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ORIGINAL ARTICLE

B7-H1 and a Mathematical Model for Cytotoxic T Cell and Tumor Cell Interaction

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Abstract The surface protein B7-H1, also called PD-L1 and CD274, is found on carcinomas of the lung, ovary, colon, and melanomas but not on most normal tissues. B7-H1 has been experimentally determined to be an antiapoptotic receptor on cancer cells, where B7-H1-positive cancer cells have been shown to be immune resistant, and *in vitro* experiments and mouse models have shown that B7-H1-negative tumor cells are significantly more susceptible to being repressed by the immune system. We derive a new mathematical model for studying the interaction between cytotoxic T cells and tumor cells as affected by B7-H1. By integrating experimental data into the model, we isolate the parameters that control the dynamics and obtain insights on the mechanisms that control apoptosis.

Keywords Tumor immunogeneity · Percent lysis

1 Introduction

B7-H1 is a surface protein which has been found on carcinomas of the lung, ovary, and colon and in melanomas but not on most normal tissues (Dong et al. 2002). An understanding of a blockade of B7-H1 has been theorized to be applicable not

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only to cancer, but also to viral infections and inflammatory bowel disease (Chen 2004). In the case of tumor immunity, experiments have shown that when the B7-H1 surface protein, also referred to as PD-L1 and CD274, is present on a tumor, cytotoxic T cells (CTLs) are less effective at inducing apoptosis in the cancer cells (Dong et al. 2002; Hirano et al. 2005). It is believed that B7-H1 forms a blockade against CTLs by interacting with PD-1 on the surface of CTLs (Azuma et al. 2008). Phase I clinical trials with an antibody which blocks B7-H1/PD-1 interactions yielded complete remission in one patient with non-Hodgkin's lymphoma (Berger et al. 2008). A better understanding of the mechanism and dynamics may allow medical researchers to develop a cancer treatment schedule specifically targeting this molecular shield, allowing the immune system to more effectively repress a tumor.

Cytotoxic T cells interact with cancer cells as to induce apoptosis via two mechanisms: Fas/FasL binding and perforin (Weinberg 2007; Alberts et al. 2008). In the case of Fas/FasL binding, the Fas ligand (FasL), which is present on some CTLs, forms a complex with Fas, a protein on the surface of a tumor cell. Through this complex, CTLs are able to send apoptotic signals to the cancer cells. In the case of perforin, the CTLs produce perforin which is thought to perforate the tumor cell surface. This allows CTLs to inject cancer cells with granzymes which induce apoptosis. This overview of the apoptosis mechanism is clearly oversimplified. For a more thorough biological review of these mechanisms we refer to Russell and Ley (2002). The Fas-FasL mechanism is theorized to be affected by the presence of B7-H1 (Dong et al. 2002), while perforin-mediated lysis, the primary means of lysis during the first four hours of culture, is expected to be less affected by the presence of B7-H1 (Hirano et al. 2005). Cytotoxicity can be measured experimentally by performing a Chromium release assay which measures percent lysis of target, or cancer, cells by effector cells, or CTLs.

Many tumor-immune system interaction models have been developed to date. None of the existing mathematical models deals directly with B7-H1. A noncomprehensive list of references include the following. Kirschner offers a tumor-immune interaction model which incorporates IL-2 (Kirschner and Panetta 1998). DePillis offers an ordinary differential equation model which utilizes "Hill"-like terms and fits percent lysis data (de Pillis et al. 2005). Thorn and Henney produced a relatively simple model using Michaelis–Menten enzyme-substrate kinetics which also utilizes percent lysis data (Thorn and Henney 1976). Models using kinetic theory have even been developed (Bellomo et al. 2003). There are a few very mechanistic models of CTL-induced apoptosis. Lai offers a model of the Fas-FasL trimer complex formation biology (Lai and Jackson 2004). Another model considers the effect of Fas/FasL binding on a population scale (Webb et al. 2002). A detailed model for the internal workings of granzyme-induced apoptosis has also been produced (Golovchenko et al. 2008). However, none of these models consider both mechanisms of CTL induced apoptosis of a target cell.

