Nonlinear Dynamics of Immunogenic Tumors: Parameter Estimation and Global Bifurcation Analysis

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SFI WORKING PAPER: 1992-09-047

SANTA FE INSTITUTE

NONLINEAR DYNAMICS OF IMMUNOGENIC TUMORS: PARAMETER ESTIMATION AND GLOBAL BIFURCATION ANALYSIS

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ABSTRACT

We present a mathematical model of the cytotoxic T lymphocyte response to the growth of an immunogenic tumor. The model exhibits a number of phenomena that are seen *in vivo*, including immunostimulation of tumor growth, "sneaking through" of the tumor, and formation of a tumor "dormant state". The model is used to describe the kinetics of growth and regression of the B-lymphoma BCL_1 in the spleen of mice. By comparing the model with experimental data, numerical estimates of parameters describing processes that cannot be measured *in vivo* are derived. Local and global bifurcations are calculated for realistic values of the parameters. For a large set of parameters we predict that the course of tumor growth and its clinical manifestation has a recurrent profile with a three to four month cycle, similar to patterns seen in certain leukemias.

Keywords: tumor, cytotoxic lymphocytes.
Running title: A model of immunogenic tumor growth.

1 Introduction

The immune response to a tumor is usually cell-mediated with cytotoxic T lymphocytes (CTL) and natural killer (NK) cells playing a dominant role. A number of mathematical models of the interactions between the immune system and a growing tumor have been developed (Thorn & Henney, 1976, 1977; DeLisi & Rescigno, 1977; Rescigno & DeLisi, 1977; Kuznetsov & Volkenshtein, 1978, 1979; Albert *et al.*, 1980; Prigogine & Lefever, 1980; Look *et al.*, 1981; Lefever & Erneaux, 1984; Grossman & Berke, 1980; De Boer & Hogeweg, 1985, 1986; Perelson & Macken, 1984; Hiernaux *et al.*, 1986; Merrill & Sathananthan, 1986; Mohler & Lee, 1989; Dozmorov & Kuznetsov, 1988). The kinetics of cell mediated cytotoxicity *in vitro* have also been described by mathematical models (Thorn & Henney, 1976, 1977; Thoma *et al.*, 1978; Merrill, 1982; Perelson & Bell, 1982; Perelson & Macken, 1984; Macken & Perelson, 1984; Callewaert *et al.*, 1988; Kuznetsov, 1979, 1981, 1984; Lefever *et al.*, 1992). With such models, numerical estimates of biologically significant parameters have been obtained, a number of phenomena interpreted, and predictions made.

The dynamics of the anti-tumor immune response *in vivo* is complicated and is not well understood. Spontaneously arising tumors are known to be of low immunogenicity and usually grow out of control in an organism. The escape from immune surveillance has been linked with a number of different mechanisms, including the selection of tumor clones resistant to cytolytic mechanisms, the loss or masking of tumor antigens, the loss of MHC class I molecules, and tumor induced disorders in immunoregulation (Brondz, 1987; Nelson & Nelson, 1987; Tanaka *et al.*, 1988). Nevertheless, cancer cells are attacked and killed by cells of the immune system (Hellström & Hellström, 1969; Herberman, 1974), and thus immune surveillance of spontaneous tumors may be effective and important in keeping cancer incidence low.

The main attempts at present to develop schemes for immunotherapy or its combination with other therapy methods are directed at lowering tumor mass, heightening tumor immunogenicity, and removal of immunosuppression induced in an organism in the process of tumor growth. Nevertheless, the majority of such attempts are not effective. One of the main reasons for this lies in the fact that even after a so-called "successful" and "clinically" complete removal of a tumor, a small quantity of "residual" tumor cells stay in an organism, which can grow into secondary tumors or "dormant" metastases (Mathe & Rejzenstein, 1986; Wheelock & Robinson, 1983).

Tumor dormancy is an operational term used to describe a state in which potentially lethal tumor cells persist for a prolonged period of time with little or no increase in the tumor cell population (Wheelock *et al.*, 1983). It is frequently presumed that tumor cells do not grow at a rapid rate during dormancy, say due to the absence of a factor needed

for progressive growth into a tumor, but an alternative possibility is that rapidly growing cells are killed at a rate equal to that at which they generated (Uhr *et al.*, 1991). Dormant states emerge not only after a radical treatment of a tumor, but also at early stages of tumor progression. In fact, there is general agreement that in the human, neoplastic cells escape from a primary tumor very early in its development. The fate of these escaping neoplastic cells will determine whether the patient lives or dies of cancer (Uhr *et al.*, 1991). The direct participation of CTL in the support of a tumor dormant state has been shown in some experimental models (Wheelock & Robinson, 1983; Wheelock, 1986). Besides CTL, other types of immune system cells, such as macrophages and NK-cells, may participate in the maintenance of a tumor dormant state. Anti-idiotype antibodies may also play a role in inducing the tumor cells into a dormant state (Uhr *et al.*, 1991). In spite of this, our understanding of the mechanisms of immune surveillance and of the tumor dormant state is still quite incomplete (Wheelock 1986; Chen *et al.*, 1990; Liu *et al.*, 1990).

Small dormant tumors, which after a long time begin uncontrolled growth, may escape from immune surveillance by the so-called "sneaking through mechanism" (Uyttenhove *et al.*, 1983; Wheelock *et al.*, 1981; Wheelock & Robinson, 1983; Wheelock, 1986). Sneaking through refers to a phenomenon in which animals, when challenged with a low dose of tumor cells fail to mount a successful anti-tumor immune response and progressive tumor growth results; challenge with medium doses of tumor cells leads to tumor rejection, and large doses break through the immune defenses and successfully generate tumors (Old *et al.*, 1962; Deichman, 1979). This effect has been reproduced in different experimental models (Deichman, 1979; Gatenby *et al.*, 1981). Experimentally, "sneaking through" can be most easily manifested after a preliminary immunization with a subimmunogenic dose of tumor antigens (Gatenby *et al.*, 1981; Deichman *et al.*, 1979). The time between initial immunization and injection of tumor cells is an important parameter (Alsabti, 1978; Deichman, 1979, Deichman *et al.*, 1979). The mechanisms responsible for sneaking through are not known and the phenomenon has been the subject of a variety of mathematical models (DeLisi & Rescigno, 1977; Rescigno & DeLisi, 1977; Grossman & Berke, 1980; De Boer & Hogeweg, 1985, 1986).

Different host defense cells are able to suppress the growth of or destroy tumor cells. However, in a number of experimental and clinical cases it has been observed that stimulation of the immune system by immunotherapy results in the stimulation of tumor cell growth rather than suppression (Prehn, 1983; Colmeraver *et al.*, 1980; Sampson *et al.*, 1977). There are even cases of human or animal tumors being stimulated by the very lymphoid cells which may also cause their lysis. For example, immune CTL in small amounts have been shown to stimulate tumor cell population growth *in vitro* (Fidler, 1973; Jeejeebhoy, 1977), and *in vivo* after an injection of immune lymphocytes and tumor cells into lethally irradiated animals (Prehn, 1972, 1983; Umiel & Trainin, 1974). Accelerated

growth of an allogeneic tumor in an animal first immunized against this tumor has been observed, immune T lymphocytes being responsible for this effect (Penn, 1984). The mechanisms responsible for immunostimulation of tumor growth are not clear, but the very fact of their existence restrains the carrying out of immunotherapy.

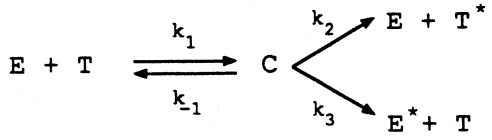
There are different explanations for the termination of a tumor dormant state, for sneaking through of tumors, and for immunostimulation effects. Often these explanations are based on the ideas of immunoselection, antigenic modulation, production by tumor cells of different types of immune cell blocking factors, generation of immunosuppressor cells, changes in autoregulatory networks in a tumor localization region, and other more complex ideas that are very difficult to prove or disprove experimentally. Here we propose that these phenomena may be the result of nonlinear dynamic interactions between the tumor the the immune system (also see Kuznetsov, 1984, 1987, 1988, 1991).

