ANTITUMOR IMMUNITY AND THE ROLE OF IMMUNE-MEDIATED ACTIVATION OF CYTOTOXIC T LYMPHOCYTES: A MATHEMATICAL MODEL

by

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Abstract

The tumor immunosurveillance theory suggests that various components of the immune system constantly survey the body for nascent tumors and respond by eliminating most of them and possibly slowing the growth of others. Four types of immune cells – CTLs, macrophages, NK and helper T cells – play the most significant roles in developing antitumor immunity. Each of these immune cell types directly recognizes and kills cancer cells. In addition, evidence suggests that macrophages, NK and helper T cells stimulate CTLs, thereby eliciting a more effective immune response.

This study develops and explores a mathematical model that addresses the immune system’s role in combating cancer. In particular, the model focuses on the extent to which stimulatory mechanisms used by macrophages, NK and helper T cells to induce CTL activity impacts tumor growth dynamics. The model incorporates the hypothesis that the ability of immune cells to infiltrate a tumor bed relies on the presence of a vascular system. I have chosen to extend a model by Nagy (2004) describing a homogeneous vascularized tumor to include a simplification of the mechanisms used by immune cells to attack cancer. The model was analyzed numerically. Results support proposed theories that maximal antitumor immunity depends on each of the four immune cell types working together as well as immune-mediated CTL stimulation. In addition, the model predicts conditions under which several dynamical behaviors, including exponential tumor growth or decay, apparent chaos and oscillatory behavior, arise. These results may provide testable predictions of tumor growth dynamics occurring at sizes that are usually not clinically detectable.
1. Introduction

1.1 The Tumor Immunosurveillance Theory

The once-disputed tumor immunosurveillance theory has recently begun to reemerge in the fields of cancer biology and immunology as a likely hypothesis for explaining the phenomenon by which the immune system is able to elicit an effective response against cancer. This theory suggests that various components of the immune system constantly survey the body for nascent tumors and respond by eliminating most of them and possibly slowing the growth of others (Diefenbach and Raulet 2002). As chronicled by Dupont (2002), Thomas (1959) and Burnett (1970) pioneered the theory of tumor immunosurveillance, claiming that lymphocytes were capable of providing protection from tumor development. Later, Stutman (1979) performed a comparative study between mice with normal T cell production and mutant mice lacking a thymus and therefore deficient in normal T cell production. His study contested the tumor immunosurveillance theory because no differences in primary tumor development were observed between the two groups of mice. However, in 1987 Maleckar and Sherman found that Stutman’s mice did not completely lack T cell function, thus casting doubt on Stutman’s study, which had originally been interpreted to refute the immunosurveillance theory (Dupont 2002).

Most recent experimental evidence has renewed interest in the tumor immunosurveillance theory. For example, significantly higher incidences of cancer are found in humans with congenital or acquired immunological deficiencies compared to healthy individuals (unless otherwise noted, these conclusions and those that follow are reviewed in Diefenbach and Raulet 2002). In addition, experiments on mice have shown
that depletion of natural killer cells (NK cells) drastically reduces resistance to transplanted tumor cell lines. Furthermore, substantially increased incidences of spontaneous tumors have been demonstrated in aged mice deficient in both T and B cells. Increased rates of tumor formation have also been observed in studies using gene-knockout mice lacking perforin, the pore forming granule responsible for the cytotoxic effects of NK cells and cytotoxic T lymphocytes (CTLs), as well as mice deficient of interferon-\(\gamma\) (IFN-\(\gamma\)), which is produced by NK cells and up-regulates MHC molecules and other proteins involved in antigen processing. Finally, researchers have identified highly specific antigenic peptides expressed by tumors and recognized by CTLs (Bruggen et al. 2002, Romeo et al. 2002, Rivoltini et al. 2002). All of these findings have contributed to the growing body of evidence in support of the tumor immunosurveillance theory.

1.2 Specific Roles of Immune Cells in Eliciting Antitumor Immunity

A vast amount of research on the complexities of antitumor immunity has been performed to date. Each particular type of immune cell known to participate in antitumor immunity recognizes and responds to cancer by its own specific mechanisms. Some immune cells, in addition to directly killing cancer, stimulate other components of the immune system in such a way that a more effective immune response is produced (Pardoll 1998, Diefenbach and Raulet 2002, Bruggen et al. 2002). With the exception of some other minor contributors, four types of immune cells – NK cells, helper T cells, CTLs and macrophages – are responsible for a significant majority of the antitumor response. Antitumor immunity results from both direct cytotoxic interactions between immune and cancer cells as well as interactions among immune cells, which stimulate a
more effective immune response as explained below. In what follows I will refer to CTLs, helper T cells and NK cells as “effector lymphocytes.”

Historically, studies have focused on the antitumor response of CTLs because most tumors express class I Major Histocompatibility Complex (MHC) antigens and not MHC class II antigens. This characteristic is significant because CTLs recognize peptide antigens presented by tumors on MHC class I molecules. If certain antigens are presented to CTLs on MHC I, then CTLs will directly kill the antigen-presenting cell (Pardoll 1998). Because this was discovered relatively early most previous studies of cancer immunity have focused on CTLs.