In this paper, we present a dynamical model for the induction of apoptosis in cancer cells by cytotoxic T cells. We consider apoptosis by two different mechanisms: Fas/FasL binding and perforin. The model, developed in Sect. 2, is fit to percent lysis data for B7-H1 transfected and mock protein transfected cancer cells exposed to cytotoxic T cells for periods of 4 and 12 hours. The results of our model fitting and an

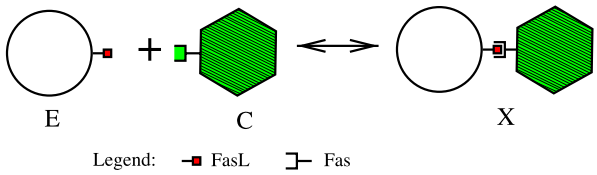

Fig. 1 Cartoon depiction of an effector cell, E, or CTL, interacting with a cancer cell, C, via Fas/FasL binding to form complex X

analysis of the parameter values are presented in Sect. 3. We were able to show how our model can be used to fit percent lysis data as well as capture desirable population trends. A formula for calculating percent lysis from cell population data is derived in the Appendix.

2 A Mathematical Model for CTL and Tumor Interaction

In this section, we consider a model of cytotoxic T cells interacting with the immune system *in vitro*. We also give parameter estimates for the model.

2.1 Model

In the experimental model of Hirano et al. (2005), activated cytotoxic T cells are cultured with cancer cells; hence, for this model we consider possible interactions between the two populations.

In defining the model, let $C(t)$ denote the "uncomplexed" cancer cells at time t, $X(t)$ denote complexes between cancer cells and CTLs at time t, and $T(t)$ denote the total number of cancer cells at time t, i.e., $C(t) + X(t)$. Let $P(t)$ denote perforin in solution and let $E(t)$ denote the CTLs or effector cell population at time t.

First consider the kinetic equation for complexes of cancer cells and effector cells. Assuming mass action kinetics, complexes form at a rate proportional to the number of cancer cells $C(t)$ and effector cells $E(t)$, i.e., $k_1 C(t)E(t)$. They dissociate at a rate $k_2 X(t)$. This association and dissociation via Fas/FasL binding are depicted in Fig. 1. Complexes are also removed from the system when the CTL induces apoptosis in the tumor cell via Fas/FasL binding; we assume this happens at rate $k_3 X(t)$. Hence, the dynamic equation for $X(t)$ reads:

$$\frac{dX}{dt} = k_1 C(t)E(t) - k_2 X(t) - k_3 X(t) \tag{1}$$

For the kinetic equation for perforin, we will assume that perforin is produced at a rate proportional to the number of effector cells $E(t)$, but only when there are very few effector cells in the system. Otherwise, we assume that the rate of perforin production saturates with respect to $E(t)$. To achieve this, we use Michaelis–Menten kinetics. We also allow the presence of cancer cells to inhibit the production of perforin if enough cancer cells are present; we account for this by dividing the produc-

tion term by $k_{m2} + C(t)$. Perforin is removed from the system when it interacts with cancer cells; this happens at rate $k_4 C(t) P(t)$. The resulting equation is:

$$\frac{dP}{dt} = \frac{k_p E(t)}{(k_{m1} + E(t))(k_{m2} + C(t))} - k_4 C(t) P(t) \tag{2}$$

Finally, we consider the cancer cells which are not in complexes, for which we assume a logistic tumor growth with rate constants k and k_5. Cells are removed from this population when a complex forms at a rate of $k_1 C(t) E(t)$ but are added back if the complex dissociates, which happens at a rate of $k_2 X(t)$. Cells which undergo apoptosis via the Fas/FasL binding are eliminated. The other mechanism by which cells are induced to apoptosis is modeled by assuming mass action kinetics for interaction between cancer cells and perforin which we consider at a rate of $k_4 C(t) P(t)$. All terms are combined into:

$$\frac{dC}{dt} = kC(t) - k_5 C(t)^2 - k_1 C(t) E(t) + k_2 X(t) - k_4 C(t) P(t) \tag{3}$$

During the first 12 hours of culturing the effector and cancer cells, we assume the total number of effector cells does not change; that is, $E(t) + X(t)$ is constant. Since CTLs form complexes with cancer cells, the number of uncomplexed effector cells $E(t)$ at any time is calculated as $E(0) - X(t)$. This assumption is experimentally justified in (Hirano et al. 2005).

