ABSTRACT

SOBERANO, LISA ALBERT. Models of Virus-Immune Dynamics and Drug Resistant Virus Infections. (Under the direction of Alun Lloyd.)

Models for the activity of a virus within a host describe the interaction between virus, the cells they infect and the attempts of the body's immune response to remove the infection. We aim to improve these models by incorporating more detailed, and more realistic, descriptions of the immune response. One particular application that is of interest involves the administration of drug treatment during acute infections, with the aim of understanding the dynamics of co-circulating wild-type and drug resistant viruses. Two models are presented that suggest improvements for the portrayal of the immune response. The first is based on existing basic infection models for acute viruses. The second is based upon recent experimental results show that a brief exposure to a pathogen can cause the $CD8^+$ cytotoxic T lymphocytes (CTLs), of the immune system to undergo a programmed set of divisions, including sustained proliferation, giving rise to effector cells, which can kill infected cells, followed by the production and maintenance of memory cells. In the co-infection model of wild-type and drug resistant viruses, a target cell limited model is also used. Previous results suggested that the level of immune response maintained during therapy is the key to suppressing the peak load of resistant virus. Our results, however, suggest that suppression of a resistant strain is not solely dependent on the immune response, but also on the availability of target cells. A spatial virus-immune model is presented in an appendix, which allows multiple target regions within the host to be infected by one or more viruses. Another model is introduced which allows multiple viruses to infect the same target region, with immune specific responses to each virus.

MODELS OF VIRUS-IMMUNE DYNAMICS AND DRUG RESISTANT VIRUS INFECTIONS

by

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A thesis submitted to the Graduate Faculty of
North Carolina State University
in partial fulfillment of the
requirements for the Degree of
Master of Science

BIOMATHEMATICS

Raleigh

2006

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My current research is in the study of infectious diseases and on the behavior of viral infections within an individual. Past research includes electronic properties and transport in silicon nanowires, performed at UIC, and the mathematical foundation of image compression, performed at UNCW.

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1 INTRODUCTION

The motivation for creating a mathematical model to describe virus activity within a host is to understand the interaction between virus, the cells they infect and the attempts of the body's immune response to remove the infection. Many researchers are attempting to understand drug resistance in an epidemiological setting. However, before drug resistance can become a problem on a population level, it will exist at a cellular level. In their study of drug resistance in acute viral infections, Wodarz and Lloyd (2004) and Lloyd and Wodarz (2005) used a model that described the interaction between virus particles, the cells that they infect, infected cells and the immune response that develops as a consequence of the infection.

Initially we concentrate on models from the literature that describe the dynamics of the virus and how it infects healthy cells. In the first part of this paper, we outline the development of Wodarz and Lloyd's model. In section 2.1, we first introduce the Nowak's "Basic Model of Virus Dynamics". In section 3.1, we explain a simplification of this model that is commonly used, and explain its origins in terms of a quasi steady state (QSS) assumption. In section 4.1, we turn to a model that explicitly accounts for a simple immune response. After analyzing each of these models in detail, we discuss the limitations of these models for the description of acute infections. The end goal is to discuss some additional models including immune cell proliferation.

In later models, we will introduce an immune response component that will help us to understand what determines whether a virus will die out, or take off within the host, causing

severe infection. If the immune system can't fight off the virus immediately, then there is the potential that the host can spread the virus to other hosts. At this point spread of infection becomes a population level issue, on how fast the virus can spread between hosts. There are models that exist for both within host and between host virus dynamics, but this research concentrates on within host models.

Our interests lay in understanding and improving existing models, in particular the Wodarz and Lloyd model (2004), from the perspective of the immune response. There are many proposed models in the field that have different descriptions of the immune response. Our goal is to incorporate the strengths from various models and produce a model that incorporates an acquired immunity, or memory, to a particular virus once the host has been initially infected with it. This model should display an increased and much quicker immune reaction when the host is attacked with the same virus after the initial infection has been cleared.