Currently three mechanisms that effector lymphocytes use to kill target cells are known. The first mechanism requires CTLs to form an immunological synapse with the target cell and then secrete cytotoxic cytokines such as interferon-γ (IFN-γ) or Tumor Necrosis Factor-α (TNF-α) (Shresta et al. 1998). The other two mechanisms are referred to as calcium-dependent or calcium-independent cytotoxicity. In the calcium dependent pathway cytotoxic granules, in particular perforin, are secreted onto the surface of target cells causing channel-like structures to form in their membranes (Shresta et al. 1998). The calcium-independent pathway, however, relies on interactions between the Fas ligand on effector lymphocytes and the Fas receptor, a member of the TNF family of receptors, on target cells (Shresta et al. 1998). The Fas ligand is synthesized within several hours after T cell receptor stimulation. Once present on the T cell surface, the Fas ligand is able to interact with Fas receptors on target cells. This interaction activates a series of downstream caspases culminating with the release of nuclease, which initiates DNA fragmentation and ultimately causes cell death (Shresta et al. 1998). This
phenomenon is better known as apoptosis or programmed cell death, in which a suicide program is activated within the target cell leading to DNA fragmentation, shrinkage of the cytoplasm, membrane changes and cell death without lysis or damage to neighboring cells (Alberts et al. 2002). Recent studies indicate that different types of effector lymphocytes operate via their own specific cytotoxic pathway. For example, Shresta et al. (1998) showed that CTLs and NK cells primarily use the perforin/granzyme system to kill target cells, whereas helper T cells rely on the Fas system.

Helper T cells, although capable of directly initiating cell death via the Fas ligand/receptor system, are restricted because they only respond to MHC class II molecules. As previously mentioned tumor cells tend not to express MHC class II. These facts may indicate that helper T cells play a limited role in the direct killing of tumor cells. However, helper T cells have been shown to be an essential component in developing maximal antitumor immunity. Helper T cells actively regulate many antigen-specific and antitumor immune responses (Pardoll 1998). Studies have suggested several mechanisms by which helper T cells help orchestrate antitumor immunity. They stimulate CTLs, via the secretion of lymphokines, to proliferate and differentiate into active effector cells. In addition, interactions between the ligand CD40L on helper T cells and the CD40 receptor on cancer cells have been shown to play a critical role in the priming of CTLs (Pardoll 1998). Also, a growing body of evidence suggests that helper T cells can stimulate other immune cells besides CTLs. Helper T cells mediate the activation of macrophages and eosinophils via Th1 and Th2 effector pathways (Pardoll 1998). These pathways result from the differentiation of naïve helper T cells into either of two types of distinct subclasses. Th1 cells will secrete IFN-γ and TNF-α activating macrophages,
whereas Th2 cells will secrete interleukins, thus initiating an innate immune response (Alberts et al. 2002). So, although helper T cells may not combat cancer directly in any significant way, they are essential for mediating critical immune stimulation pathways.

While many of the functions of CTLs and helper T cells in antitumor immunity are known, the role of macrophages is not yet well understood. One study has suggested that macrophages may comprise up to half of a breast cancer tumor’s mass (O’Sullivan and Lewis 1994). However, their significance remains unclear. Macrophages are known to produce reactive oxygen intermediates such as nitric oxide and superoxides within the tumor bed in response to Th1 effector stimulation (Pardoll 1998). In addition, in cases where tumors have down-regulated MHC class II molecules, macrophages may stimulate helper T cells by ingesting antigens released by the tumor and displaying them to helper T cells. In response, helper T cells secrete lymphokines, thus magnifying the immune response by stimulating the proliferation and differentiation of other immune effector cells that will attack target tumor cells (Pardoll 1998, Bruggen et al. 2002).

Another immune cell type known to play a considerable role in antitumor immunity are the NK cells. NK cells can distinguish pathological from normal cells in a variety of ways. As mentioned previously NK cells can recognize tumor specific antigens and induce target cell death. Once an NK cell recognizes a cancer cell, it secretes the pore forming granule perforin into the region of the immunological synapse, thus causing target cells to lyse (Diefenbach and Raulet 2002, Shresta et al. 1998). In addition, some researchers have hypothesized that NK cells recognize cells that fail to express class I MHC molecules (Ljunggren and Karre 1990, Diefenbach and Raulet 2002). Interestingly, advanced tumors frequently down-regulate class I MHC molecules (Garrido et al. 1995).
This theory, known as the “missing self hypothesis,” suggests that NK cells use their ability to recognize class I MHC down-regulation to identify cancer cells. In support of this hypothesis, Ljunggren and Karre (1990) found that transplant rejection due to a mismatch between donor and host was a result of failed expression of normal self class I MHC molecules in the host, which induced NK cell elimination of the transplants.

NK cells may also recognize specific proteins up-regulated and presented by cancer cells. Studies have indicated that cells in distress, including tumor cells, tend to express Rae1 and H60 ligands, which are not expressed in normal cells. The Rea1 and H60 ligands are recognized by the NKG2D receptor, which is regularly expressed by NK cells, activated CTLs and activated macrophages (Diefenbach and Raulet 2001, 2002). Studies performed on mice revealed that the induced self ligands Rae1 and H60 on tumor cells significantly enhanced antitumor responses by NK cells and CTLs (Diefenbach and Raulet 2001, 2002).

