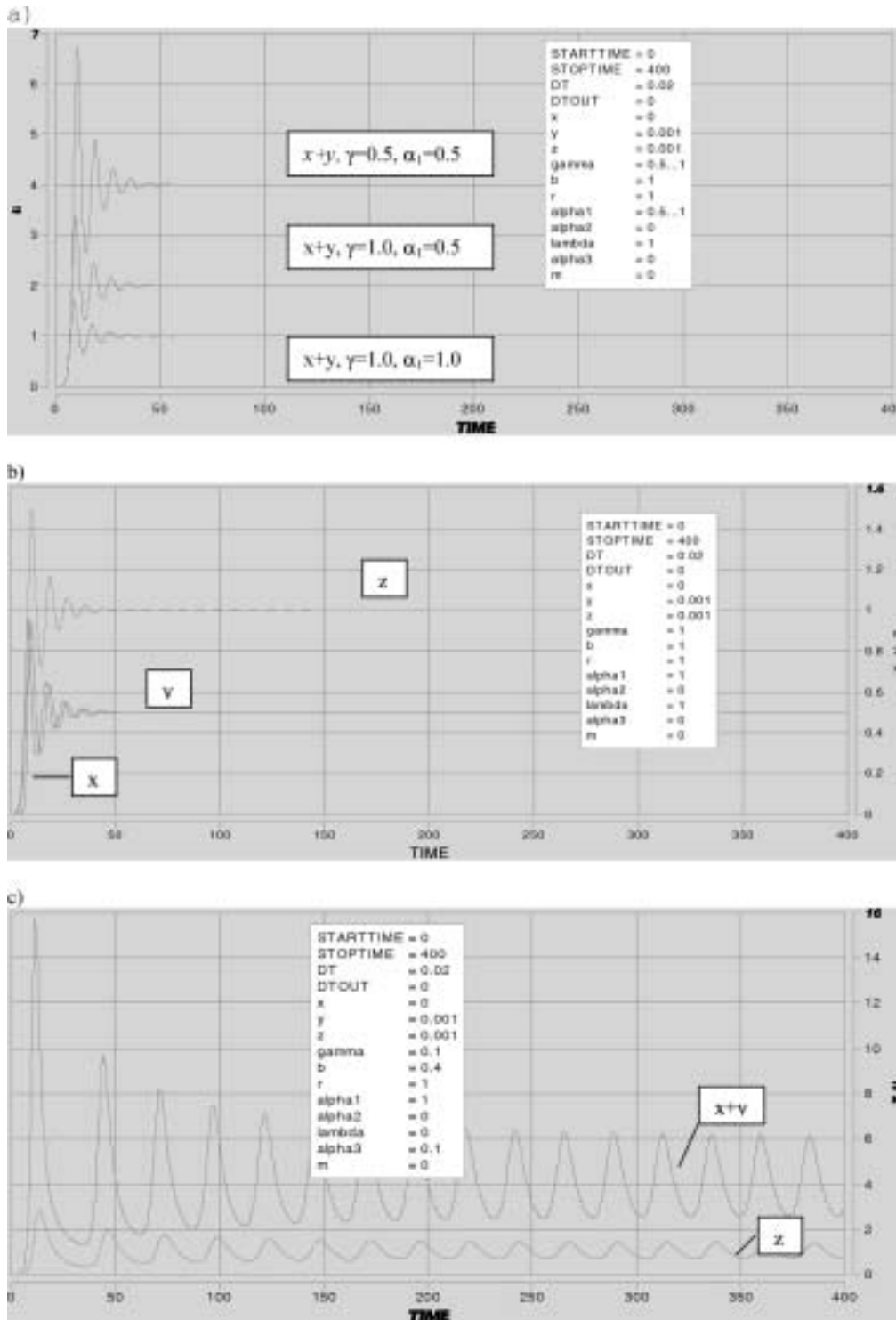


Figure 3. Simulations of model a with parameter values as marked on the figures. The values given for x, y and z are their initial values. In (a), the total tumor burden $u = x + y$ is shown for $\gamma = \alpha_1 = 1.0$; $\gamma = 1.0$, $\alpha_1 = 0.5$ and $\gamma = 0.5$, $\alpha_1 = 0.5$. The tumor undergoes decaying oscillations and converges on a dormant steady state. The figure illustrates that decreasing α_1 or γ increases the size of the tumor proportionally. (b) shows x (the density of quiescent cells), y (the density of proliferating cells) and the antibody concentration, z , separately when $\gamma_1 = 1.0$. In (c), with different parameter values, the tumor burden oscillates much more strongly. These simulations and those in Figures 4–7 were performed using the fourth-order Runge–Kutta method in the “Berkeley Madonna” package.

this case the tumor size is dependent only on the parameters of antibody production and decay, that is, rather counterintuitively, it is independent of such parameters as the replication and death rates of the tumor cells. If the antibody production is fast and its decay slow, then the tumor will be small. If the production is slow or its decay fast then the tumor will be large. The ratio of proliferating to quiescent cells in the dormant steady state is the same as for model a.

We conclude that, in this model too, the system may oscillate, indeed it is likely to do so (although again, it may be that the tumor burden actually drops so low that the tumor is completely cleared). Figure 4 shows the oscillations in total cell density and antibody concentration that ensue for the parameter values shown in the legend. Note that for these example parameter values, there are no quiescent cells.