1.1 Virus Basics

"Poisonous Slime" is the Latin translation for virus. It is interesting to note that most viruses that exist are harmless to life, but "Poisonous Slime" seems to be a fitting name for a microscopic agent that can harm its host and potentially cause serious infections and even death. Charles Siebert suggests that viruses have been "viewed as evolutionary latecomers: genomic scraps that fell out onto the floor back when life was assembling itself into more complex arrangements" (Siebert 2006). However, viruses have an organized program by which they seek out a host cell to invade in order to replicate. There are many intricacies to

viruses, how they replicate, how they spread within a host, and how the host responds to the threat of viral infection.

Viruses are composed of a nucleic acid core, not a nucleus, containing genetic material, either deoxyribonucleic acid (DNA) or ribonucleic acid (RNA) (Levine 1992, Nowak and May 2000). This core is surrounded by a coat of repeating protein units (Levine 1992, Nowak and May 2000). In addition, some viruses have a lipid (fatty) envelope surrounding the protein bound genetic material (Levine 1992, Nowak and May 2000).Viruses come in many shapes and sizes and cannot replicate without a host cell. Although the details differ between viruses, they infect living cells of a host and take over the cell's machinery in order to multiply. The basics of the replication process are that the virus enters the target cell, releases its genetic material, which then replicates itself creating new genetic material. Proteins then bind the new material, which is then released from the host cell, producing new virus particles.

There are many different types of viruses that can attack a human host. Although the models we discuss here can be applied to most mammals, we will assume the host is a human host. Viruses range from acute viruses as simple as a common cold, which can be caused by rhinovirus, to persistent and severe, life threatening viruses such as the Human Immunodeficiency Virus (HIV). Other diseases caused by viruses include the flu (influenza virus), small pox (variola virus), Severe Acute Respiratory Syndrome**,** SARS, (a coronavirus), herpes (herpes simplex virus HSV-1 or HSV-2), liver diseases (hepatitis viruses A, B, or C), genital warts (human papilloma virus, HPV), and some cancers.

Each virus has an affinity for certain cell types, which is why viral infections tend to affect a particular region of the body. The cells that a virus has an affinity for are referred to as target cells. For example, rhinovirus and the flu invade nasal epithelial cells and other respiratory epithelial cells, HIV infects CD4 T-cells, and HPV infects epithelial cervical cells. So, when modeling a virus, we must keep in mind that the system is modeling the particular region or cell type of the host that the virus will infect.

The effectiveness of drug treatment of viruses resides in the fact that the drug creates some mechanism that prevents replication of the virus. For example, with the influenza A virus, drugs like adamantine and rimantadine, block the virus' M_2 ion channel protein, located in the viral envelope, preventing fusion of the virus and host cell's membranes, thus preventing the uncoating of the virus and the exposure of the virus to the host cells' cytoplasm. The M_2 protein of the influenza A virus can mutate, which disables the M_2 inhibitor drugs from being effective (Bright et al. 2005). The more frequently these M_2 inhibitor drugs are used, the higher the possibility the virus will mutate, creating a survival of the fittest scenario. Resistance to these drugs has been reported in semi-closed settings where the antiviral treatment was used. (Bright et al. 2005). The incidence rate of influenza strains, (including avian flu strains) resistant to the most commonly used antiflu drugs is at about 12% worldwide. In a global survey of the influenza virus, Bright et al. surveyed over 7,000 isolated strains of the influenza A virus. From this survey, they found that in China, the incidence of drug resistant strains in 2004 was 73.8% whereas the incidence in Japan was 4.3% and the U.S. was 1.9% (Bright et al. 2005). Although there is no statistical significance

linking these rates to this fact, it is interesting to note that antiflu drugs such as adamantine and rimantadine are sold over the counter in China, whereas in the U.S. these drugs are prescribed by licensed physicians only for flu or flu-like symptoms. In the event of an global influenza pandemic, it is likely that these drugs mentioned will not be effective due to mutations of the Influenza A virus, since the drugs will have potentially lead to a selection of drug resistant strains over time.