Recall that the total number of cancer cells in solution $T(t)$ is $C(t) + X(t)$. In order to calculate percent lysis, we must compare this cancer cell population to one which has been cultured in the absence of CTLs, a population which we denote by $T^*(t)$. As with the previous cancer cell population, we assume logistic growth, giving us the following equation:

$$\frac{dT^*}{dt} = kT^*(t) - k_5\left(T^*(t)\right)^2 \tag{4}$$

Note that the kinetic coefficients are the same for $T^*(t)$ and the logistic growth terms of $C(t)$.

The initial conditions are chosen to correspond to the experimental data in (Hirano et al. 2005). In each experiment, the initial concentration of P815 cancer cells is 10^4 cells per 200 μL well. The cancer cells in each culture have either been transfected with B7-H1 or transfected with a mock surface protein. CTLs were added to each culture so that ratio of CTLs to cancer cells is fixed at various effector cell to target cell ratios. We denote these fixed ratios in boldface as $\mathbf{E/T}$. At the end of the culture period, either 4 or 12 hours, the percent lysis of the tumor cells by the CTLs is then determined by chromium release assay.

We begin fixing our initial conditions by scaling our variables so that $C(0) = 1$. The initial effector cell concentration is then determined by a scale factor $\mathbf{E/T}$. To correspond to the experimental data, values of $\mathbf{E/T}$ for time t of 4 hours are 0.25, 0.5, 1, 2, 4, 8, and 16. Values of $\mathbf{E/T}$ for time t of 12 hours are 0.08, 0.15, 0.25, 0.6, 1.25, and 2.5. In this way, the initial effector cell concentration $E(0)$ is equal to $\mathbf{E/T}$ times $C(0)$. We assume that no Fas-FasL complexes X are present in the system at

Table 1 Initial conditions for model

Description	Equation
"Uncomplexed" cancer cells	$C(0) = 1$
Complexes of CTLs and cancer cells	$X(0) = 0$
All cancer cells in system	$T(0) = 1$
CTLs, or Effector cells	$E(0) = (\mathbf{E}/\mathbf{T})C(0)$
Perforin activity	$P(0) = \frac{k_6 E(0)}{k_m + E(0)}$
Cancer cells in absence of CTLs	$T^*(0) = 1$

time zero, that is $X(0) = 0$. Hence, we also assume $T^*(0) = C(0) + X(0) = 1$. We assume that the initial amount of perforin in the system is proportional to the number of effector cells for low levels of effector cells but is constant for large levels of effector cells. To do this, we use a Michaelis–Menten model for the initial condition as can be seen in Table 1. Initial conditions of perforin activity were not available experimentally.

To check the validity of our model, we use percent lysis data found in Hirano et al. (2005). Percent lysis can be calculated from our model:

$$\%\ \text{lysis at time } t = 100\frac{T^*(t) - T(t)}{T^*(t)} \tag{5}$$

Our derivation of this expression and our assumptions can be found in Appendix.

2.2 Parameters

In our model, we assume that all of the parameters, except for three, are the same when considering the interactions of both B7-H1$^+$ and mock-transfected cancer cells with cytotoxic T cells. The presence of B7-H1 is expected to reduce the effectiveness of CTLs in inducing apoptosis in the cancer cells by means of forming a blockade; hence we allow k_3, the rate parameter for apoptosis induced by the Fas-FasL mechanism and k_4, the rate parameter for apoptosis induced by perforin and granzymes, to differ depending on the presence or absence of B7-H1. Experimental studies (Ebelt et al. 2009; Jeong et al. 2008) have shown that in the presence of B7-H1, the production of perforin is inhibited. Assuming that this has to do with the extent of the blockade and the number of cancer cells present, we also allow k_{m2}, the Michaelis–Menten term for perforin production inhibition by the presence of cancer cells, to differ based on the presence or absence of B7-H1. Note that the resulting units of perforin activity are in terms of cellular concentration. In this way, we account for this small particle on the scale in which it affects cancer cells. The actual amount of perforin in the system can be determined by scaling with the average number of perforin molecules required to lyse a cancer cell.