Model c

An alternative model considers the case when antibody is produced at a constant rate independent of the amount of antigen presentation and antibody is depleted by binding to the cancer cells. The equations are given by:

$$\begin{aligned} \dot{x} &= \alpha_1 zy - \alpha_3 zx - \lambda x + my \\ \dot{y} &= ry - \alpha_1 zy - \alpha_2 zy - my \\ \dot{z} &= \gamma - bz - \alpha_1 zy - \alpha_2 zy - \alpha_3 zx \end{aligned} \tag{5}$$

This has non-zero steady state

$$\begin{aligned} z &= \frac{r - m}{\alpha_1 + \alpha_2} \\ y &= \frac{[\lambda(\alpha_1 + \alpha_2) + \alpha_3(r - m)][\gamma(\alpha_1 + \alpha_2) + bm - br]}{(r - m)[\lambda(\alpha_1 + \alpha_2)^2 + r\alpha_2\alpha_3 + 2r\alpha_1\alpha_3 - \alpha_1\alpha_3m]} \\ x &= \frac{(\alpha_1 r + \alpha_2 m)[\gamma(\alpha_1 + \alpha_2) + bm - br]}{(r - m)[\lambda(\alpha_1 + \alpha_2)^2 + r\alpha_2\alpha_3 + 2r\alpha_1\alpha_3 - \alpha_1\alpha_3m]} \end{aligned} \tag{6}$$

when $0 < r - m < \frac{\gamma}{b}(\alpha_1 + \alpha_2)$. However, this steady state is unstable when it exists. The steady state $x = y = 0, z = \frac{\gamma}{b}$ is stable for $r - m < \frac{\gamma}{b}(\alpha_1 + \alpha_2)$. Thus, for sufficiently small r , small tumors regress and large ones grow. For $r - m > \frac{\gamma}{b}(\alpha_1 + \alpha_2)$ all tumors grow ad infinitum. Thus, this model cannot explain dormancy.

The models presented so far do not explain the role of the initial immunization. They allow antibody production to be stimulated by the tumor cells. In reality, this does not occur without prior immunization. The idiotype present on the cancer cells is not sufficiently immunogenic. We now present a model which can account for the necessity of immunization.

Model illustrating the need for immunization

The new model is a slight variation on the second model discussed above. We make two changes. First, we assume that cancer cell growth will saturate even in the absence of antibody, albeit at a high (lethal) level. Second, and most importantly, we assume that the dynamics of antibody production by interaction with the tumor cells and of antibody decay are slow,

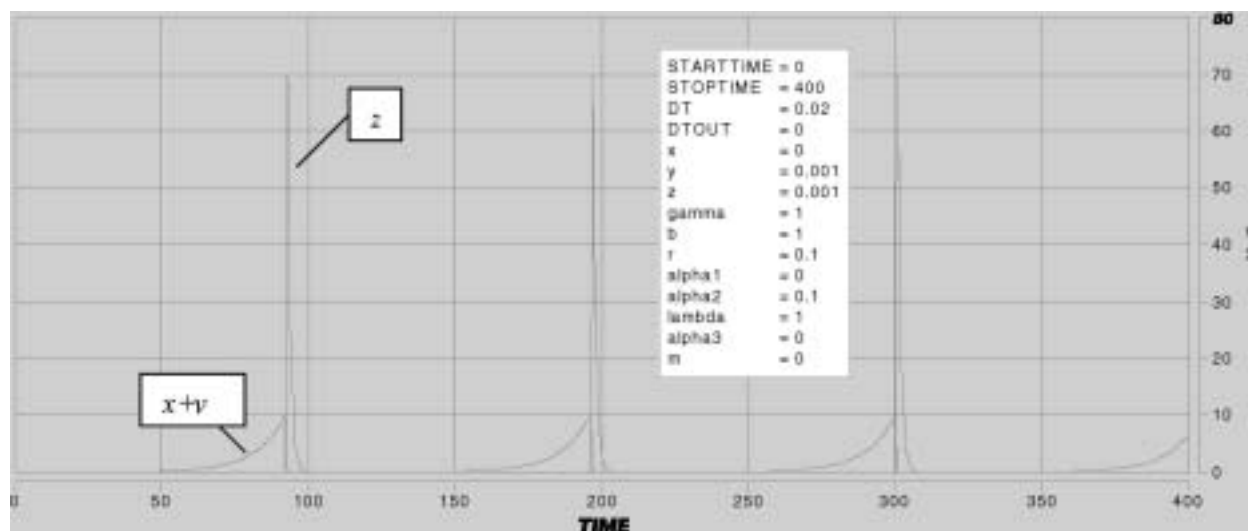


Figure 4. Simulation of model b with parameter values as marked on the figure. As is typical for this model, the cell densities ($x + y$ = total density of tumor cells) and antibody concentration (z) undergo oscillations. As in Figure 3c, the levels drop so low that in reality the tumor and antibody are probably eliminated.

but that antibody can be produced at a faster rate following stimulation by the vaccine (consisting of the idiotype and an adjuvant, which makes it more immunogenic). For now, we ignore the effects of cell cycle arrest and assume that all the cells are proliferating. Thus, the antibody acts by inducing apoptosis. We will reintroduce quiescent cells later. The equations are given by:

$$\begin{aligned} \dot{y} &= ry(1 - y/K) - \alpha_2zy \\ \dot{z} &= \gamma iz + \varepsilon z(\gamma y - b), \end{aligned} \tag{7}$$

where K is the carrying capacity of the cancer cells and we now assume that the decay rate of the antibody is given by εb and its production rate on interaction with the cancer cells is given by $\varepsilon\gamma$, where ε is small. We also assume that, in the presence of idiotype, i , the antibody is produced at a faster rate, γ .

Because we assume that the idiotype from the vaccine will only be present transiently,³ we assume that the major effect of the γiz term will be to

elevate the level of antibody at some short time after the vaccination. In experiments, the vaccine is given at 24, 17 and 7 days before tumor cells are injected [5]. Thus, we assume that the effect of vaccination is to make the antibody level on injection of tumor cells non-negligible. Thus, as a first approximation, we simulate the equations with $i=0$, but with non-negligible initial titers of antibody on tumor injection (corresponding to experiments with pre-immunization) and compare them with simulations in which the antibody titer is negligible (no prior vaccination).