There is also evidence that NK cells may stimulate CTLs in response to cancer. Bruggen et al. (2002) discovered the first tumor specific antigen, MAGE-3.B40, to be exclusively presented by tumor cells expressing an immunoproteosome. Under normal conditions, a complex in the cytosol called the proteosome degrades peptides derived from internal proteins that are later presented by class I molecules. These peptides are then transported to the endoplasmic reticulum where they combine with newly synthesized class I molecules and are then sent to the cell surface. However, once exposed to IFN-γ, cells switch to an immunoproteosome, which has slightly different catalytic activity than the standard proteosome. The resulting peptides presented by immunoproteosome-expressing cells are preferentially recognized and eliminated by
CTLs. NK cells have been shown to secrete IFN-γ in regions of tumor sites (Ljunggren and Karre 1990, Diefenbach and Raulet 2002). Consequently, IFN-γ, can cause target cells to begin up-regulating tumor specific MHC class I molecules, possibly as a result of the switch by cells to instigate expression of the immunoproteosome. Thus, these results provide a possible mechanism by which NK cells magnify the antitumor response by stimulating aberrant cells to initiate expression of antigens recognized by CTLs.

1.3 Objectives and Conclusion

Substantial evidence exists suggesting that multiple mechanisms are used by NK cells, helper T cells, CTLs and macrophages to develop antitumor immunity. Each mechanism directly attacks tumor cells. In addition, NK cells, macrophages and helper T cells appear to either directly or indirectly stimulate CTLs. Figure 1 illustrates the complexity of these interactions. In order to study this intricate system of antitumor immunity, we have developed a mathematical model that describes interactions between cancer and the immune system. I am particularly interested in analyzing the significance of the stimulatory signals NK cells, macrophages and helper T cells use to enhance CTL activity against cancer. In addition I want to determine under what conditions the immune system is capable of eliciting an effective response that eliminates an otherwise viable tumor. Furthermore, I want to determine circumstances in which tumors can evade the immunosurveillance system, resulting in life threatening disease. Finally, it is my goal to investigate potentially novel tumor and immune system behaviors produced by this model.
Interactions between cancer and immune cells depend on a functioning tumor vascular system. In addition to supplying the tumor with blood, nutrients, oxygen, and a way to remove waste, the vascular system also supplies immune effector cells with a means to infiltrate and attack cancer cells within the tumor bed. Thus, I have chosen to extend a model by Nagy (2004), which describes the growth dynamics of a vascularized tumor, to now include a simplification of mechanisms previously discussed that are used by immune cells to kill cancer. This model highlights the importance of each of the four types of immune cells working together as well as the immune-mediated activation of CTLs in establishing maximal antitumor immunity. In addition, the model predicts conditions under which several dynamical behaviors arise, which may provide testable
predictions of tumor growth dynamics occurring at sizes that are usually not clinically detectable.

**Table 1.** Dependent variables used in the model.

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Meaning</th>
<th>Units</th>
</tr>
</thead>
<tbody>
<tr>
<td>$x(t)$</td>
<td>Mass of parenchyma cells</td>
<td>gm</td>
</tr>
<tr>
<td>$y(t)$</td>
<td>Mass of immature vascular endothelial cell precursor</td>
<td>gm</td>
</tr>
<tr>
<td>$z(t)$</td>
<td>Length of mature tumor microvessels</td>
<td>MU*</td>
</tr>
<tr>
<td>$v(t)$</td>
<td>Tumor microvessel length density</td>
<td>MU/gm</td>
</tr>
<tr>
<td>$m(t)$</td>
<td>Mass of macrophages, NK and helper T cells</td>
<td>mg</td>
</tr>
<tr>
<td>$n(t)$</td>
<td>Mass of CTLs</td>
<td>mg</td>
</tr>
</tbody>
</table>

*Microvessel Units – one unit of microvessels is equal to the mean length of microvessels in one gram of undiseased tissue.

**Table 2.** Tumor parameters: notation and meaning.

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Meaning</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\Phi(v)$</td>
<td>Per capita growth function of parenchyma cells</td>
</tr>
<tr>
<td>$C(v)$</td>
<td>Tumor O$_2$ concentration</td>
</tr>
<tr>
<td>$h(v)$</td>
<td>TAF secretion rate of parenchyma cells</td>
</tr>
<tr>
<td>$\alpha$</td>
<td>VEC precursor response rate to TAF</td>
</tr>
<tr>
<td>$\beta$</td>
<td>VEC precursor maturation/death rate</td>
</tr>
<tr>
<td>$\gamma$</td>
<td>Mature microvessel construction rate</td>
</tr>
<tr>
<td>$\delta$</td>
<td>Microvessel remodeling rate</td>
</tr>
<tr>
<td>$A$</td>
<td>Maximum parenchyma cell reproduction rate</td>
</tr>
<tr>
<td>$B$</td>
<td>Maximum parenchyma cell mortality rate</td>
</tr>
<tr>
<td>$\hat{c}_1$</td>
<td>O$_2$ sensitivity of parenchyma reproduction rate</td>
</tr>
<tr>
<td>$\hat{c}_2$</td>
<td>O$_2$ sensitivity of parenchyma mortality rate</td>
</tr>
<tr>
<td>$r$</td>
<td>Strength of angiogenesis signal produced by parenchyma cells</td>
</tr>
<tr>
<td>$\xi$</td>
<td>O$_2$ sensitivity of parenchyma TAF secretion rate</td>
</tr>
</tbody>
</table>
Table 3. Immune parameters: notation and meaning