The parameter values utilized in model simulations can be found in Table 2. The values were obtained by minimizing the sum of square errors between the 4 hour and 12 hour percent lysis data and percent lysis as calculated by our model also at the 4 and 12 hour marks. The minimization was performed using the MATLAB function

Table 2 Parameter values for model simulation

Param.	B7-H1$^+$	B7-H1$^-$	Interpretation	Units
k	0.035		cancer growth rate	hr^{-1}
k_1	0.0001		complex formation	$2 \cdot 10^{-2}$ μL/(cell hr)
k_2	0.0001		complex dissociation	hr^{-1}
k_3	0.0001	190	apoptosis by complex	hr^{-1}
k_4	3.0	2.2	apoptosis by perforin	$2 \cdot 10^{-2}$ μL/(cell hr)
k_5	0.003		k divided by Cancer carrying capacity	$2 \cdot 10^{-2}$ μL/(cell hr)
k_6	0.63		maximum initial amount of P	50 cells/μL
k_p	0.097		maximum rate of P production	2500 cells^2/(μL^2 hr)
k_m	1		initial M-M term for P & E	50 cells/μL
k_{m1}	2.2		M-M term for P & E	50 cells/μL
k_{m2}	80	0.1	M-M term for P & C	50 cells/μL

lsqnonlin. In order to obtain parameter values which fit both the B7-H1$^+$ and B7-H1$^-$ data, the sum of square errors being minimized was the weighted sum of square errors over both data sets. The weight for each term in the sum is the square of the inverse of the experimental error at that point.

Note that the parameter values for k_3 and k_{m2} differ as expected. With k_4 being the parameter for Fas/Fas-L induced cytolysis, it has been experimentally predicted that the presence of B7-H1 cancer cells would inhibit this pathway thus making the value of k_3 for B7-H1$^+$ less than that of k_3 for B7-H1$^-$. Hirano et al. suggest that their results imply that the perforin/granzyme pathway of cytolysis is not inhibited by B7-H1 (Hirano et al. 2005). Two other recent papers (Ebelt et al. 2009; Jeong et al. 2008), have experimentally shown that perforin production is greatly decreased in the presence of B7-H1$^+$ cancer cells. In combination, these references imply that the initial amount of perforin within effector cells can be used to induce apoptosis in both B7-H1$^+$ and B7-H1$^-$ cancer cells, but after the first 4 hours or so, the effector cells in the presence of B7-H1 will not be able to produce as much perforin. This is reflected in our values of k_{m2}. It is interesting that the value of k_4 for B7-H1$^+$ is slightly larger than that k_4 for B7-H1$^-$. We will examine this further in the next section.

3 Results and Discussion

In Fig. 2, we present the percent lysis calculation results of our model in comparison to experimental data. There are two noteworthy trends in the data. First, note that the experimental data at 4 hours is not that different for B7-H1$^+$ and B7-H1$^-$; however, after 12 hours the expected percent lysis for B7-H1$^+$ is approximately half of the B7-H1$^-$. Secondly, it is noteworthy that as **E**/**T** increases, the experimental percent lysis appears to saturate. That is, even if the effector cells are at a very high ratio to the cancer cells, the CTLs are not sufficiently capable of suppressing the tumor in these ratios. This may imply the existence of another mechanism of cancer cell avoidance of lysis by CTLs. It is important to note that with only three parameters

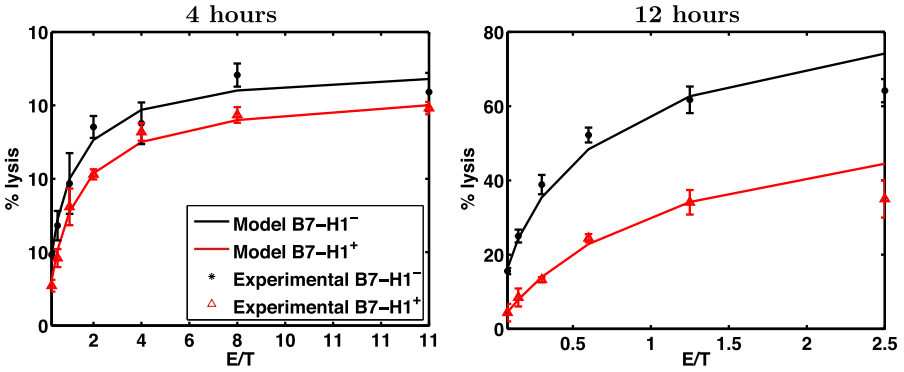

Fig. 2 Percent lysis experimental data fit by model at 4 and 12 hours for both B7-H1$^+$ and B7-H1$^-$ for various effector to target cell ratios. The experimental data is from Hirano et al. (2005)