We display the results of simulations with $r=0.1$, $\gamma = \alpha_2 = b = 1.0$, $\varepsilon = 0.001$, $K = 10.0$ and $y(0) = 1.0$ in Figure 5. The simulation time is 2000.⁴ In the first simulation, shown in Figure 5a, the initial antibody titer is non-negligible, $z(0) = 0.1$.

We see that the tumor growth is controlled to a low level, $y \approx 1$. In the second simulation, shown in Figure 5b, the initial antibody titer is negligible, $z(0) = 0.001$. In this case, the tumor grows very large,

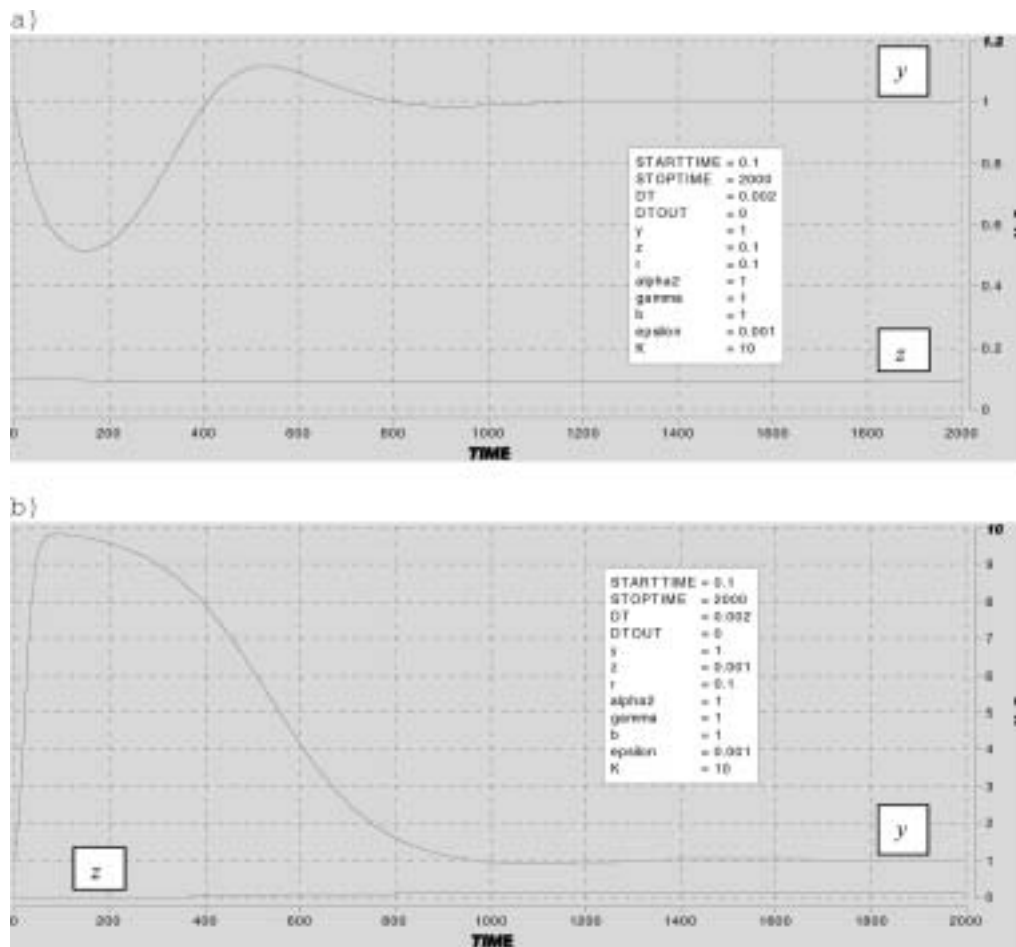


Figure 5. Simulations of the model illustrating the need for vaccination, with only proliferating cells, Equation (7). The parameter values are given on the figure. In (a), the initial antibody titer is non-negligible, $z = 0.1$, and the tumor (y = density of proliferating cells, which is the total tumor burden in this model) is controlled to a small dormant size. In (b), the initial antibody titer is negligible and the tumor grows to a large (presumed lethal) size.

$\gamma \approx 10$. We assume that this high tumor burden for an extended time is lethal, so that although ultimately the immune response would have controlled the tumor, by that time it is too late.

Thus, this new model, with slow antibody kinetics, illustrates the role of the vaccine, which is to raise initial antibody titers and thus prevent the growth of a very high tumor burden. The saturation term in the tumor cell growth is probably biologically realistic and serves to prevent the system from oscillating too much.

We now consider analytically this system after vaccination, when the idiotypic antibody has disappeared but there are raised levels of anti-idiotypic antibody. Considering the system of Equations (7) without the term γiz , we see that because ε is small, the tumor growth kinetics happen on a faster time scale than the B-cell (antibody) response to the tumor. Thus, the tumor cell level will reach its first turning point when the antibody has a value close to its initial value. So we obtain approximately, $0 = ry(1 - y/K) - \alpha_2 z(0)y$ and so $y \approx K[1 - \alpha_2 z(0)/r]$. Thus, if $z(0)$ is much larger than r/α_2 , we should get dynamic clearance of the tumor (in practice in our ODE (ordinary differential equation) model, because we are making a continuum approximation, y can never reach zero exactly), as the tumor level should drop very low before the antibody has decayed significantly (see Discussion). For $z(0) < r/\alpha_2$, the tumor should grow until it reaches a size $\approx K[1 - \alpha_2 z(0)/r]$ and then start to shrink. In the case of the simulation in Figure 5b this approximate calculation predicts a peak tumor size of approximately 9.9. This is very close to the value found in the simulations. For $z(0) \approx r/\alpha_2$, the

calculations are more complicated, because second-order (non-linear) terms become important.