In this paper, we analyze a simple mathematical model of the cytotoxic T lymphocyte (CTL) response to a growing tumor cell population. This model differs from most others in the literature in that it takes into account the infiltration of the tumor by CTLs as well as the possible effects of effector cell inactivation. A variant of this model has been studied by Kuznetsov (1991). Here we focus on the qualitative behavior of the system using techniques from bifurcation theory. We apply the model to the analysis of the mechanisms of tumor dormancy and sneaking through. Interestingly, we find that a non-zero rate of effector cell inactivation is required to obtain sneaking through. We also find that sneaking through, tumor dormancy and the immunostimulation of tumor growth, effects which have been analyzed separately, according to our model, may all be related.

2 Mathematical Model

It has been found in numerous studies both *in vivo* and *in vitro* that the growth of a tumor cell population is exponential for small quantities of tumor cells but growth is slowed at large population sizes. The inhibition of growth may be caused by the competition of cells for metabolites and/or growth factors, or by growth inhibiting factors produced by the tumor cells. In many cases of nonexponential tumor growth, the kinetics is well described by the logistic or Gompertz equation (Emanuel, 1981; Swan 1977).

Consider a tumor whose cells are "immunogenic", and thus subject to immune attack by cytotoxic effector cells, *e.g.*, CTL or NK cells. The interaction between effector cells (EC) and tumor cells (TC) *in vitro* can be described by the kinetic scheme:



where E, T, C, E^*, T^* are the local concentrations of effector cells, tumor cells, effector cell–tumor cell conjugates, inactivated effector cells, and "lethally hit" TC cells, respectively. Lethally hit tumor cells are destined to perish. They also have been called cells "programmed to die". The inclusion of inactivated effector cells is an unusual feature of our model. NK cells, and to a lesser extent CTL, in culture seem to have a limited ability to repeatedly kill target cells (Abrahms and Brahmi, 1988; Callewaert *et al.*, 1988; Kuznetsov *et al.*, 1988). This might be due to exhaustion of molecules responsible for the cytotoxic effect or other regulatory effects, possibly due to the release of molecules from the tumor cell when the TC and EC are conjugated. The parameters k_1, k_{-1}, k_2 and k_3 are nonnegative kinetic constants: k_1 and k_{-1} describe the rates of binding of EC to TC and detachment of EC from TC *without* damaging cells; k_2 is the rate at which EC-TC interactions irreversibly program TC for lysis; and k_3 is the rate at which EC-TC interactions inactivate EC.

We propose the following system of differential equations as a model for the interactions between EC and a growing immunogenic tumor *in vivo*:

$$\frac{dE}{dt} = s + F(C,T) - d_1 E - k_1 ET + (k_{-1} + k_2)C \tag{1a}$$

$$\frac{dT}{dt} = aT(1 - bT_{tot}) - k_1 ET + (k_{-1} + k_3)C \tag{1b}$$

$$\frac{dC}{dt} = k_1 ET - (k_{-1} + k_2 + k_3)C \tag{1c}$$

$$\frac{dE^*}{dt} = k_3 C - d_2 E^* \tag{1d}$$

$$\frac{dT^*}{dt} = k_2 C - d_3 T^* \tag{1e}$$

where E, T, C are the number of unbound EC, unbound TC, and EC-TC complexes, respectively, located at the site of the tumor, say the spleen, and E^* and T^* represent the number of inactivated EC and lethally hit TC at the tumor site. The total population of unhit TC cells in the spleen is $T_{tot} = T + C$.

The parameter s is the "normal" (non-enhanced by TC presence) rate of flow of mature EC into the region of TC localization; and d_1, d_2 and d_3 are positive constants representing the rates of elimination of E, E^* and T^* cells, respectively, resulting from their destruction or migration from the TC localization area. We assume that the tumor

does not metastasize and thus that there is no migration of TC or EC-TC complexes. The maximal growth rate of the TC population is a. This parameter incorporates both multiplication and death of TC. The maximal carrying capacity of the biological environment for TC (*i.e.* the maximum number of cells due, for example, to competition for a resources such as oxygen, glucose *etc.*) is b^{-1}.

The function $F(C,T)$ characterizes the rate at which cytotoxic effector cells accumulate in the region of TC localization due to the presence of the tumor. Both EC multiplication due to stimulation by TC and enhanced EC migration into this region from surrounding tissues (*e.g.*, nearby lymph nodes) may contribute to the process of EC accumulation. The analysis of Kuznetsov (1979) suggests the following explicit form for this stimulated accumulation of effector cells:

$$F(C,T) = \frac{fC}{g+T} \tag{2}$$

where f and g are positive constants. Note that this function depends on C, the concentration of EC-TC conjugates but does not depend explicitly on E. This functional form is consistent with a model in which one assumes that the accumulation of effector cells is due to signals, such as released cytokines, generated by effector cells in conjugates. Further note that the rate of stimulated accumulation has some maximum value as T gets large (see below where we argue that $C \approx KET$). This is consistent with limitations in the rate of transport of effector cells to the tumor. The rate limitation could occur in the circulation, in the rate of exit from the circulation, or in the rate of movement through the tissue to the tumor.

Equations 1d and 1e are "slave" to Eqs. 1a-1c because the variables T^* and E^* have no effect on each other or the other variables in the system. In the remainder of this paper we analyze Eqs. 1a - 1c, which dictate the behavior of this system.

The formation and dissociation of cellular conjugates C occurs on a time scale of several tens of minutes to a few hours. A time interval of this order is also observed before the lysis of lethally hit tumor cells (Fishelson & Berke, 1981; Kuznetsov, 1981; Brondz, 1987). However, the multiplication as well as influx of effector cells into the spleen occurs on a much slower time scale, probably tens of hours. This motivates the application of a quasi–steady-state approximation to Eq. 1c (*i.e.*, $\frac{dC}{dt} \approx 0$) which yields the following relation:

$$C \approx KET \tag{3}$$

where $K = k_1/(k_2 + k_3 + k_{-1})$. Experimental observations (Brondz, 1987; Fishelson & Berke, 1981) indicate that EC-TC conjugates usually comprise a small portion of the total number of effector or tumor cells (up to 1-10%). This motivates the approximation

$T_{tot} \approx T$ which, along with Eqs. 2 and 3, simplifies Eqs. 1a and 1b to:

$$\frac{dE}{dt} = s + \frac{pET}{g+T} - mET - dE \tag{4a}$$

$$\frac{dT}{dt} = aT(1-bT) - nET \tag{4b}$$

where the parameters $p = fK$, $m = Kk_3$, $n = Kk_2$, and $d = d_1$.

3 Parameter Estimates

Experimental data that could serve as a basis for testing mathematical models of the immune response to tumors *in vivo* are scant. In order to examine if the model, given by Eqs. 4a and 4b, is adequate we have used the results of experiments on the dynamics of growth of a BCL_1 lymphoma in the spleen of chimeric mice (Siu *et al.*, 1986; Uhr *et al.*, 1991), as well as some additional information referred to in the present study.

A number of authors (Strober *et al.*, 1979; Krolick *et al.*, 1979; Weiss *et al.*, 1983; Siu *et al.*, 1986; Uhr *et al.*, 1991) have presented an accurate quantitative description of an experimental model for the interaction *in vivo* between cytotoxic EC and BCL_1 tumor cells as a function of time. BCL_1 was the first B cell lymphoma described in mice (Slavin and Strober, 1978). It arose spontaneously in an elderly BALB/c mouse. The clinical characteristics of BCL_1 resemble the prolymphocytic form of chronic lymphocytic leukemia in humans, and hence has been used as a model for that disease (Krolick *et al.*, 1979).

BCL_1 has a number of advantages as a model tumor system. First, the cells having been derived from B cells still carry surface immunoglobulin, which can be detected with anti-idiotype reagents. Second, the tumor grows primarily in the spleen and thus studies of tumor growth and tumor-host interactions can focus mainly on this organ. Tumor cells in the spleen can be quantitated by idiotypic analysis, and transfer of spleen cells from tumor bearing animals to syngeneic recipients leads to transfer of the tumor. In fact, transfer of a single viable BCL_1 cell causes progressive disease in about 50% of recipients (Krolick *et al.*, 1979).