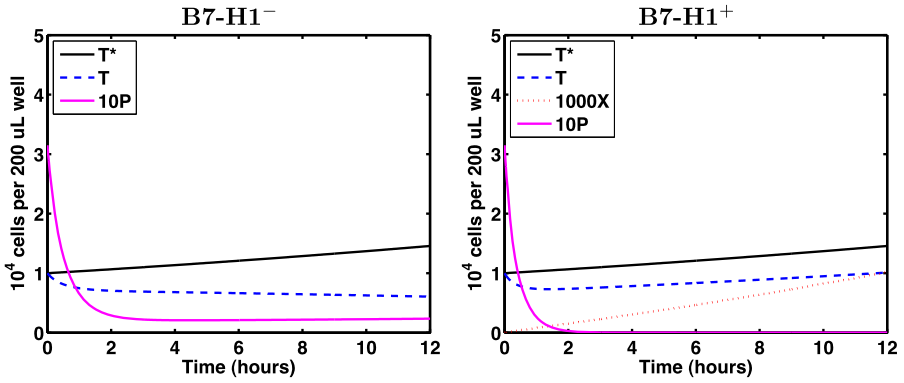

Fig. 3 Population dynamics for cancer cells, transfected with either B7-H1$^+$ or a mock protein in the presence of effector cells T and in the absence of effector cells T^*. The concentration of the Fas-FasL complexes is also plotted but very small relative to T. For the initial conditions, we chose $E/T = 1$

differing between the B7-H1$^+$ and B7-H1$^-$ equation sets, our model captures both of the these desirable trends.

In Fig. 3, we observe the cell population dynamics over the course of 12 hours. In *in vivo* experiments in Hirano et al. (2005) lasting 25 to 50 days, it was observed that a tumor cell population which was transfected with a mock protein, instead of B7-H1, would increase in number initially, but then gradually decrease as CTLs managed to lyse the population. In a 12-hour *in vitro* experiment in Hirano et al. (2005), the mock transfected cancer cells gradually decreased in number while B7-H1-positive cancer cells linearly increased in number. In looking at our population dynamics, we also see this behavior as the total cancer cell population for B7-H1$^-$ cells cultured with cytotoxic T cells, T, decreases with time. The B7-H1$^+$ cancer cell population decreases initially due to the large amount of perforin in the system, but then increases gradually. Note that the amount of perforin in the system, representative of the CTLs ability to induce apoptosis via this mechanism, decreases to a negligible amount for CTLs

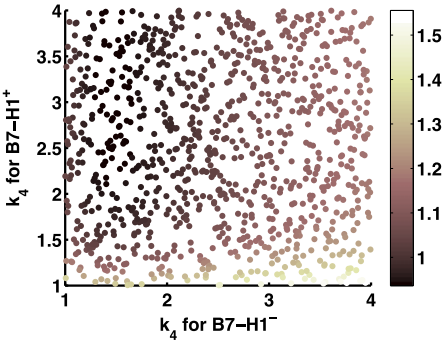

Fig. 4 Latin hypercube sampling analysis of parameter k_4, apoptosis rate for perforin interacting with cancer cells, where all other parameters are fixed at values given in Table 2. The value being plotted is the weighted sum of square errors for the plotted parameter values divided by the weighted sum of square errors for the parameter values in the table

in the presence of B7-H1 but does not go to zero for CTLs in the absence of B7-H1. The complex dynamics are only significant in the presence of B7-H1 and are shown on the corresponding graph. The negligible amount of complexes present in the B7-H1$^-$ system can be accounted for by comparing the rates at which complexes form, dissociate, and induce apoptosis via Fas/FasL binding. The rate parameter for Fas k_3 is much larger than both k_1 and k_2, implying that almost every complex that forms is almost immediately assigned to inducing apoptosis via the Fas/FasL mechanism.