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Meaning</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\lambda$</td>
<td>Cancer cell mediated activation rate of macrophages, NK and helper T cells</td>
</tr>
<tr>
<td>$\mu_1$</td>
<td>Inactivation rate of macrophages, NK and helper T cells</td>
</tr>
<tr>
<td>$\eta_1$</td>
<td>Immune-mediated CTL activation rate</td>
</tr>
<tr>
<td>$\eta_2$</td>
<td>Tumor-mediated CTL activation rate</td>
</tr>
<tr>
<td>$\kappa_1$</td>
<td>Rate at which macrophages, NK and helper T cells kill cancer cells</td>
</tr>
<tr>
<td>$\kappa_2$</td>
<td>Rate at which CTLs kill cancer cells</td>
</tr>
<tr>
<td>$\hat{\mu}_2$</td>
<td>Base CTL suppression rate</td>
</tr>
<tr>
<td>$\hat{\mu}_2 + a$</td>
<td>Maximum suppression of CTLs in the absence of a tumor</td>
</tr>
<tr>
<td>$\mu_2(x)$</td>
<td>Suppression rate of CTLs</td>
</tr>
<tr>
<td>$b$</td>
<td>Sensitivity of the suppression mechanism to the presence of tumor cells</td>
</tr>
</tbody>
</table>

2. The Model

The model I wish to consider is the following:

\[
\frac{dx}{dt} = \Phi(v)x - (\kappa_1 m + \kappa_2 n)zx, \tag{1}
\]

\[
\frac{dy}{dt} = \Psi(v)y, \quad \Psi(v) = \alpha h(v) - \beta, \tag{2}
\]

\[
\frac{dz}{dt} = \gamma y - \delta vz, \tag{3}
\]

\[
\frac{dm}{dt} = \lambda mx - \mu_1 m, \tag{4}
\]

\[
\frac{dn}{dt} = \eta_1 mn + \eta_2 nx - \mu_2(x)n. \tag{5}
\]
The normal growth rate of a single parenchyma cell type in a vascularized tumor is represented by equation (1), which is an adaptation of a previous model by Nagy (2004) that includes mortality from immune attack. This equation has been extended to include the tumor cell death rate as a result of their interactions with two groups of immune cells. The first group consists of macrophages, NK and helper T cells and the second group consists solely of CTLs (Fig. 1). The mass (gm) at time \( t \) of a single parenchyma cell type is represented by the variable \( x(t) \). The mass of VECs is symbolized by \( y(t) \) and variable \( z(t) \) represents the length of mature tumor microvessels. Variable \( v(t) \) expresses tumor vascularization (perfusion) in microvessel units per gram of parenchyma. Specifically,

\[
v = \frac{z}{x}.
\]

(6)

Variable \( m(t) \) signifies the mass (mg) of macrophages, NK and helper T cells within the tumor, and \( n(t) \) denotes the mass (mg) of CTLs within the tumor. Parameters \( \kappa_1 \) and \( \kappa_2 \) represent the per capita rates at which macrophages, NK and helper T cells as a group and CTLs kill cancer cells, respectively. These rates depend on the total length of existing tumor microvessels, \( z(t) \). Here I follow Nagy (2004) in scaling \( z \) such that one unit of microvessels is equal to the mean length of microvessels in one gram of undiseased tissue.

The function \( \Phi(v) \) expresses per capita growth rate of a single cell type as a function of blood supply. The parenchyma growth function is modeled as

\[
\Phi(v) = \frac{AC^p}{\hat{c}_1^p + C^p} - B \left( 1 - \frac{C^q}{\hat{c}_2^q + C^q} \right),
\]

(7)
which is similar to the model of Gammack et al. (2001) and used in Nagy (2004), where 
\( C = C(v) \) denotes tumor oxygen partial pressure \( (P_{O_2} \), measured in mmHg). The first 
term on the right-hand side of equation (7) expresses the parenchyma growth rate, and 
parenchyma death is modeled by the second term. Parameters \( A \) and \( B \) express the 
maximum parenchyma cell reproduction and mortality rates, respectively. Parameter \( \hat{c}_1 \) 
measures the effect of \( O_2 \) sensitivity on parenchyma reproduction rate, while \( \hat{c}_2 \) reflects 
the \( O_2 \) sensitivity of parenchyma mortality.

I assume the dependence of oxygen concentration on vascularization is

\[
C(v) = \frac{C_m v}{k + v},
\]

where the constant \( C_m \) denotes the maximum \( P_{O_2} \) possible in a patient’s tissues and 
should be approximately 95 mmHg under normal conditions (Nagy 2004). Parameter \( k \) 
measures how quickly \( P_{O_2} \) responds to changes in perfusion and is approximately equal 
to 1.375 perfusion units (Nagy 2004).

Equation (2) above expresses the dynamics of immature vascular endothelial cells 
(VECs). The term \( \alpha h(v) \) models the proliferation rate of VEC precursors. Parameter \( \alpha \) 
measures the VEC precursor response to tumor angiogenesis factors (TAF). The function 
\( h(v) \) describes the angiogenesis signal produced by parenchyma cells and is modeled by 
the equation

\[
h(v) = rC(v)e^{-\xi C(v)}. \tag{9}
\]

Parameter \( r \) measures the angiogenesis signal produced by cancer cells, and \( \xi \) expresses 
how the production of angiogenesis signal is affected by changes in oxygen supply. The
disappearance rate of VECs, either by dying or becoming mature microvessels, is symbolized by the term $\beta y$. Parameter $\beta$ expresses both the VEC maturation and death rates, and therefore can be referred to as per capita VEC disappearance rate.

The remodeling rate of mature microvessels is expressed by equation (3) above. Term $\gamma y$, where $\gamma$ is constant, expresses the rate at which new microvessels arise from activated VECs. The second term on the right in equation (3) signifies mature microvessel remodeling rate. Parameter $\delta$ is a constant that represents the basic microvessel remodeling rate, but microvessel remodeling also depends on perfusion as described by Nagy (2004).