If we use the quasi steady state approximation, assuming that ε is small, then the long-term dynamics are controlled by $y \approx K[1 - \alpha_2 z/r]$ and $\dot{z} = \varepsilon z[\gamma K(1 - \alpha_2 z/r) - b] = \varepsilon z[\gamma K - b - \gamma K \alpha_2 z/r]$. Thus, on coarse-grained time scales, z moves monotonically towards its steady state value of $\frac{r}{\alpha_2} \left(1 - \frac{b}{\gamma K}\right)$.

Hence, peaks and troughs in y will deviate less from b/γ in time. This means that, if we are interested in whether the tumor will kill the mouse or be dynamically eliminated, we need to focus attention on the first peak or trough in y .

Suppose the mouse will die if the level of tumor cells exceeds y_* , then we require an initial antibody titer of $z(0) > \frac{r}{\alpha_2} \left(1 - \frac{y_*}{K}\right)$ to prevent death. If $z(0)$ is significantly larger than r/α_2 , then complete clearance of the tumor (rather than initiation of dormancy) should be possible.

We now show how the vaccine can lead to a non-negligible initial titer of antibody. We assume that at time $t = -20$, there is a vaccination which introduces a titer of idiotypic, $i(-20) = 4.6$. We then assume that the idiotypic decays exponentially at rate $p = 1.0$ over time. We show the decay of the idiotypic and the concomitant growth in the antibody titer. The initial conditions at $t = -20$ are $z(-20) = 0.001$, as in the unvaccinated case, and $i(-20) = 4.6$. The results are shown in Figure 6. We can see from the figure that, at time $t = 0$, the antibody level is approximately 0.1.

We could predict from our model the level and schedule of idiotypic injection required for a certain initial antibody titer,⁵ but this part of the model is preliminary and we believe that it should be possible to estimate this directly from experiments (measuring

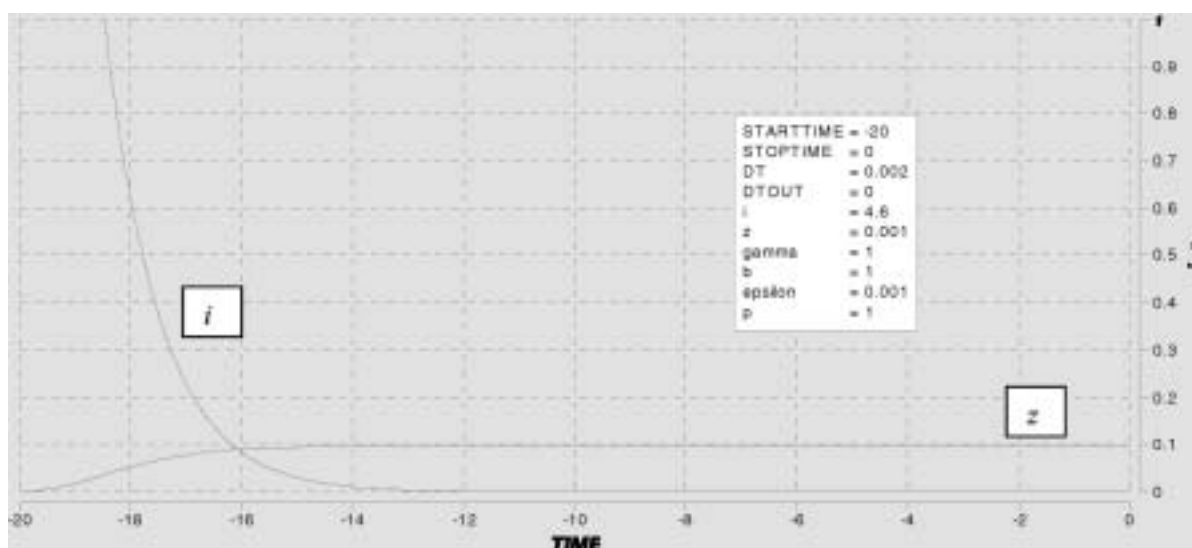


Figure 6. Simulation showing the decay of idiotypic (i) at rate p following vaccination and the concomitant rise in concentration of the antibody (z) prior to challenge with tumor cells. This simply illustrates how the vaccine can lead to non-negligible initial antibody titers.

the splenic antibody level in the mice at various time points post-vaccination and looking at the effects of second and third vaccines).

It is straightforward to reintroduce the quiescent cells and the structure of the dynamics is not changed significantly. In Figure 7 we show the results of a simulation of the following system:

$$\begin{aligned} \dot{x} &= \alpha_1 z y - \alpha_3 z x - \lambda x + m y \\ \dot{y} &= r y (1 - y/K) - \alpha_1 z y - \alpha_2 z y - m y \\ \dot{z} &= \varepsilon z [\gamma (x + y) - b] \end{aligned} \quad (8)$$

with $\alpha_2, r, \gamma, K, \varepsilon, b$ and $y(0)$ as for Figure 5 and $\alpha_1 = 0.1, m = 0.01, \lambda = 0.01, \alpha_3 = 1.0$ and $x(0) = 0.0$.

In the first simulation, shown in Figure 7a, $z(0) = 0.1$. As with the system (7) with just proliferating cells, the high initial antibody titer allows tumor growth to be controlled. In the second simulation, shown in Figure 7b, $z(0) = 0.001$.

As in Figure 5b, the tumor burden becomes very large and we assume lethal.

A paper providing fuller details of the mathematical analysis and computational simulations of the models given by Equations (7) and (8) is in preparation. A discussion of parameter estimation in model (8) is given in the Appendix.