In normal mice, tumor growth can be detected in the spleen at 3 weeks; growth plateaus by 6 weeks and by 3 months all mice succumb to the tumor (Siu *et al.*, 1986). However, what appears to be a protective immune response can be generated by appropriate experimental manipulation. BALB/c mice can be made chimeric by first having their bone marrow destroyed by lethal irradiation, and then having bone marrow derived

from mice of a different H-2 haplotype transferred in to restore immune system function. As shown in Fig. 1, BCL_1 tumor first grows and then regresses in chimeric mice, and by 12 weeks tumor cells are no longer detectable by anti-idiotypic antibody staining and fluorescence activated cell sorter (FACS) analysis. The initial growth kinetics of the tumor in chimeric animals is very similar to that found in the case of normal BALB/c mice. However, the data in Fig. 1 suggests that in chimeric mice the introduction of large doses of tumor cells invokes a gradually developing strong immune response that leads to the elimination of tumor cells.

The data shown in curve 1 in Fig. 1a describes the growth of the BCL_1 lymphoma in non-chimeric animals. We assume that this represents the normal growth of the tumor in the absence of an immune response. In this case the model reduces to:

$$\frac{dT}{dt} = aT(1 - bT) \tag{5}$$

This equation represents a logistic growth model. Values of $a = 0.18$ day^{-1} and $b = 2.0 \times 10^{-9}$ cells^{-1} give a predicted growth curve that closely approximates the data (see Fig. 1).

The other parameters of the model have been estimated as follows. First consider s, the normal rate of influx of effector cells into the region of tumor localization. The spleen of a BALB/c mouse contains approximately 10^8 splenocytes. The frequency of CTL precursors reacting to alloantigens forms several tenths of a percent of the total number of lymphocytes (Brondz, 1987). We thus assume that the number of CTL precursors reactive to the tumor, $E_p = 3.3 \times 10^5$ cells. The lifetime of T lymphocytes from the spleen and the blood is not known precisely but can be estimated to be approximately 30 days or more (Reynolds *et al.*, 1985; Gray & Leanderson, 1990). We assume that in the absence of tumor the initial number of CTL, $E(0) \approx E_p$. We further assume that a steady state is established, so that from Eq. (1a), $s \approx E_p d = 1.3 \times 10^4$ cells day^{-1}. The other parameters, p, g, m, n and d, have been estimated from the experimental data given in Fig. 1 using techniques described elsewhere (Kuznetsov, 1991; Kuznetsov *et al.*, 1992). With $a = 0.18$ day^{-1}, $b = 2.0 \times 10^{-9}$ cells^{-1}, and $s = 1.3 \times 10^4$ cells day^{-1}, we estimate:

$p = 0.1245$ day^{-1}, $g = 2.019 \times 10^7$ cells,
$m = 3.422 \times 10^{-10}$ day^{-1}cells^{-1}, $n = 1.101 \times 10^{-7}$ day^{-1}cells^{-1}
and $d = 0.0412$ day^{-1}.

Parameters are estimated to four decimal places because, as we show below, the initial condition for the experimental data given in Fig. 1c lies close to the separatrix (see Fig. 3) and very small changes in the parameters give noticeable differences in the predicted tumor growth curve.

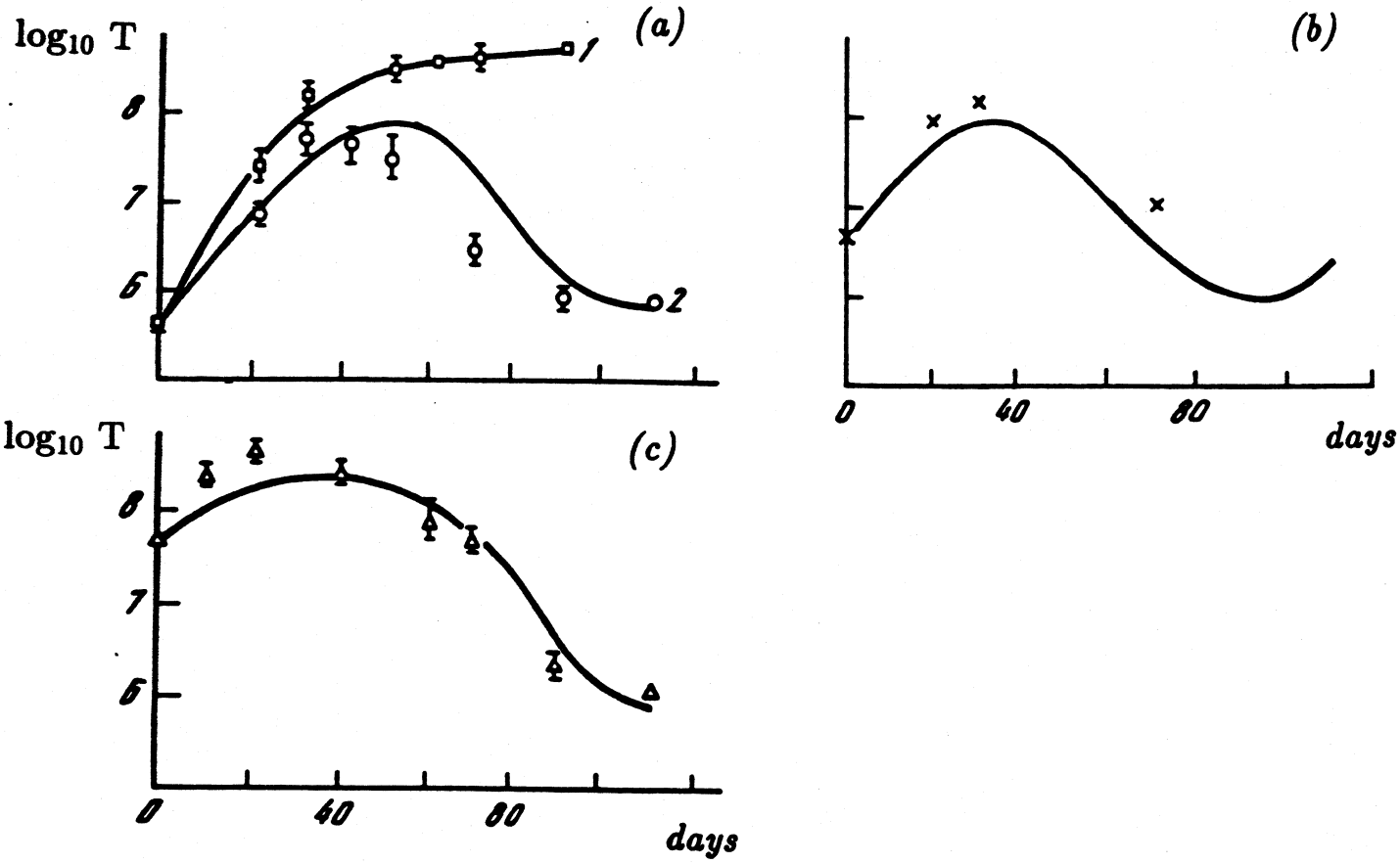


Figure 1: *Growth of BCL_1 tumor in the spleens of chimeric mice as determined by Siu et al., 1986). Control BALB/c mice received* 0.5×10^6 *viable BCL_1 cells (curve 1 in (a)). Chimeric mice received:* 5×10^5 *(curve 2 in (a));* 5×10^6 *(b); and* 5×10^7 *(c) viable BCL_1 cells. Experimental data [o,□,△,×] represents the mean of two experiments except in (b) where only one experiment was performed. Theoretical predictions [—] are for* 3.3×10^5 *initial effector cells at the parameter values presented in the text.*

The theoretical curves predicted by Eqs. 4a and 4b with these parameters values approximate the experimental values (see Fig. 1). Because all three experimental curves are fit with one set of parameter values no single experiment is fit optimally. Also, it is interesting to note that the predicted regrowth in the tumor population seen in Fig. 1*b* at 100 days has been observed in recent experiments in which 10^6 BCL_1 cells were injected into BALB/c mice (Uhr *et al.*, 1991).