Equation (4) models the activation and inactivation of macrophages, NK and helper T cells in response to the presence of a tumor. The first term characterizes the activation of macrophages, NK and helper T cells. The activation rate is proportional to the number of interactions between tumor and immune cells. Parameter $\lambda$ is the rate at which tumor cells activate macrophages, NK and helper T cells. The second term expresses the inactivation rate of macrophages, NK and helper T cells. Parameter $\mu_i$ represents the per capita inactivation rate of macrophages NK and helper T cells.

The activation and suppression of CTLs is described by equation (5). The first term symbolizes the rate of activation of CTLs by macrophages, NK and helper T cells. Parameter $\eta_i$ represents rates at which macrophages, NK and helper T cells activate CTLs. This term uses mass action because the immune-dependent activation rate of CTLs is assumed to be proportional to the interaction rate between CTLs and macrophages, NK and helper T cells, with proportionality $\eta_i$. The second term expresses the activation rate of CTLs as a result of interactions with cancer cells. The rate of CTL activation,
represented by parameter $\eta_2$ is proportional to the number of parenchyma cells in the tumor. The final term $\mu_2(x)n$ corresponds to the suppression rate of adaptive immune cells as a function of tumor mass. In particular,

$$\mu_2(x) = ae^{-bx} + \hat{\mu}_2,$$  \hspace{1cm} (10)

which expresses the suppression rate of CTLs. I modeled suppression as a decreasing function of $x$ for the following reasons. First, as a tumor increases in mass, I would expect CTL suppression to decrease in order to maintain a strong immune response. However, once the tumor is destroyed, a large percentage of activated CTLs will remain in circulation. Therefore, suppression should increase in order to down-regulate the activity of CTLs. Parameter $a + \hat{\mu}_2$ designates the maximum suppression rate of CTLs in the absence of a tumor. Parameter $b$ represents the sensitivity of the suppression mechanism to the presence of tumor cells, and the base level of CTL suppression is symbolized by parameter $\hat{\mu}_2$.

3. Parameterization

In order to explore the model’s behavior, I planned to analyze the model numerically. Prior to analyzing the behavior of the model, it was necessary to first estimate parameter values. Many of the biological aspects of this model are currently unavailable in literature or still poorly understood. However, rough approximations can be made for most of the parameters. For tumor and vascularization parameters I selected values that produced a viable tumor according to Nagy’s homogeneous tumor model (2004). I then made estimates of at least the appropriate order of magnitude for all immune parameters. Reasonable values for parameters $\lambda$, $\eta_1$, and $\eta_2$, the activation rates
of immune cells, should be the same order of magnitude as the values used by Nagy (2004) for the reproduction rate of cancer cells. Thus, these parameters ranged from 0.01 – 0.1. Correspondingly, the inactivation rate of macrophages, NK and helper T cells should also have values similar to the death rate of cancer cells. Specifically, values for parameter $\mu_1$ ranged from 0.01 – 0.05. The suppression rate of CTLs was more difficult to parameterize. Parameter $b$ the sensitivity of the suppression mechanism in the presence of a tumor should be fairly large, ranging from 1 – 2, to allow an immediate immune response in the presence of a tumor. The base CTL suppression rate ($\hat{\mu}_z$), on the other hand, should be small, ranging roughly from 0.001 – 0.01. This range would guarantee a slow disappearance of CTLs in the presence of a tumor. Parameter $a$ corresponds to maximum CTL suppression in the absence of a tumor and thus should range from 0.05 – 0.5 in order ensure that CTLs are sufficiently down regulated when no tumor is present. Finally, I determined that parameters $\kappa_1$ and $\kappa_2$, the rates that our two groups of immune cells kill cancer, should range between 0.1 – 1. This range accounts for the increased rate of cancer cell death as a result of interactions with immune cells.

4. Analysis

The first simulation was performed in order to verify that the model was capable of replicating results obtained by Nagy (2004). For this simulation all immune system parameters were set equal to zero. Fig.2a shows a tumor with a sufficiently large VEC density able to produce a viable tumor as predicted by Nagy (2004). Once I verified that the model produced the correct dynamics in the absence of the immune system, I then added the initial conditions and estimated parameter values for the immune system.
equations. The resulting dynamics would represent a starting point from which further exploration could be referenced.

Fig. 2b shows that the presence of an immune system dramatically influences the tumor dynamics. In the absence of an immune system a tumor with standard parameter values and initial conditions (Nagy 2004) grows exponentially reaching a mass of 80 g in 180 days (Fig. 2a). Once the immune system is introduced, maximum tumor mass reaches a peak of only 6 gm in roughly the same amount of time (Fig. 2b). At the inflection point in the tumor’s initial growth curve, macrophages, NK cells and helper T cells begin to respond (Figs. 2b and d). At the peak tumor size we also see a stimulation of CTLs. The combined effect of this immune response nearly kills the tumor completely. From that point on, however, the tumor size continues to oscillate in what appears to be a stable limit cycle.
I now began studying the effects of individual immune parameters on tumor and immune dynamics by selectively varying each parameter independently. Throughout the remaining analysis all tumor parameters and initial conditions remain equal to the values described in Fig. 2. The only values varied were immune parameters and initial conditions. Results yielded several biologically and mathematically important behaviors, including apparent chaos, exponential tumor growth or decay, and oscillatory behavior, each of which I will address in detail below.