To assure that we actually have attained reasonable parameter values for the rate of perforin-induced apoptosis k_4, we performed a Latin hypercube sampling experiment varying B7-H1$^+$ and B7-H1$^-$ k_4. It is noteworthy that these parameters could have been optimized individually to obtain similar results. Instead, we feel this approach gives the reader a better feel for the parameter space and relative errors, as it is similar to how the initial parameter estimates were done, by optimizing over the entire parameter space simultaneously and in this way, the parameters B7-H1$^+$ k_4 and B7-H1$^-$ k_4 are very much related. The parameter values for B7-H1 positive and negative systems were allowed to vary between 1 and 4. The value plotted in Fig. 4 is the sum of errors squared for the plotted parameter set divided by the sum of errors squared for the parameter set in Table 2. The errors are the difference in percent lysis between the experimental values and the predicted values and the summation is over both 4 and 12 hours at their respective prescribed **E**/**T** ratios. Values close to one are depicted by black dots, while larger relative errors are shown with lighter dots. As can be seen in Fig. 4, the values for perforin-induced apoptosis rates do not necessarily have to be different in order to get a reasonable, albeit suboptimal, fit to the percent lysis data. This supports the assumption in (Hirano et al. 2005) that B7-H1 does not affect or decrease the rate of cytolysis by perforin.

It is noteworthy that our model inaccurately treats perforin as a molecule with some uniform concentration in solution; perforin *in vivo* would be localized to neighborhoods between CTLs and cancer cells. However, given that the solution is cultured at relatively high cell concentrations *in vitro* with only effector and target cells present, a uniform perforin concentration is a reasonable first approximation to a global average of activity due to perforin and granzymes. Regardless of the applicability of this assumption, the model does provide means of considering apoptosis by two separate mechanisms. These mechanisms are not mathematically equivalent under quasisteady state approximations, that is assuming dX/dt and dP/dt are both

zero. Such considerations of utilizing both apoptosis mechanisms are a likely key to the development of a more accurate and more mechanistic model describing CTL and cancer cell interaction.

Considering that perforin-induced apoptosis is the primary mechanism of apoptosis during the time series considered, our results seem to agree with experimental data that B7-H1 has little effect on the rate of perforin-induced apoptosis but does inhibit perforin production. In fact, if the parameters for k_4 of B7-H1-positive and B7-H1 negative, are equated, the curve fits are achieved with comparable accuracy as is displayed in Fig. 2. It is important to note that our assumptions about the rate limiting mechanisms, perforin production and Fas-FasL complex formation, might not be accurate. For instance, it is possible that apoptosis by these means is not rate limited by binding mechanism but is instead limited by signal transduction. Indeed, de Pillis et al. (2005) conclude that the law of mass action is not a sufficient way to model cytolysis as a whole. Regarding the parameter estimates, considering that the data we used is strictly *in vitro* and we are interested in extending our model to fit *in vivo* data, a more comprehensive analysis of the parameter space might be necessary.

The formula which we derive in Appendix for calculating percent lysis from cell population data is simple, intuitive, and computationally cheap. While the formula may not be as precise as it could be, the development and validation of a more involved model may require experimental research. It is also noteworthy that accuracy associated with the use of this formula will depend on the assumed model dynamics of cancer cells in the absence of CTLs. In our case, we modeled cancer cell growth and death with a logistic growth model. This assumption could possibly be improved by using a cancer growth model which more accurately fits a growth curve for P815 cancer cells grown *in vitro*; however, this data was unavailable for the experiment.

4 Conclusions

We have provided a model for CTL-induced apoptosis of cancer cells which is more mechanistic than those currently available while still being relatively elementary. We were able to show how the model can be used to fit percent lysis data for *in vitro* interactions between CTLs and cancer cells, transfected with either B7-H1 or a mock protein. Only three of the parameters were assumed to be different: the rate parameter for Fas-mediated apoptosis, the rate parameter for perforin-induced apoptosis and a Michaelis–Menten-like parameter for perforin production inhibition by cancer cells. The model's projected cell population data behaved as desired with the mock-transfected cancer cell population decreasing in size and the B7-H1-transfected cancer cell population increasing in size. While the model may require more work to ensure realistic parameter values, the idea of incorporating both mechanisms of apoptosis and a formula for calculating percent lysis were presented.