4 Nondimensionalization

We non-dimensionalize Eqs. 4a and 4b by choosing an order-of-magnitude concentration scale for the E and T cell populations, E_o and T_o, respectively. As suggested from the experiments discussed above: $E_o = T_o = 10^6$ cells. Time is scaled relative to the rate of tumor cell deactivation; *i.e.*, $\tau = nT_o t$. Then the model can be re-expressed as:

$$\frac{dx}{d\tau} = \sigma + \frac{\rho xy}{\eta + y} - \mu xy - \delta x \tag{6a}$$

$$\frac{dy}{d\tau} = \alpha y(1 - \beta y) - xy \tag{6b}$$

where

$$x = \tfrac{E}{E_o}, \quad y = \tfrac{T}{T_o}, \quad \sigma = \tfrac{s}{nE_oT_o}, \quad \rho = \tfrac{p}{nT_o},$$

$$\eta = \tfrac{g}{T_o}, \quad \mu = \tfrac{m}{n} = \tfrac{k_3}{k_2}, \quad \delta = \tfrac{d}{nT_o}, \quad \alpha = \tfrac{a}{nT_o}$$

$$\text{and} \quad \beta = bT_o.$$

Values for these seven non-dimensional parameters are obtained from the dimensional parameter values estimated in Section 3 to be approximately:

$$\sigma = 0.1181, \quad \rho = 1.131, \quad \eta = 20.19, \quad \mu = 0.00311,$$
$$\delta = 0.3743, \quad \alpha = 1.636 \quad \text{and} \quad \beta = 2.0 \times 10^{-3}.$$

5 Steady States

Let us consider the steady states of the reduced model described by Eqs. 6a and 6b. Solutions of practical interest will have non-negative populations x and y. We assume

that the parameters are also non-negative. Information regarding both the vector field and the steady states of Eqs. 6a and 6b can be obtained by examining the nullclines; *i.e.*, the curves along which $dx/dt = 0$ and $dy/dt = 0$. There are steady states for the system at the intersections of these nullclines. There is a single nullcline for Eq. 6a,

$$x = \frac{\sigma}{\delta + \mu y - \frac{\rho y}{\eta + y}} \equiv f(y) \tag{7}$$

There are two nullclines for Eq. 6b. One is $y = 0$. The other can be expressed as

$$x = \alpha(1 - \beta y) \equiv g(y)$$

This is simply a straight line with a negative slope. A steady state with coordinates $(x, y) = (\sigma/\delta, 0)$ is given by the intersection of $f(y)$ and $y = 0$. The stability of this steady state depends upon the relative values of the parameters for the system. Depending upon the relation of $f(y)$ and $g(y)$ there may be from zero to three *additional* steady states for the system (see Fig. 2). Setting $f(y)$ equal to $g(y)$ yields a third-order polynomial for the y values of these steady states:

$$C_3 y^3 + C_2 y^2 + C_1 y + C_0 = 0 \tag{8}$$

where

$$C_0 = \eta\left(\frac{\sigma}{\alpha} - \delta\right), \qquad C_1 = \frac{\sigma}{\alpha} + \rho - \mu\eta - \delta + \delta\eta\beta,$$
$$C_2 = -\mu + (\mu\eta + \delta - \rho)\beta \quad \text{and} \quad C_3 = \mu\beta.$$

In order for Eq. 8 to have three real roots, it follows from Descartes' rule of signs that there must be three sign changes among the coefficients. Sturm's method (*cf.* Beaumont & Pierce, 1963) provides more precise conditions for the number of real, distinct roots of Eq. 8. Table 1 presents a summary of the number of steady states as determined by the signs of the coefficients of Eq. 8 and the signs of the key quantities appearing in the Sturm sequence of Eq. 8.

6 Phase Space

For the parameter values estimated in Section 4, there are four steady states predicted by the model. The phase portrait is shown in Fig. 3. The steady states labeled B and D are both stable. Steady state B is characterized by a relatively low TC level and we refer to it as the "dormant tumor" steady state. On the other hand, steady state D, characterized by a relatively high tumor and low effector cell level, corresponds to relatively "uncontrolled" tumor growth or "tumor escape". The one-dimensional stable

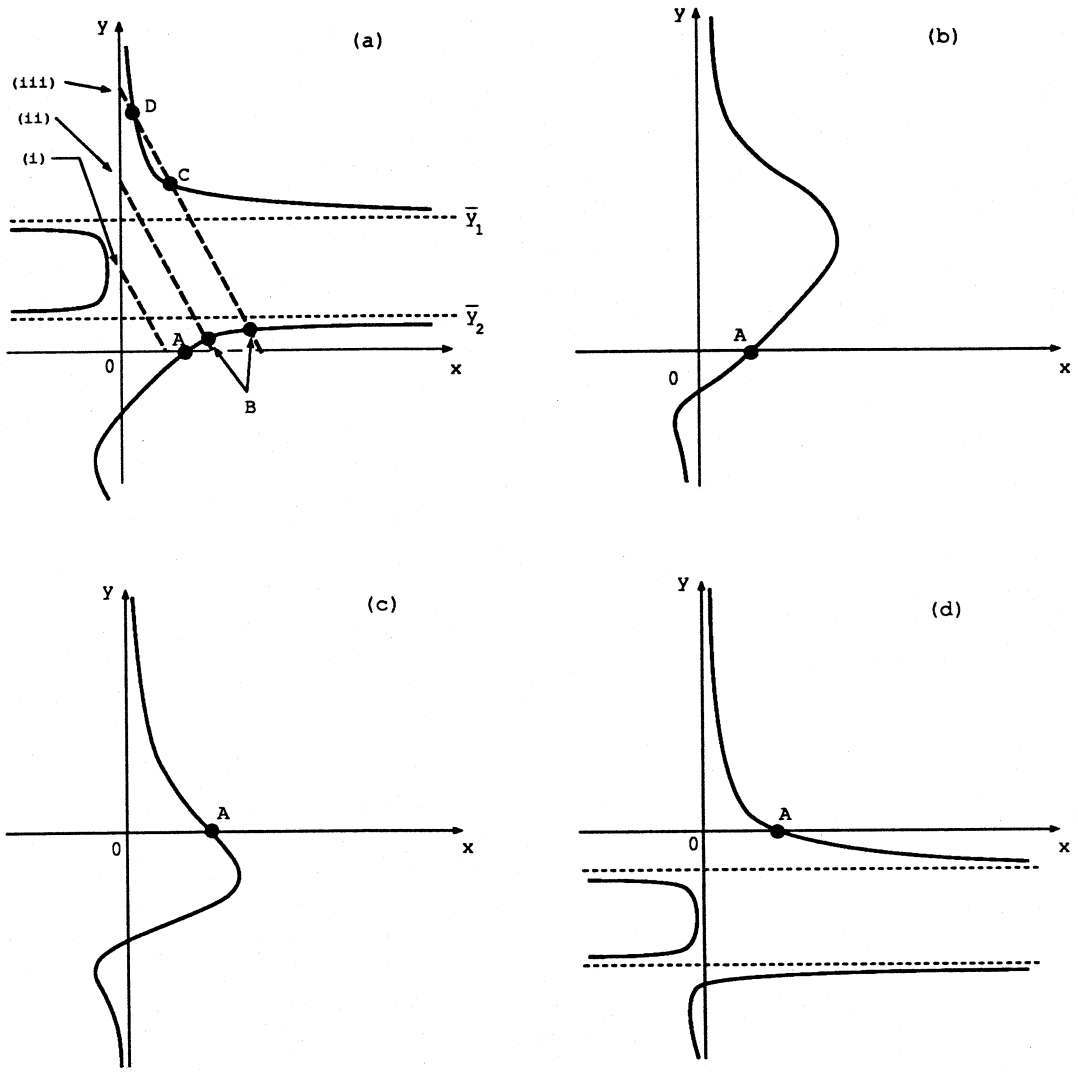


Figure 2: *Four qualitatively different forms for $f(y)$ are depicted. These cases are for parameter values such that: (a) $\rho \geq \left(\sqrt{\eta\mu} + \sqrt{\delta}\right)^2$; (b) $\left(\sqrt{\eta\mu} - \sqrt{\delta}\right)^2 < \rho < \left(\sqrt{\eta\mu} + \sqrt{\delta}\right)^2$ and $\rho > \eta\mu$; (c) $\left(\sqrt{\eta\mu} - \sqrt{\delta}\right)^2 < \rho < \left(\sqrt{\eta\mu} + \sqrt{\delta}\right)^2$ and $\rho < \eta\mu$; and (d) $\rho \leq \left(\sqrt{\eta\mu} - \sqrt{\delta}\right)^2$, respectively. The horizontal asymptotes $\bar{y}_1$ and $\bar{y}_2$ for $f(y)$ in (a) and (d) are given by:*

$$\bar{y}_{1,2} = \frac{\rho - \eta\mu - \delta \pm \sqrt{(\rho - \eta\mu - \delta)^2 - 4\eta\mu\delta}}{2\mu}$$

Three possible orientations for $g(y)$ in relation to $f(y)$ are labeled (i), (ii) and (iii) in (a) which lead to zero, one and three additional positive steady states, respectively. Labeling: $f(y)$ [—]; $g(y)$ [- - -]; horizontal asymptotes for $f(y)$ [- - -]; and steady states [•].