Discussion

Models a and b show how signaling by antibodies to an idiotype present on the surface of cancer cells can limit the growth of a tumor. In cases where a dormant tumor is maintained, the size of the tumor is determined by the rapidity and strength of the antibody response ($\frac{b}{\gamma}$) and may also depend on the parameters of the proliferating cells, which determine the steady state level of the antibody ($z = \frac{r-m}{\alpha_1 + \alpha_2}$). Thus, in the case when antibody production is proportional to the number of antigen-presenting

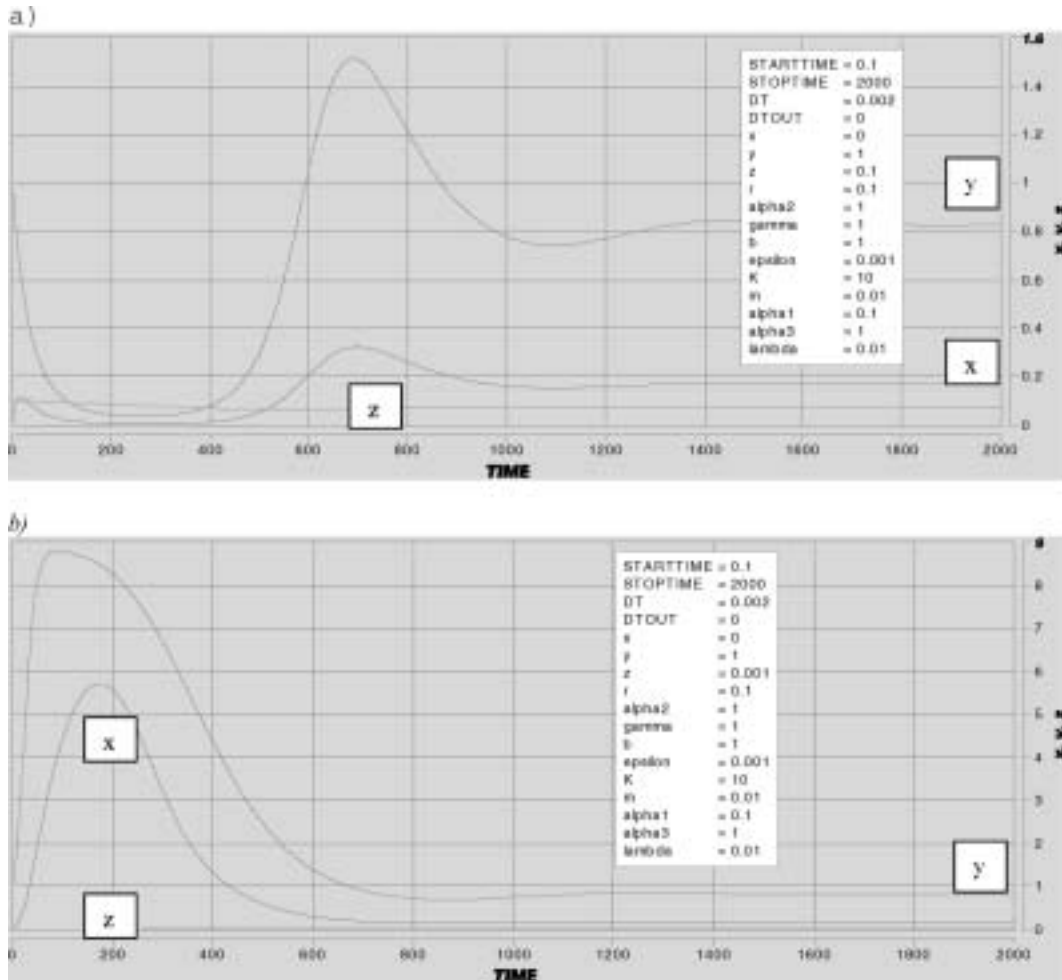


Figure 7. Simulations of the model (8), illustrating the need for vaccination, but this time including quiescent cells (x) as well as proliferating cells (y). The parameter values are given on the figure. As in Figure 5, in (a), the initial antibody titer is non-negligible, $z = 0.1$, and the tumor is controlled to a small dormant size. In (b), the antibody titer is negligible and the tumor grows to a large (presumed lethal) size.

cells, but not proportional to the amount of antibody already present, dormant tumors are large if the proliferating cells replicate rapidly and undergo apoptosis and cell cycle arrest at a low rate. More surprisingly, these parameters, such as the tumor cell replication rate, do not affect the size of the dormant tumor in the case when antibody is produced at a rate proportional to the amount of antibody already present as well as the number of antigen-presenting (tumor) cells. In this case the size of the dormant tumor is entirely determined by the antibody kinetic parameters ($x + y = \frac{b}{\gamma}$). These models show that, because greater numbers of cancer cells stimulate more antibody production, which speeds up the death of the cancer cells, antibody signaling can cause cell death to balance cell proliferation.

The models produce a number of experimentally verifiable predictions. For example, if antibody is produced at a rate proportional to the number of antigen-presenting cells, but not proportional to the amount of antibody already present, then inhibiting apoptosis in the cancer cells should increase the size of the dormant tumor by a factor of $1 + \frac{\alpha_1}{\alpha_2}$. Similarly, blocking the initiation of cell cycle arrest by the antibody, if this is possible, should increase the size of the dormant tumor by a factor of $1 + \frac{\alpha_1}{\alpha_2}$. Both of these procedures should have a similar effect on the steady state level of the antibody. In both of the first 2 models, increasing the rate of production of the antibody or reducing its decay rate should decrease the size of the dormant tumor proportionally.

A major difference between the dynamics of models a and b is that model a will typically result in a dormant tumor, provided the antibody decay rate is sufficiently large, whereas model b will typically lead to oscillations in the size of the tumor and the antibody concentration. It may be that a more biologically realistic assumption is that the production rate of antibody-producing B cells (and hence the antibody itself) is enhanced by the pre-existence of larger numbers of these specific B cells, but not indefinitely so. It would be interesting to investigate this, as it clearly affects the dynamics. It may be that to accurately model stimulation of the antibody response by the tumor, it is necessary to explicitly consider the anti-idiotypic antibody and the cells that produce it separately.