Another prime example of emergence of drug resistant virus is in an HIV-1 infection setting. The HIV-1 virus mainly attacks $CD4^+$ cells which, as discussed in section 1.2, are helper cells in the immune system. $CD4^+$ cells usually communicate to the immune system to produce more of an immune response, including antibodies and $CD8^+$ cells that will clear the infection. Since HIV-1 is infecting the helper cells, the CTL response is not very good in fighting off the virus. The challenge with treating the virus is that a single-drug treatment regime can lead to a completely drug resistant viral population within a few weeks. This type of treatment will lead to rapid, but short lived decline in plasma virus load, however several weeks later the plasma viral load is back to its pre-treatment level (Nowak and May 2000). It is suggested that the reason for the increase in viral load after treatment has started is due to the fact that more $CD4^+$ target cells are available for HIV-1 to infect. The threat of this one-drug treatment regime is that the virus will selectively mutate enabling it to avoid the action of the drug. Although HIV-1 infected patients have recently benefited from combination therapy, which has decreased viral load beyond detection for years in many patients, it has become clear that combination therapy will not lead to elimination of HIV from the patient.

Rather than killing off the virus, recent advances in anti-retroviral therapy have considered enhancing the immune response in order to manage the virus (Wodarz 2001).

1.2 Immune System

The immune system is a network of cells, tissues and organs in the body that work together to identify and fight away foreign substances in the body (NIH 2003). These foreign substances could be invaders such as bacteria, viruses, parasites, and fungi. The foreign substances are also known as antigens. Some cells and other organisms display markers, which are protein segments, known as peptides, located on the surface or through the surface of the cell (Riviere 1999). For a virus, this surface is a bi-layer lipid membrane (Riviere 1999, Danielli and Davson 1935). Following infection of a cell, peptides that are derived from the virus and bound to MHC class I molecules are expressed on the surface of the infected cells (Antia et al. 2003).

Due to these peptides, a healthy immune system will be able to distinguish between the body's own cells (self) and foreign cells (non-self) (NIH 2003). If the peptide marks a particle of type self, then the host's body or immune system should not attack the particle. However, if the protein is recognized as a pathogen, or non-self, then the host's body will build up the immune system to attack that pathogen. However in some abnormal situations, the immune system can mistake self for non-self, launching an attack against the host's own cells and tissues. This false detection results in an autoimmune disease (NIH 2003).

There are some pathogens that do not mark themselves clearly, which makes it difficult for the immune system to fight off that pathogen (Staines et al.. 1993). For instance, Human

Immunodeficiency Virus (HIV) and malarial parasites are pathogens that subvert the immune system by hiding inside of the host cells (Staines et al.. 1993). Although an immune response is made specific to the peptides, the response does not interact very effectively with the virus hidden within the cell. In addition to hiding, theories have been suggested that various mutations of the HIV virus may develop, which enable it to avoid an immune response (Wodarz and Nowak 2000), preventing complete elimination of the virus. In the case of the malarial parasite, it is outside of the red blood cells at a certain point in its lifecycle, but it changes the proteins displayed on its exterior, so the immune system does not recognize the invader in order to attack quick enough before the parasites are hiding inside of red blood cells again (Staines et al.. 1993).

The cells of the immune system are generated from precursor cells that are produced in the bone marrow, where all blood cells are generated (Janeway et al. 1999, NIH 2003, Staineset al. 1993). There are many types of immune cells, but our discussion will concentrate on lymphocytes and macrophages. Macrophages assist the immune system in combating many types of pathogens that have remained unchanged in the course of evolution (Janeway et al. 1999). Although this helps in the defense against bacteria, it doesn't defend very well against viruses whose surface molecules undergo a rate of evolution much faster than the host can adapt to. However, lymphocytes have evolved to conquer this. When maturing in the bone marrow and the thymus, many lymphocytes are created that carry receptors for one specific type of pathogen, rather than non-specific receptors. This assures that there are millions of lymphocytes in the body with specificity to millions of different types of pathogens (Janeway et al. 1999).