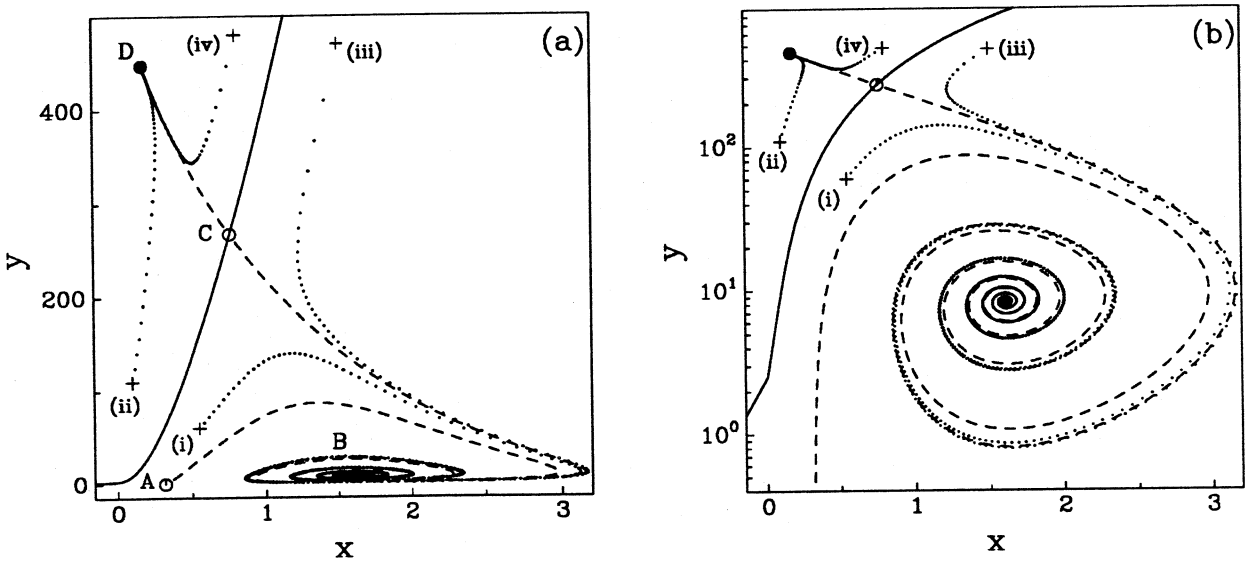


Figure 3: *Phase portrait for the model at the dimensionless parameter values presented in Section 4. Portrait (a) is redrawn with a logarithmic ordinate in portrait (b). Labeling: stable steady states [•], saddle steady states [○], one-dimensional stable manifold of steady state C [—], one-dimensional unstable manifolds of steady states A and C [– – –], initial conditions for transients [+], and the evolution of transients in time increments of 1 day [· · ·].*

SS	C_0	C_1	C_2	S	T	R
1	+	+	+			
			−	+	+	+
					−	
		−		−		
2	−	+	−	+	+	+
					−	
			+			
		−				
3	+	+	−	−	+	+
		−				
4	−	+	−	−	+	+

Table 1: *The number of positive steady states (SS) as determined by the signs of the coefficients of Eq. 8 and the signs of the quantities* $S = C_2C_1 - 9C_3C_0$, $T = \frac{2C_2S}{R} - \frac{3C_3S^2}{R^2} - C_1$ *and* $R = 2C_3^2 - 6C_3C_1$ *from the Sturm sequence. Blank entries correspond to coefficients which may take positive, negative or zero values.*

manifold of steady state C partitions the basins of attraction for each of these attractors. Initial conditions beginning below and the right of this separatrix (*e.g.*, initial conditions (i) and (iii) in Fig. 3) asymptotically approach the dormant tumor steady state B. For initial conditions above the separatrix (*e.g.*, initial conditions (ii) and (iv) in Fig. 3), the tumor escapes immune regulation. Thus the model is capable of explaining both tumor dormancy and escape from immunoregulation. In the next sections we delineate the parameter regimes in which these behaviors, as well as "sneaking through" can be expected.

7 Bifurcation Analysis

Naturally, the parameter values studied above are only estimates. In this section we explore critical parameter values where the qualitative behavior predicted by Eqs. 6a and 6b changes.

The Dulac-Bendixson criterion (*cf.*, Wiggins, 1990) can be used to show that there are no closed orbits for the system of Eqs. 4a and 4b for positive values of E and T. To

illustrate, consider the function $M = 1/xy$ and calculate

$$\begin{aligned} L &\equiv \frac{\partial}{\partial x}(M\frac{dx}{dt}) + \frac{\partial}{\partial y}(M\frac{dy}{dt}) \\ &= -\left(\frac{\sigma}{x^2 y} + \frac{\alpha\beta}{x}\right). \end{aligned}$$

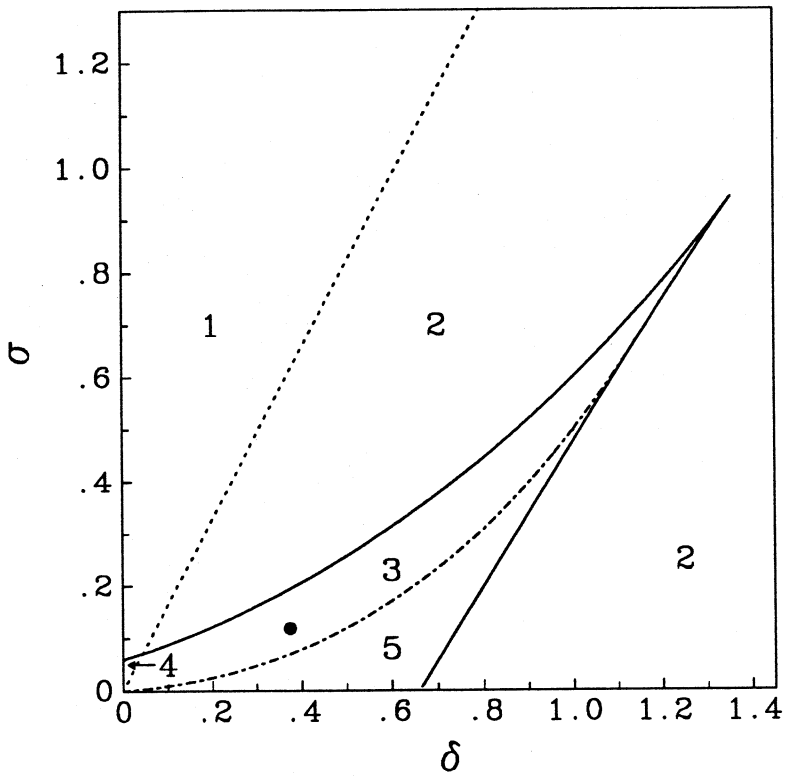
Since the parameters are positive, $L < 0$ over the domain of interest and the Dulac-Bendixson criterion is satisfied. It follows then that there are no limit cycles or homoclinic connections observed for the system. Similarly, no Hopf bifurcations giving rise to limit cycles occur.

To better understand the behavior predicted by Eqs. 6a and 6b, we have mapped out qualitatively different regions of behavior as a function of the parameters δ and σ at the fixed values of the other parameters. These results are presented in Fig. 4*a*. Curves in this diagram represent codimension-one bifurcations which partition parameter regions of qualitatively different dynamic behavior. Representative phase portraits for regions 1 through 5 are depicted in Figs. 5*a* - 5*e*, respectively. The transcritical bifurcation curve and the saddle-node bifurcation curve involve different pairs of steady states. They appear to intersect in Fig. 4 only because the solution $\times$ parameter ($\mathbb{R}^2 \times \mathbb{R}^2$) space is being projected onto a plane.