Since this model focuses on a stimulatory mechanism by which macrophages, NK and helper T cells are able to activate CTLs, the first parameter I chose to vary was the activation rate of macrophages, NK and helper T cells in response to the presence of a tumor ($\lambda$). After increasing $\lambda$ from 0.05, as in Fig. 2, to 0.5 apparent chaotic behavior was observed (Fig. 3). However, a much smaller maximum tumor mass (0.8 gm) is obtained by this hundred-fold increase in $\lambda$. In addition, macrophages, NK and helper T cells appear to be approaching extinction. One might predict that such an increase in the

Figure 3. Apparent chaotic dynamics produced by model (1). Upper panel – tumor variables. Lower – panel immune variables. $\lambda = 0.5$, and all other parameters as in Fig. 2.
rate of activation of macrophages, NK and helper T cells, from \( \lambda = 0.05 \) to \( \lambda = 0.5 \), would result in a large increase in levels of these immune cells as well as a more efficient activation of CTLs. As expected a spike in activated CTLs is observed at around 4000 days. However, this peak in CTL activation is abnormally large (\( \sim 5 \times 10^5 \)) and it remains unclear what exactly drives this rapid activation of CTLs because such low levels of macrophages, NK and helper T cells are present. Furthermore, the largest time spans between tumor decay and re-growth occur where significant activation of CTLs has taken place. It is also unclear what factor is causing the chaotic tumor growth and decay during time periods when very small amounts of immune cells are present. However, one possibility is that the chaos is driven by time-delays introduced by immune-mediated activation of CTLs. In addition, it appears as though this chaotic behavior is dependent on the presence of both groups of immune cells. As shown in Fig. 4, in the complete absence of CTLs, chaos is replaced by stable oscillations when \( \lambda = 0.5 \).

**Figure 4.** Stable oscillations resume in the absence of CTLs. Upper panel – tumor variables. Lower panel – immune variables. \( \lambda = 0.5 \), \( n = 0 \), and all other parameters as is Fig. 2.
The critical value of $\lambda$ at which chaos arises is between 0.25 and 0.26. As shown in Fig. 5a, at $\lambda = 0.25$ irregular tumor growth behavior begins, but eventually settles down to stable oscillations at 3000 days. However, panel (b) reveals that at $\lambda = 0.26$ chaos occurs.

The biological relevance of this chaotic behavior is unclear; however, it may be mathematically important. Further mathematical analysis will be required to understand the full significance of this behavior.

Because $\lambda$ represents the activation rate of macrophages, NK and helper T cells, and these cells not only participate in the direct killing of cancer cells but also drive the activation of CTLs, one might predict that large increases in this parameter completely eliminate an otherwise viable tumor. As predicted, when $\lambda = 2$ the tumor is eliminated (Fig. 6a). An otherwise viable tumor never becomes a clinical entity, reaching a maximum tumor mass of merely 0.2 gm. Only by decreasing the rate of activation of
Figure 6. Exponential tumor decay and growth. Upper panels – tumor variables. Lower panels – immune variables. Panel (a) shows tumor elimination. \( \lambda = 2 \) and all other parameters as in Fig. 2. Panel (b) reveals exponential tumor growth in the complete absence of CTLs and low activation of stimulatory immune cells. \( n = 0 \), \( \lambda = 0.00005 \), and all other parameters as in Fig. 2.

Macrophages, NK cells and helper T cells to \( \lambda = 0.00005 \) and removing CTLs \( n = 0 \) was exponential growth observed, with a tumor reaching a mass of 4000 gm in approximately 250 days, but then it eventually approached a stable limit cycle (Fig. 6b).

I next focused my attention on the behavior of parameters \( \kappa_1 \) and \( \kappa_2 \), the rates at which macrophages, NK and helper T cells as a group and CTLs kill cancer, respectively. By setting \( n = 0 \) I was able to examine the sole affect of \( \kappa_1 \) against cancer.

Under standard parameter values and initial conditions, maximum tumor mass reached approximately 6 gm (Fig. 7a). In addition, 0.06 mg of macrophages, NK and helper T cells were activated by interactions with cancer cells. By increasing \( \kappa_1 \) from 0.2 to 2.5 similar oscillatory behavior was produced as that in Fig. 7a; however, a smaller maximum tumor mass of 4 gm was obtained (Fig. 7b). Furthermore, only 0.05 mg of macrophages, NK and helper T cells were activated. Thus, by increasing \( \kappa_1 \),
macrophages, NK and helper T cells more efficiently kill cancer requiring fewer immune cells to achieve the same level of control. Similarly, decreasing \( \kappa_1 \) increased the number of immune cells required to stabilize a tumor.

Variations in \( \kappa_2 \) produced similar behaviors as those found with \( \kappa_1 \). In this case I set \( m = 0 \), meaning that no macrophages, NK or helper T cells were present. Any increase in \( \kappa_2 \) resulted in the activation of fewer CTLs and more efficient elimination of tumor cells. Likewise, by decreasing \( \kappa_2 \) more CTLs were needed to stabilize the tumor.

Interestingly, this behavior changed dramatically once I began to study the effectiveness of the stimulatory mechanisms by which macrophages, NK and helper T cells activate CTLs. To focus on immune-mediated CTL activation, I set \( \eta_2 = 0 \) in the following series of investigations. In addition, I assume that the rate which CTLs kill cancer cells (\( \kappa_2 \)) is an order of magnitude or more, larger than the rate which
macrophages, NK and helper T cells kill cancer cells ($\kappa_i$). This assumption was made because CTLs are believed to play the major role in the direct immune attack on cancer. The resulting behavior, (Fig. 8a), reveals chaotic dynamics similar to those obtained in earlier simulations. Increasing $\kappa_z$ from 0.2 to 10 (Fig. 8b) lowers the number of CTLs required to obtain a similar sized tumor as that found in Fig. 8a. Furthermore, under these conditions the tumor is eliminated at much lower values of $\lambda$ than previously found in earlier simulations. Tumor elimination was achieved when $\lambda = 2$. However, with $\kappa_z > \kappa_i$ and the tumor-mediated CTL activation rate set to zero ($\eta_z = 0$), complete tumor elimination was attained at $\lambda = 0.13$ (Fig. 9).