For the models illustrating the need for vaccination, the only stable steady state for sensible parameter values corresponds to dormancy with a prolonged immune response $\left(y = \frac{b}{\gamma}, z = \frac{r}{\alpha_2} \left(1 - \frac{b}{\gamma K} \right) \right)$. Convergence to a steady state with no immune response ($z = 0, y = K$) is also possible, but only for parameter values such that this state has a lower tumor burden than the dormant state ($K < b/\gamma$). Because in experiments mice invariably die in the absence of an

immune response, but can remain alive for considerable times with dormant tumors, this is unrealistic.

We have shown here, however, that dynamic clearance of the tumor in this model is likely if $z(0) \gg r/\alpha_2$. We have also shown that for $z(0) < r/\alpha_2$, damped oscillations are likely. Although these should converge to the dormant state given above, death is also possible if the height of the first peak in tumor burden is sufficiently large. This leads to an approximate criterion for determining outcome (dormancy/death) given by $z(0) < \frac{r}{\alpha_2} \left(1 - \frac{y_*}{K} \right)$, where y_* is the supposed maximal tolerated tumor burden.

The analysis mentioned in the previous 2 paragraphs has been performed for the model without quiescent cells because it is more analytically tractable. A paper describing this analysis in detail and results relating to the model with quiescent cells re-included is in preparation.

The final models (7) and (8) are more realistic than the others, as they illustrate the need for a vaccine to induce dormancy in the tumors. When a dormant state is induced in these models, the total tumor burden should be $x + y = \frac{b}{\gamma}$ and, hence, once again surprisingly, only depends on the kinetics of the antibody. Small initial antibody titers lead to massive tumor burdens, which presumably kill the host. Intermediate initial antibody titers lead to dormancy with the tumor burden given above and very large (potentially toxic) initial antibody titers lead to dynamic clearance of the tumor. In fact, in simulations with very high initial antibody titers the tumor burden drops very low, but then may shoot back up to very high levels. This is because a very low tumor burden means that the antibody is no longer being stimulated and may decay to near zero. This then gives the very tiny tumor burden opportunity to grow. Realistically though, the tumor burden probably drops so low that in fact no cells are left (the value is non-zero only because we consider a continuum approximation, where the density of tumor cells can take an arbitrarily low value). Thus, realistically these cases actually correspond to total clearance of the tumor. We cannot say, however, whether this is obtainable at non-toxic antibody titers.

It should be noted that in this paper we have not estimated parameters, but have simply shown the results of example simulations. While the results are moderately robust, we intend in future to attempt to obtain parameter estimates from experiments/the biological literature to determine the behavior of the system for realistic values of the parameters (see the Appendix).

An issue of potentially great clinical importance is escape from dormancy. This may occur in a number

of ways. First, the antibody may disappear. This may be due to depletion of antibody-producing cells, perhaps because they have been displaced by or have had their resources taken up by the cancer cells. Also, the antigen-presenting cancer cells may develop a method of shielding their antigen presentation, such that it does not result in stimulation of antibody production. Second, the cancer cells may cease to bind the antibody and thus to receive a signal to undergo apoptosis or cell cycle arrest. (It is known in some cases that the escape from dormancy involves the down-regulation of antibody receptors on the surface of the cancer cell.) Lastly, the downstream signaling pathways of the antibody–cancer cell receptors may be disrupted. Hence, despite antibody binding, the cancer cells do not receive signals to die or become quiescent. If any of these changes takes place, then the terms $-\alpha_1zy$, $-\alpha_2zy$ and $-\alpha_3zx$ disappear from the equations and the proliferating cell numbers increase exponentially and indefinitely or to saturation and the tumor grows. Mutations that could lead to escape from dormancy are fairly likely in the cancer cells, because they are dividing rapidly and may also be genetically unstable (see for example [36]). Indeed, we have shown mutations in signaling pathways in half of the mice that escape from dormancy. Thus, when the “escapee” spleen cells were injected into Id-immune mice, they grew progressively [15]. Data on the escape of tumors from dormancy are consistent with a single random mutation, as the rate of escape is constant in time (see Figure 8).

Dormancy is akin to the “equilibrium process” of Dunn et al.’s cancer immuno-editing hypothesis [37], although these authors are considering the cellular (CD4+ and CD8+ T cell and NK cell) response to tumors. We postulate that in BCL1 too

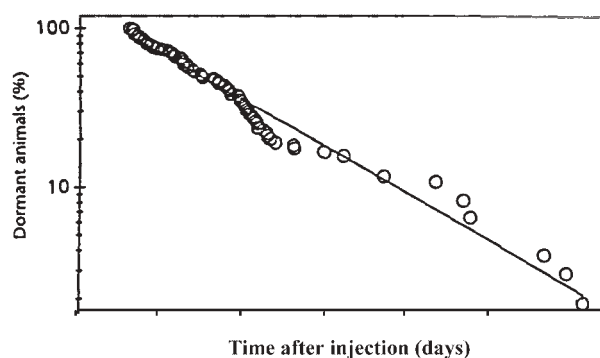


Figure 8. The loss of dormancy with time after BCL1 challenge. Dormant mice ($n = 114$) were examined weekly by palpation for splenic enlargement. The latter correlates with the number of Id+ cells per spleen. The straight line was generated by computer analysis of the data; the regression coefficient is 0.985. The figure is adapted from [6].

this dormant state will involve a process of mutation and selection leading ultimately to escape. Assessing the plausibility of this hypothesis will depend on the generation of well-defined mathematical models and estimation of the values of model parameters. We assert that this should allow us to determine whether the time scales of maintenance of dormancy and escape are plausible given the assumptions of the models.

An area worthy of future work, both experimental and theoretical, is how escape from dormancy may be prevented. An answer to this question could mean that vaccination or antibody injection would provide a cure or permanent long-term control therapy for many types of cancer. We intend to address this issue in the future.