The loci of saddle-node and transcritical bifurcations presented in Fig. 4 are termed "local" bifurcation curves because they are characterized by a qualitative change in the linearized stability evaluated at the steady state. A qualitatively different codimension-one bifurcation which is not observable from the local linearization forms the transition between regions 3 and 5. This "global" bifurcation is a *heteroclinic connection* where one side (the lower-left portion) of the one-dimensional stable manifold of steady state C and the non-negative portion of the one-dimensional unstable manifold of steady state A coincide. The effect of this bifurcation is to radically change the territory claimed by competing attractors and is illustrated schematically in Fig. 6. This bifurcation has biological significance (see below) and has been previously called a "vital barrier" (Kuznetsov, 1983, 1988).

Parameter estimates place our system in region 3 (marked • in Fig. 4). In this region, all trajectories beginning with high numbers of effector cells, x, and low numbers of tumor cells, y, (corresponding to the unshaded region in Fig. 6*b*) asymptote to the dormant tumor state.

Our parameter estimates place the system close to the "vital barrier". Thus, with a slight change in parameters the system can be in region 5. Interestingly, many experimentally observed phenomena are characteristic of the behavior predicted in region 5. In region 5 transients beginning with high effector cell levels, *i.e.* high x levels (*e.g.*,



Transition	Brief Description
1 - 2, 4 - 3	Transcritical bifurcation involving steady states A and B
1 - 4, 2 - 3	Saddle-node bifurcation involving steady states C and D
2 - 5	Saddle-node bifurcation involving steady states C and B
3 - 5	Heteroclinic connection involving steady states A and C. Associated with a significant change in the basins of attraction for the attractors.

Figure 4: *The two-dimensional transition structure (including both local and global bifurcations) as a function of δ and σ at the fixed values of the other dimensionless parameters presented in Section 4. The two-parameter diagram contains five distinct regions, labeled* 1-5. *The table summarizes the transitions between each of these regions. Fig. 5 shows a representative phase portrait for each of these regions. Labeling of bifurcation curves: saddle-node [—]; transcritical bifurcation [- - -]; and heteroclinic connection [– – – – – -]. The estimated parameter values for our model: [•].*

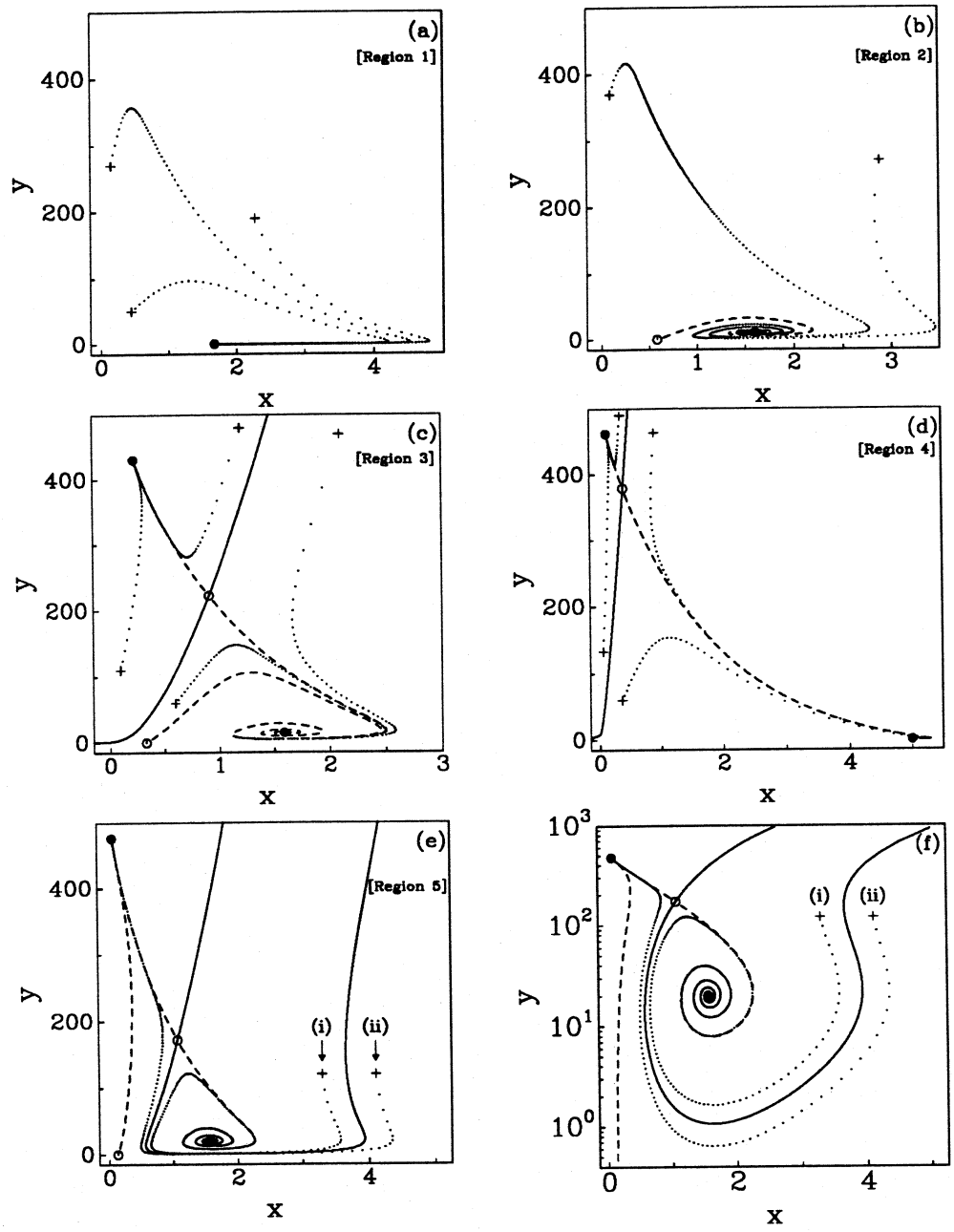


Figure 5: *Representative phase portraits for each of the regions in Fig. 4. Portraits (a) through (e) (which represent regions 1 through 5, respectively) are at (δ, σ) values of (0.1908,0.318), (0.545,0.318), (0.545,0.182), (0.009,0.045) and (0.545,0.073), respectively. Portrait (e) is redrawn with a logarithmic ordinate in portrait (f). Labeling: stable steady states [•], steady states with saddle stability [o], one-dimensional stable manifolds of saddles [—], one-dimensional unstable manifolds of saddles [– – –], initial conditions for transients [+], and the evolution of transients in time increments of 1 day [· · ·].*

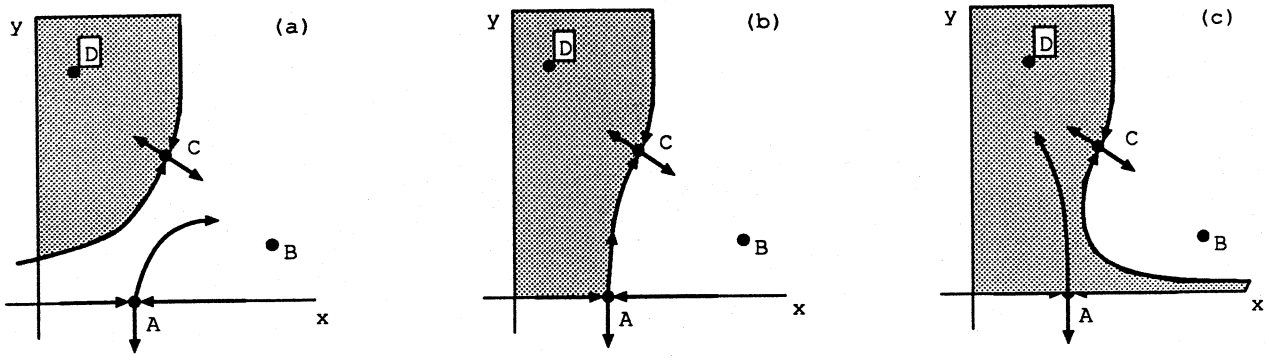


Figure 6: *Representative phase portraits: (a) before, (b) during, and (c) after; the heteroclinic connection between steady states A and C. Portions of the basins of attraction for attractors B and D are the white and shaded regions, respectively.*

initial condition (ii) in portrait 5*e*) quickly approach an apparent dormancy where the tumor presence is reduced but not eliminated. However, the tumor level persists over time as the effector cell level gradually drops. Eventually the tumor escapes and the system approaches steady state D. This is an illustration of the "sneaking through" phenomenon. An external stimulation to an immune system in region 5, which may seem intuitively to aid the immune response (immunostimulation, *e.g.*, perturbing from initial condition (i) to (ii) in Fig. 5*e*) can actually be detrimental. In addition, sufficiently small tumors (below the separatrix in portrait 5*e*) are predicted to eventually escape immune regulation.