For future work, in order to make inferences with the model at time intervals longer than twelve hours, we intend to relax the assumption that the CTL population is constant and incorporate T cell dynamics. It would also be useful to incorporate data specifically involving perforin, Fas/FasL and cell knockouts of each. Additionally, it might be possible to reduce the dimension of the parameter space by making

quasisteady state approximations for the concentrations of perforin and CTL-cancer complexes. We would also like to extend our model to *in vivo* data, including the upregulation of B7-H1 on tumor cells and interaction with an antibody; however, dynamic data for B7-H1 upregulation is not currently available.

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Appendix: Percent lysis

Percent lysis experiments are done by Chromium release assay; the goal of such an experiment is to provide a scale by which to compare the effectiveness of different lytic agents or effector cells in lysing target cells. In order to conduct an experiment to determine percent lysis, the target cells must be incubated with radioactive chromium. The cells take in some of the chromium and the rest is washed away before the experiment begins. After some period of time t, percent lysis is calculated with the following formula:

$$\%\ \text{lysis} = 100\frac{(\text{experimental } {}^{51}\text{Cr release}) - (\text{spontaneous } {}^{51}\text{Cr release})}{(\text{maximum } {}^{51}\text{Cr release}) - (\text{spontaneous } {}^{51}\text{Cr release})} \tag{6}$$

Each of the variables in the equation for percent lysis (6) is calculated at time t. The spontaneous ^{51}Cr release at time t is determined by assessing how much chromium is in solution at time t where the target cancer cells have not been in the presence of effector cells. This is a measure of how much chromium has been spontaneously released by the cells after time t. The maximum ^{51}Cr release is determined by exposing the target cancer cells, again in the absence of effector cells, to a lytic agent and then measuring the amount of chromium in the system. The experimental ^{51}Cr release is determined by measuring the amount of chromium in solution in which target cells have been exposed to effector cells for a time duration t. Mathematical formulae to predict percent lysis have been developed, e.g., see Thorn and Henney (1976), but these models do not take into account the full dynamics of the system.

To relate this to our model, we need to make a couple of assumptions. We assume that the target cells have a uniform concentration of chromium, and let $\rho(t)$ denote the amount of chromium present per viable cell at time t. We also assume that the $\rho(t)$ is identical for the two target cell populations we are considering, i.e., those in the absence of effector cells, $T^*(t)$, and those in the presence of effector cells, $T(t)$. Noting that we previously assumed that target cells have the same growth dynamics and associated growth parameters in our model, it seems reasonable to assume that $\rho(t)$ is reduced only by spontaneous release and mitosis. If a target cell dies, by apoptosis or otherwise, this should not affect the amount of chromium in the remaining viable cells. In this sense, we are assuming that once chromium leaves the cell the amount that diffuses back into the cell is negligible.

Using our assumptions, we can derive a new equation for percent lysis. The maximum amount of chromium released minus the amount released spontaneously is equal to the amount of chromium that is present in viable cells, which are in the absence of effector cells, that is,

$$\left(\text{maximum } {}^{51}\text{Cr release}\right) - \left(\text{spontaneous } {}^{51}\text{Cr release}\right) = \rho(t)T^*(t) \tag{7}$$

A similar expression can be determined for the amount of chromium which is present in viable cells that are in the presence of effector cells. If the maximum release minus the spontaneous release tells us how much chromium could possibly be present in the cells at time t and we know how much has been removed from the cells by lysis at time t using the experimental release minus the spontaneous release, then we can calculate the amount of chromium present in the cells at time t by taking the difference between how much chromium could possibly be in the cells and how much has been removed from the viable cell population as can be seen below.

$$\left[\left(\text{max. } {}^{51}\text{Cr release}\right) - (\text{spont. rel.})\right] - \left[(\text{exp. rel.}) - (\text{spont. rel.})\right] = \rho(t)T(t) \tag{8}$$

Combining (6), (7), and (8), we have obtained an equation for percent lysis that we can now use with our model variables:

$$\%\text{ lysis at time } t = 100\frac{T^*(t) - T(t)}{T^*(t)} \tag{9}$$

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