The models also suggest another possible aim of therapy, namely dynamic clearance of the tumors. Thus, it would be interesting to investigate experimentally the highest admissible (non-toxic) initial antibody titers. If it is not possible to make the titers high enough for tumor elimination, then a possible alternative is to lower the threshold required by reducing $\frac{r}{\alpha_2}$ in the model for proliferating cells or, more realistically, $\frac{r-m}{\alpha_1+\alpha_2}$, the equivalent in the full model with proliferating and quiescent cells. This might most plausibly be achieved by reducing r or increasing m by means of concomitant chemotherapy. Thus, the model suggests that high initial antibody titers and/or combined immuno- and chemotherapy are the most likely methods of completely eliminating the tumors.

Another interesting extension to the work in this paper would be to look at how the normal homeostatic mechanisms that control the numbers of various types of B cell [38–43] contribute to dormancy in the tumor. These mechanisms are clearly not sufficient, because the vaccine is required to control tumor growth, but they may nonetheless contribute in a significant way.

We have focused in this paper on the antibody immune response to the tumor. There is evidence that T cells (in particular CD8+ cells) may also be required to enhance this antitumor effect in the BCL1 lymphoma via the secretion of cytokines [44]. Mathematical models of the T-cell response to this tumor have previously been studied [16,17]. In addition to modeling the antibody response to the tumor rather than the T-cell response, our models differ from those of Kuznetsov et al. in another major way. Namely, our models explicitly consider separate compartments for the proliferating and quiescent cells. Specifically, we take into account the initiation of cell cycle arrest. Considering cell cycle arrest could significantly modify the dynamics and we hope that the use of 2 separate compartments will facilitate

parameter estimation. In future we intend to model the interaction between the humoral (B-cell) and cellular (T-cell) compartments of the immune system in controlling tumor growth.

We now further compare our models with those of Kuznetsov and co-workers. In [16] and [17], the authors consider the possibility that tumor cells may kill immune cells, whereas we do not do this explicitly. In a sense, however, this possibility is dynamically equivalent in our system to saying that antibody may be used up in the process of antibody-driven cell death (or initiation of cell cycle arrest). In the models of Kuznetsov and co-workers, this happens if and only if the tumor cell is not killed. We consider only the cases in which antibody is always used up in killing the cells (model c) or antibody is never used up in killing the cells (models a, b and our more realistic models that address the need for immunization). We believe the latter dynamics to be more realistic because the level of antibody may be primarily controlled by the level of specific B cells (if we assume that the kinetics of antibody secretion and degradation are fast), and these cells may never interact with tumor cells directly.

Although the models of Kuznetsov and co-workers have no quiescent cells, dynamically one could compare the complexes of [16] with our quiescent cells (to see this, compare our model b with equations 1a, 1b and 1c of [16] and note that equations 1d and 1e decouple). However, experiments show that the majority of cells in the dormant tumors are quiescent, whereas Kuznetsov and co-workers have a minority of complexes of cells.

Their term for stimulation of immune cells by the tumor is similar to ours, in that it increases in proportion to the product of the densities of tumor cells and immune cells. However, they also consider saturation in the density of tumor cells. This may well be more realistic, but we believe that it does not make a significant difference to our simulations, as the dormant tumors are relatively small.

In [16], the authors do not explicitly consider vaccination. This is dealt with, however, in [17]. The model discussed in [17] is very similar to that of [16], the major difference being that this latter paper considers a time delay of 28 days between implantation of the tumor and the growth of an immune response (this is the time that they estimate it takes a CD8+ T cell to mature). We do not consider such a delay. The modeling of vaccination differs from our models in that it looks at the effect of vaccination following the establishment of a dormant tumor. This is very interesting because it is relevant to immunotherapies for existing tumors. We consider vaccination prior to tumor implanta-

tion because we are interested in the establishment of dormancy in the mouse BCL1 lymphoma for which prior vaccination or injection of antibodies is necessary in syngeneic mice. Presumably the authors of the 2 papers described do not consider this because they are modeling the growth dynamics of the tumor in chimeric mice where an immune response can be raised without vaccination, as the immune system derives from mice of a different haplotype to those from which the tumor derives [45].

This paper has presented what were designed to be first attempts at modeling the mechanism by which dormancy is induced by the interaction between antibody and the tumor. We find that for a dormant state to exist, there must be a feedback from the size of the tumor to the strength of the immune response to it. This is needed to give rise to a stable steady state. Thus, there must be some boosting of the antibody quota (or, in alternative models, of the T-cell density) by the cancer cells themselves. In order to assess the plausibility of these models and to use them to make quantitative predictions, the parameters of the models must be estimated (see the Appendix). Further extensions of these models should address spatial effects, with a distinction between absolute cell number and cell density, should consider the enhancement of the antitumor effect by T cells, should establish whether it is necessary to consider the antibody and the B cells that produce it separately and might look at the influence of the normal homeostatic mechanisms that control B-cell numbers in limiting growth of the tumor. A subsequent study of the process of escape from dormancy and how this may be prevented would have potentially important clinical implications.

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Notes

- [1] For simplicity of analysis, we neglect a term $-lx$ in the first equation and $+lx$ in the second equation representing the rate at which quies-

cent cells re-enter the cell cycle. This term will only be significant if $x \gg y$. This rarely seems to be the case in simulations and indeed when $y \ll x$, $\dot{x} < 0$, so x decays. Thus, the only time when this might be important is in sharp oscillations.