8 Discussion

Given the limited data of Fig. 1, the numerical estimates of the model parameters for the response to BCL_1 cells should only be regarded as preliminary. However, we have some confidence in our estimates of the parameters α and β characterizing tumor growth in the absence of an immune response, since the logistic model is biologically reasonable and the two parameters that need to be estimated from the data determine the slope and asymptote of the tumor growth curve. As shown in Fig. 1, the logistic curve with our parameter estimates fits the data well over a wide range of initial tumor cell concentrations. If independent measurements of other parameters were available, they could help guide interpretation of the processes of interaction between the host immune system and a growing tumor. However, most of the relevant parameters have not been

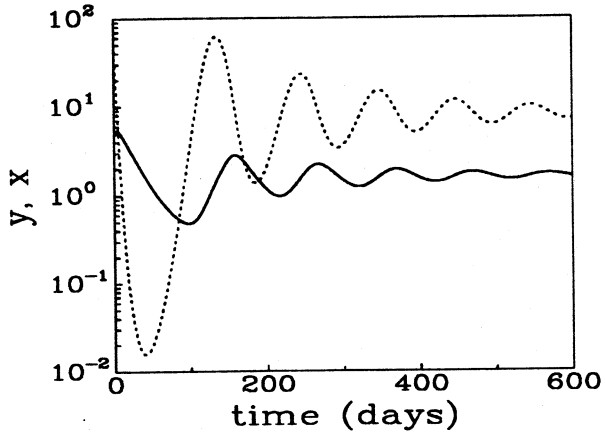


Figure 7: *The time course of BCL_1 leukemia y [—] and effector x cell levels [- - -] in chimeric mice, illustrating a decaying oscillation to the dormant tumor state. The dimensionless initial number of leukemia cells is $y = 50$ and the dimensionless initial number of effector cells is $x = 5$. Dimensionless parameter values are those presented in Section 4.*

measured *in vivo* and thus we have used bifurcation theory to study the behavior of the model as parameters are varied.

Using our parameter estimates, we can make a number of biologically interesting predictions about the interactions of the immune system with a growth tumor. In Fig. 3 a phase portrait of the system is presented at the parameter values estimated in Section 4 . Transients in the vicinity of steady state B exhibit decaying oscillations. A time series for an initial condition in this neighborhood is presented in Fig. 7. The result is of interest from the standpoint of predicting the behavior of the tumor over greater time-scales than those measured in Siu *et al.* (1986). Cyclic fluctuations in the number of leukocytes has been found in a number of cases in the development of chronic human myeloleukemia (Menta and Agarwal, 1980) and chronic bovine lymphoid leukemia (Kukain *et al.*, 1982). This pattern of spontaneous relapse and remission is reminiscent of observations in non-Hodgkin's lymphoma (Krikorian *et al.*, 1980). It is also interesting that the predicted time scale of oscillations in the model, 3 or 4 months, is in rough agreement with the time for recurrent clinical manifestations of certain human leukemias. In addition, recurrent patterns of tumor remission and regrowth have been seen in the BCL_1 system for mice challenged with 10^6 BCL_1 cells (Uhr *et al.*, 1991).

Another prediction of the model follows from Fig. 3. In a mouse with a typical effector cell concentration of 0.5×10^6 ($x = 0.5$), when more than approximately 1.5×10^8 tumor

cells ($y = 150$) are injected into the animal, our model predicts that the resultant tumor growth should not be controlled by the immune system. However, if fewer tumor cells are injected, then tumor growth is predicted to be controlled. This prediction of an "immunological barrier" value can be easily checked experimentally.

In reports by Strober *et al.* (1979) and Weiss *et al.* (1983) data are presented that suggest that immunological mechanisms induce the formation of a dormant state of BCL_1 tumor in chimeric mice. In this state potentially lethal tumor cells persist in the animal with little or no increase in their population. Our model predicts the existence of a dormant state (steady state B in Fig. 3).

In certain animals it is not possible to detect leukemia cells several months after the administration of BCL_1 cells, even if 10^6 splenocytes from these regressor mice are transplanted into non-chimeric BALB/c mice (Weiss *et al.*, 1983). In this regard it is of interest to note that total regression may be obtained with the model given by Eqs. (6a-6b) only when there is a sufficiently pronounced change in the parameters from our estimated values, for example, a six-fold increase in the σ/δ ratio. In this case the rate of growth of the tumor cell population will be less than their death rate ($\alpha < \sigma/\delta$) and solutions emerge in which the tumor cell population y approaches zero.

As a body ages the probability of transformed cells arising is thought to increase and the reliability of immune surveillance mechanisms probably decreases. Within the framework of our mathematical model such changes may be interpreted as: (1) an increase in the initial number of tumor cells at the time the immune system encounters the tumor, and a reduction of the threshold for its macroscopic growth, or (2) the reduction of the area in parameter space which corresponds to the total elimination of a primary tumor (*i.e.* where solutions y tend to 0) and the growth of areas in which either the system is in dynamic balance or the tumor is not controlled by the system. In either of these variants of the system even single tumor cell may develop into a small "dormant" tumor. The number of such tumors is thus expected to increase with the organism's age until some stochastic event causes a change in the tumor growth rate, access to lymphocytes, or some other parameter changes such that a bifurcation border is crossed and the uncontrolled tumor growth begins.

It follows from our bifurcation analysis that at certain parameter values solutions are possible that can be interpreted as tumor sneaking through and immunostimulatory effects. Sneaking through refers to a phenomena in which low doses of tumor cells can escape immune defenses and grow into a large tumor, whereas larger doses of tumor cells are eliminated. Numerical continuation has shown that the parameter values we have estimated for the system lie close to what has been called a "vital barrier" transition (Kuznetsov, 1983, 1988). On one side of the barrier sneaking through is possible, whereas on the other side it is not. The possible behaviors of our model are very sensitive to the

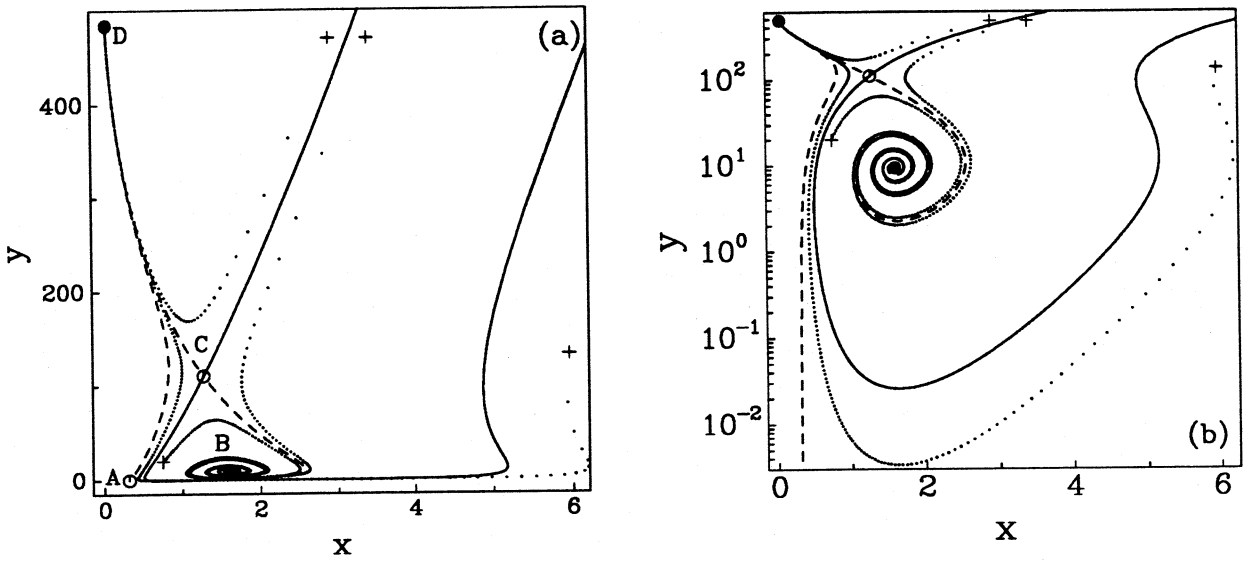


Figure 8: *A typical phase portrait for μ slightly greater than* 0.005014. *For this portrait $\mu = 0.0055$ and the other dimensionless parameter values are those presented in Section 4. Portrait (a) is redrawn with a logarithmic ordinate in portrait (b). Labeling: stable steady states [•], saddle steady states [○], one-dimensional stable manifold of steady state C [—], one-dimensional unstable manifolds of steady states A and C [– – –], initial conditions for transients [+], and the evolution of transients in time increments of 1 day [· · ·].*