Because of the likely relationship between $\kappa_i$ and $\kappa_z$ described above I decided to re-run some of the initial simulations. With all immune parameters and initial conditions
set as in Fig.2, except for $\kappa_1 = 0.02$ and $\kappa_2 = 0.2$, we can clearly see CTL activation at the peak of macrophage, NK and helper T cell activation in the presence of a tumor (Fig. 10). This result supports the assumption that $\kappa_2$ should be a few orders of magnitude greater than $\kappa_1$ because the behavior appears to be intuitively more realistic for the following reasons. Evidence suggests that the immune-mediated activation of CTLs is a key factor
in developing antitumor immunity. The dynamics observed in Fig. 10 illustrates the expected sequential activation of stimulatory immune cells and then CTLs in response to a tumor, after which the tumor size oscillates in what appears to be a stable limit cycle.

Oscillatory tumor growth around an increasing mean can arise in this model (Fig. 11). This behavior is important because it has been detected clinically (Cotran et al. 1999). When no macrophages, NK and helper T cells are initially present \((m = 0)\) and tumor-mediated CTL activation is zero \((\eta_z = 0)\), the tumor oscillated around an increasing mean mass even though the CTL population is oscillating around a declining mean. This behavior is a result of an increase in the number of interactions between cancer cells and CTLs when the tumor expands. As the tumor increases in size the ratio between cancer cells and CTLs increases dramatically because more cancer cells are available for CTLs to interact with. Gradually, the decreasing CTL population is able to kill off enough cancer cells that a less damaging ratio is restored. Thus, the tumor begins to decrease in size; however, this also increases CTL suppression, which begins down-
regulating CTL activity. Consequently, the tumor is able to begin growing again, reaching sizes larger than had been obtained during its previous peak.

Alternatively, this behavior may also be explained by microvessel dynamics, at least in part. As the tumor size approaches a local maximum, the microvessel length is increasing at an increasing rate. As a result, more CTLs are able to infiltrate the tumor bed, thereby increasing the rate at which they kill cancer cells, leading to the eventual decline in tumor size. However, before the tumor is completely eliminated microvessel density crashes. Consequently, fewer CTLs can infiltrate the tumor, so the tumor begins to grow again.

5. Discussion

There currently exists an extensive body of research showing that the immune system may possess the ability to elicit an effective attack on a tumor, resulting in tumor suppression or elimination. In addition, macrophages, NK and helper T cells may play a dual role in antitumor immunity, by either directly killing cancer cells or stimulating a more efficient CTL response. The goal of this study was to investigate a mathematical model that describes the interaction between cancer and immune cells and interactions occurring among different immune cell types. Combined, these interactions make up a complex system of antitumor immunity. The model presented in this thesis was designed to generate insight into how these interactions affect tumor growth.

The model consists of five coupled differential equations. I assumed that cells of the immune system rely on the presence of a vascular system in order to infiltrate a tumor. Thus, I extended a model by Nagy (2004) describing a homogeneous vascularized tumor to include mechanisms used by immune cells to attack cancer. Equations (1), (2)
and (3) were adapted directly from Nagy (2004). Equation (1) represents the per capita growth rate of a single cancer cell type as a function of blood supply. The development and maintenance of a vascular system is a continuous process of blood vessel remodeling. Equation (2) describes the growth and maturation or death of immature VECs. Immature VECs will eventually develop into mature microvessels. Thus, equation (3) expresses the rate at which new microvessels arise from activated VECs as well as the rate at which mature microvessels are remodeled in the tumor.

Immune cells must be activated in order to attack cancer cells. Therefore, to account for the independent activation of two groups of immune cells, two new equations were formulated. Equation (4) expressed the activation rate of macrophages, NK and helper T cells as a result of interactions with cancer cells and the natural suppression of these immune cells. In addition to directly killing cancer cells, macrophages, NK and helper T cells also stimulate CTLs. Thus, equation (5) expressed the activation rate of CTLs due to interactions with macrophages, NK and helper T cells as well as from direct interactions with cancer cells. I also assumed the presence of an intrinsic CTL suppression mechanism. In the presence of a tumor, little CTL suppression will occur. However, once the tumor has been eliminated a large number of activated CTLs will remain in the body. At this point CTL suppression should increase to down-regulate immune activity. Therefore, I modeled the CTL suppression mechanism as a decreasing function of tumor mass.

The behavior of this model supports the hypothesis that the stimulatory roles of macrophages, NK and helper T cells play an important role in establishing an effective antitumor response. Under standard parameter values and initial conditions,
macrophages, NK and helper T cells become activated in response to initial tumor growth (Figs. 2b and 10). Cytotoxic T lymphocytes are then stimulated via interactions with activated macrophages, NK, and helper T cells in addition to direct interactions with cancer cells. Although complete tumor elimination does not occur, exponential tumor growth that would have occurred if the immune system were absent is prevented. Instead, the tumor oscillates, reaching roughly one-third of its initial maximum mass. Thus, under estimated standard parameter values and initial conditions the model suggests that a tumor will not develop into a clinical entity.