- [2] This is because large tumor sizes lead to large quantities of antibody which induce net death of the proliferating cells. The first equation of (1) implies that x cannot become large while y remains small.
- [3] We assume that the idio type from the vaccine decays fairly rapidly. In reality it may persist for several months (J. W. Uhr et al., unpublished data). It would be interesting to investigate the significance of this persistence in protecting against uncontrolled tumor growth. This will require appropriate estimation of the relative time scales and parameters of the models (see the Appendix).
- [4] At this stage we have not estimated realistic parameters (see the Appendix) and so our parameters are in arbitrary units, in particular a simulation time of 2000 will not generally correspond to 2000 h or days.
- [5] If we consider a time of length T before injection of cancer cells (we define $t = 0$ to be when cancer cells are injected), in which titers, i_j , of idio type are injected at times $-T + t_j$, then $\ln(z(0)/z_{\text{init}})$ will be equal to $-\epsilon b T + \sum_j i_j [1 - e^{-p(T-t_j)}]$, where z_{init} is the antibody level at $t = -T$, before any immunization.

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Appendix

Notes on parameter estimation in the final model

We will not discuss parameter estimation for the other models, only for the model given by Equations (8), as this is, in our view, the most realistic.

The parameter ε is not very important in determining the outcome (clearance/dormancy/death) of a mouse's tumor, provided it is small. It also does not affect the size of a dormant tumor, if such a tumor arises. Therefore, provided it is true that the immune response kinetics are slow relative to the kinetics of tumor growth, it is not of primary importance to estimate this parameter.

Because the only true parameters from the equation for the antibody concentration are $\varepsilon\gamma$ and εb , we have freedom to choose to set one of b and γ to an arbitrary value. We could choose, for example, to set $b = 0.2 \text{ days}^{-1}$. This is approximately the rate of division of cancer cells estimated by Kuznetsov et al. [16,17] for their model and, as such, gives us an order of magnitude estimate for the time scales of the tumor cell kinetics. We want γ and b to have similar sizes to the parameters for the tumor cells, as it is in this case that $\varepsilon \ll 1$ validates use of the quasi steady state approximation to solve the model equations.

We can estimate b/γ as this is the predicted size of a dormant tumor, given that the outcome is dormancy.

Thus, $b/\gamma \approx 10^6$ cells per volume of the spleen, and hence $\gamma \approx 2 \times 10^{-7} \times (\text{spleen volume}) \text{ cell}^{-1} \text{ s}^{-1}$.

In order to estimate the parameters of the tumor growth model accurately, it would be necessary to have time course data on the fraction of proliferating tumor cells within the growing tumor.

From the early growth kinetics of unvaccinated mice (making the assumption that $z(0) \approx 0$), we can approximate $r - m$ (\approx the initial exponential growth rate of the proliferating compartment of the tumor). We are assuming that the immune response is sufficiently slow that in the unvaccinated mice, the level of z will not have changed very much in the time taken for the mouse to be killed. If we can estimate an asymptotic value of the size of the tumor and the asymptotic proportion of proliferating cells (by asymptotic we mean the sizes that would be reached at long times if death of the mouse did not stem tumor growth), then we can estimate $(r - m)K/r$. The initial linear growth rate of the quiescent cells will be approximately $my(0) - \lambda x(0)$. If the immune response is so slow in starting in unvaccinated mice that we can essentially assume the antibody level would be zero right up until the growth plateau of the tumor (this plateau is hypothetical—it may not precede death of the mouse), then the plateau will have $x = my/\lambda$. Time course data on the sizes of the proliferating and quiescent tumor populations will automatically give us $y(0)$ and $x(0)$ and thus estimates of the initial

growth rate of quiescent cells and estimates for the plateau level of quiescent cells will give us estimates for both m and λ . Thus, in total from time course data on the levels of proliferating and quiescent cells in tumors in unvaccinated mice, we should be able to estimate r , m , λ and K . These estimates will also require an estimate of the total size of the spleen and we need to assume that the spleen itself does not vary too much in size during the time window of growth that we use for estimation purposes.

We can apply a similar process to data from a vaccinated mouse. Assuming that we have time course data on the sizes of both quiescent and proliferating cell populations within the tumor, and once again assuming that the expansion of antibody-producing cells (and, hence, increase in antibody level) after tumor challenge is sufficiently slow that we can assume $z = z(0)$ for the initial tumor growth phase (long enough to predict in the 2 tumor cell populations), then using the same measurements as before (initial growth rates and projected plateaus in the 2 tumor cell populations from the data) we can estimate

$$(1) \quad \begin{aligned} &K/r[(r - m) - (\alpha_1 + \alpha_2)z(0)] \\ &\text{instead of } (r - m)K/r \end{aligned}$$

$$(2) \quad [r - m - (\alpha_1 + \alpha_2)z(0)] \text{ instead of } r - m$$

$$(3) \quad [m + \alpha_1 z(0)] \text{ instead of } m$$

and (4) $[\lambda + \alpha_3 z(0)]$ instead of λ .

Hence, using both unvaccinated and vaccinated mouse data, we should be able to obtain estimates of $\alpha_3 z(0)$, $\alpha_1 z(0)$ and $\alpha_2 z(0)$ and have 1 equation left over to test the consistency of the model.

If we can measure or independently estimate the concentration of antibody at the site of the tumor (the spleen), then clearly we can obtain estimates for α_1 , α_2 and α_3 . This is not at all straightforward, whereas serum antibody level can be fairly accurately measured.

Thus, if $z(0)$ can be measured in addition to the values of x and y at various times, and the assumptions about the time scales of processes outlined above hold, then it should be possible to estimate all parameters of the model, except for ε , by the means described. These measurements would have to be made in unvaccinated mice and in vaccinated mice with 1 non-zero value of $z(0)$. The model could then be used to predict the outcome of disease in mice with different initial antibody titers and the model could be tested by comparison with experimental results for a different value of $z(0)$. In particular, it should be possible to predict threshold values in the initial antibody titer required for a dormant tumor to develop instead of the tumor killing the mouse and for the tumor to be cleared completely instead of reaching a dormant state.

[NB The simulations shown in this paper use arbitrary units of time, cell density and antibody concentration.]