parameter $\mu = k_3/k_2$. For example, as μ increases the one-dimensional manifolds of steady states C and A cross in a heteroclinic connection at $\mu = 0.005014$. After the heteroclinic connection, the phase portrait of the system (*e.g.*, for $\mu = 0.0055$ in Fig. 8) is qualitatively similar to portrait 5*e* and exhibits the phenomena of sneaking through and immunostimulation leading to tumor escape. On the other hand, the possibility of tumor escape is lost as μ decreases since steady states C and D collide in a saddle-node bifurcation at $\mu = 0.002633$. After the saddle-node, the phase portrait of the system is qualitatively similar to portrait 5*b* (*e.g.*, for $\mu = 0.0021$ in Fig. 9).

It is clear that small fluctuations of parameter values take place *in vivo*. Neither the effector cell or tumor cell populations are homogeneous. Differing subpopulations will have different parameter values characterizing their behavior. Because of the sensitivity of the model's behavior to parameter values, we predict the ultimate instability of the dormant state for BCL_1. Moreover, we predict that the times of clinical manifestation of tumor growth should be stochastic. From our numerical experiments, shown in Fig. 6,

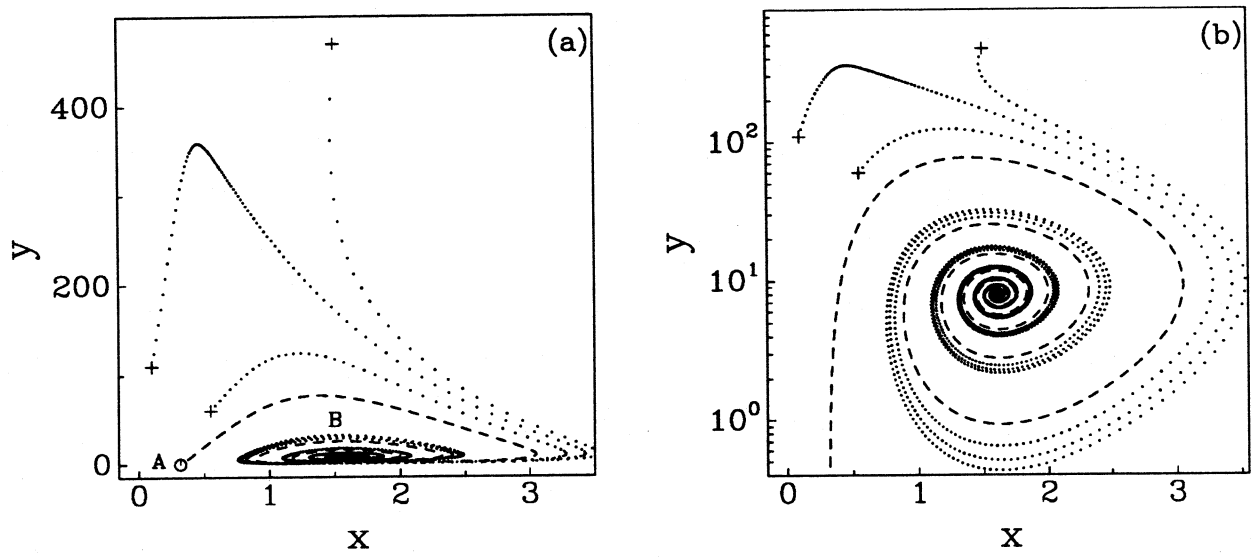


Figure 9: *A typical phase portrait for μ slightly less than* 0.002633. *For this portrait $\mu = 0.0021$ and the other dimensionless parameter values are those presented in Section 4. Labeling: stable steady states [•], saddle steady states [○], one-dimensional unstable manifold of steady state A [– – –], initial conditions for transients [+], and the evolution of transients in time increments of 1 day [· · ·].*

we see that small changes of initial conditions in the sneaking through region lead to very large differences in the time period needed for the apparence of large tumors. These results correspond to observations on the emergence from the dormant state for BCL_1 lymphoma (Krolick *et al.*, 1979; Strober *et al.*, 1979; Siu *et al.*, 1986; Uhr *et al.*, 1991) as well as for other experimental models of the tumor dormant state (Weinhold *et al..*, 1979a,b; Uyttenhove *et al.*, 1983; Hiernaux *et al.*, 1986).

The above observations make it clear that μ is a critical parameter in the model. Our estimate, $\mu = 0.003$, is sufficiently small to make one wonder if in the biological system μ is not actually zero. Recall that $\mu = k_3/k_2$, and hence μ small or zero implies that $k_3 \simeq 0$, where k_3 is the rate at which effector cells are inactivated due to interaction with the tumor. Thus with small μ, our model predicts that BCL_1 tumors do not efficiently inactivate effector cells. The argument given above, however, shows that the phenomena of sneaking through and immunostimulation of tumor growth depend on μ being above 0.002633. Thus, according to our model, a rather small rate of effector cell inactivation is needed to generate the phenomena seen *in vivo*. Our model thus suggests that it would be worthwhile to accurately monitor the ability of different tumors to inactivate effector cells and correlate that ability with observation of sneaking through and immunostimulation.

The mathematical model presented here shows that the phenomena of sneaking through, immunostimulation and the presence of dormant tumors may be related effects. Although we have used the paradigm of CTL as the effector cells in our presentation, other effector cells, such as NK cells or the effector cells in antibody dependent cellular cytotoxicity reactions, may be involved in immune surveillance against tumors. Thus the detailed mechanisms leading to the phenomena of sneaking through, immunostimulation and dormant tumors may be rather diverse. Thus, it is still necessary to determine the prevailing effector mechanism(s) leading to the stabilization and regression of a tumor growth *in vivo* and to obtain detailed experimental data regarding the kinetics of the relevant immune processes. Nevertheless, we feel that the model that we have presented is generic and relies on properties that any effector cell population should exhibit. Thus, the transitions in the behavior of the system as parameters are varied should have a universal character, that we expect will be seen in a variety of biological situations. It is for this reason that bifurcation diagrams, such as the one given in Fig. 4, may be quite helpful in interpreting experimental data.

9 Conclusions

A quantitative model has been proposed for the interaction between CTL and cells in a growing tumor. The model adequately describes the kinetics of growth and regression

of a BCL_1 lymphoma in the spleen of chimeric mice over a wide initial tumor cell concentration range and indirectly indicates that cytotoxic effector cells are responsible for the antitumor reactivity seen in this experimental model. Local and global bifurcations for realistic values of the parameters were calculated, and show that there may be a connection between the phenomena of immunostimulation of tumor growth, "sneaking through" of tumor, and formation of the tumor "dormant" state.

According to our model, the limited growth of even the high initial BCL_1 tumor cell concentrations in the chimeric animals is associated with a high rate of accumulation of specific, highly-active, cytotoxic lymphocytes in the spleen and with the absence of suppression of the cytotoxic activity of these cells by the tumor. A threshold number of tumor cells, equivalent to 8×10^7 cells for the analyzed experimental model, is predicted above which immunologically uncontrollable tumor growth should be found and below which attenuation of the disease with periodic exacerbations and clinical manifestation every 3-4 months occur.

One can speculate that CTL, which in our model do not totally eliminate even highly immunogenic tumors, could promote the accumulation of a multitude of dormant tumors in body tissues with increasing age, and hence in part explain the increasing frequency with which tumors become visible as an animal ages.

Acknowledgements: One of the authors (V.K.) acknowledges the assistance of Yu. I. Skal'ko with some of the calculations, and wishes to thank the Santa Fe Institute for their hospitality. We also thank Dr. Steven Skates for valuable comments about a preliminary draft of the manuscript. This work was performed under the auspices of the U.S. Department of Energy. It was supported in part by NIH Grant AI28433, and by the Santa Fe Institute through their Theoretical Immunology Program.

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