One might reasonably question the stable oscillatory behavior that results in this model, as these types of oscillations do not appear to occur clinically. However, we must consider the fact that this model is describing tumor dynamics at tumor sizes that would not be observed clinically. Thus, the stable oscillatory behavior revealed represents a theoretical prediction of the growth dynamics that may be occurring in 2-3 gm tumors.

Further evidence supporting the critical role of macrophages, NK and helper T cells in developing antitumor immunity was shown in situations where CTLs were not present. In the absence of CTLs, the remaining cell types were able to effectively suppress tumor development (Fig. 7). Dangerous exponential tumor growth only occurred when activation of macrophages, NK and helper T cells was severely suppressed or absent (Figs. 6b and 11). These findings are consistent with the increased incidences of tumor development in diseases such as AIDS. It is well known that HIV enters helpers T cells by using the CD4 molecules present on the surface of helper T cells (Alberts et al. 2002). HIV eventually causes depletion of helper T cells, resulting in an extremely suppressed immune system. Consequently, an infected patient is susceptible not only to a
wide variety of microbial infections, but also to various forms of cancer, including Kaposi’s sarcoma and cervical cancer (Alberts et al. 2002). Thus, while an effective antitumor response can be elicited in the absence of CTLs, the presence of activated macrophages, NK and helper T cells appear to be essential to suppressing an otherwise viable tumor. These observations suggest that the presence of activated macrophage, NK and helper T cells may play a more significant role than CTLs in establishing an effective antitumor response.

Although effective immune responses can be maintained with sufficient activation of macrophages, NK and helper T cells, the model suggests that these cells and CTLs share the burden of eliminating tumors completely, a result consistent with previous studies. For example, Diefenbach and Raulet (2001) performed a study in which they transplanted tumor cells expressing Rae1 and H60 ligands into mice to determine which immune cell types expressing the NKG2D receptor were capable of completely eliminating the tumor. Their results indicated that at the lowest dosage ($10^4$) of tumor cells, either CTLs or NK cells were able to independently mediate tumor rejection. However, at the largest dosage ($10^6$) of transplanted tumor cells both CTLs and NK cells were required to achieve complete tumor elimination. Consistent with these studies, model (1) indicates that complete tumor elimination occurs only in circumstances where both groups of immune cells were present and activated (Figs. 6a and 9). It should be noted that two distinct conditions are required for complete tumor elimination: (1) sufficiently large activation of stimulatory immune cells (Fig. 6a); and (2) sufficient cytotoxic action of CTLs (Fig. 9). The first condition eventually results in the stimulation
of a large number of CTLs. In any case, tumor elimination required both CTLs and the stimulatory mechanisms used by macrophages, NK and helper T cells.

Thus far I have addressed several results that support the theory that each class of immune cells plays a critical role in developing an antitumor immune response. In addition, several unexpected tumor growth behaviors were observed. When macrophages, NK and helper T cells were absent from the model, the resulting tumor would have been fatal. Under these conditions the tumor oscillated around an increasing mean (Fig. 1). Specifically, this means that although the tumor exhibited oscillatory behavior, its overall mass increased over time. In addition to a lack of other immune cell types, the activation rate of CTLs due to interactions with cancer cells was zero. Thus, only the initial CTLs present were capable of killing cancer cells and no new CTLs were activated, resulting in a monotonically decreasing CTL population (Fig. 11).

So what causes this increasing oscillatory growth behavior of the tumor? I suggest that as the tumor increases in mass, more cancer cells are available for CTLs to interact with. Eventually, the remnant CTL population is able to kill enough cancer cells that the tumor begins to decrease in size. Eventually, as CTLs continue to die, the tumor is able to overcome CTL suppression and resume growth. Ultimately, however, the ratios between cancer cells and CTLs become so great that the tumor is able to grow unhindered by the few remaining CTLs.

In addition to the increasing oscillations mentioned above, I also obtained several incidences of chaotic immune system and tumor growth dynamics (Figs. 3, 5, and 8). This behavior is difficult to understand biologically; however, it may represent another theoretical prediction of potential behavior when tumor masses are too small to be
detected clinically. From the figures we can see that expected behaviors, such as occurrences of large time spans between tumor decay and re-growth resulted after large activations of immune cells had taken place. However, it is unclear what causes the sporadic tumor growth and decay when no immune cells appear to be activated (Figs. 3 8a). One aspect common to each of the simulations that exhibited apparent chaos is that even chaotic activation of immune cells is able to suppress tumor development at masses smaller than could be detected clinically.

I believe that the tumor and immune system behaviors exhibited by this model, such as that of chaos and stable oscillations, may provide reasonable predictions of what is occurring in tumors too small to detect. These events may be taking place without our knowledge throughout our lifetime, and not until a tumor is able to overcome the suppression mechanisms provided by the immune system will it develop into a clinically relevant tumor. This conclusion represents the main contribution of this thesis – the model behavior unequivocally supports the immnosurveillance theory.

In addition, this model presented behaviors consistent with previous research. In particular, the occurrence of tumors capable of killing a patient were observed in situations where the stimulatory immune cells, macrophages, NK and helper T cells were severely suppressed or absent. This situation is commonly associated with patients suffering from HIV. Thus, this model provides a possible explanation for the tumor dynamics occurring in immune suppressed individuals based on the interactions between cancer and immune cells. Furthermore, results obtained from this study support the hypothesis that the presence of activated CTLs as well as activated stimulatory immune cells are required to elicit maximal antitumor immunity.
Literature Cited