There are different types of lymphocytes with different functions. Some lymphocytes produce antibodies and secrete them into the blood and lymphatic circulation. These antibodies find the antigens that the lymphocytes originally recognized then surround the antigen and eventually destroy or remove the antigen (Staines et al. 1993). Another type of lymphocyte produces messengers, rather than antigens, that travel to other areas of the immune system in order to communicate to the system to produce antibodies.

Some lymphocytes gain specificity to certain antigens when traveling through other lymphoid organs, such as the thymus; these lymphocytes are then called T lymphocytes or T-cells. Most T-cells fall into the subset of either $CD4^+$ or $CD8^+$ cells, distinguished by the presence of either the CD4 or CD8 glycoprotein on the surface of the cell. $CD4^+$ cells are referred to as helper cells since they bind to an antigen, and attract other immune cells and antibodies to the area, causing inflammation. $CD8^+$ T-cells are best known for their ability to attack and destroy certain antigens. These T-cells are referred to as cytotoxic T lymphocytes (CTLs) or killer cells. B lymphocytes do not receive specificity in the thymus, but they mature in the bone marrow and later settle in secondary lymphoid tissues, the lymph nodes and the spleen (Staines 1993). B cells bind to antigens and engulf the antigen, digesting it into fragments, and displaying it on the cell surface. This allows helper T-cells to bind to the structure, attracting more production of immune cells. The type of immune response in a host depends on the antigen and its route of entry into the body (Staines et al.. 1993). Helper cells mostly handle the communication for the immune system, calling for more CTLs specific to kill off the virus.

In addition to such a complex communication and response system, the immune system also has its own circulation throughout the body, called the lymphatic circulation (Figure 1). It is

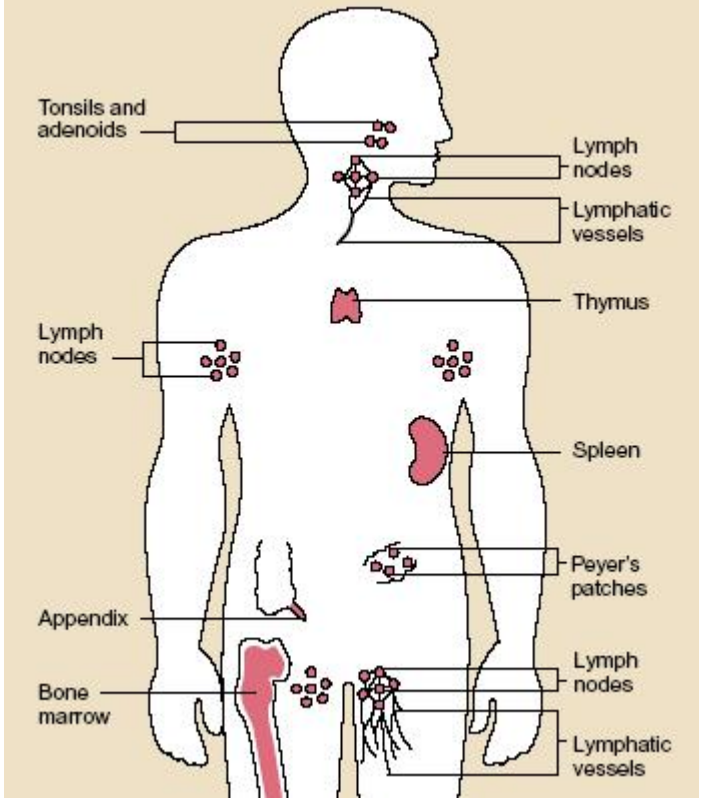


Figure 1 Organs of the Immune System Positioned Throughout the Body (NIH 1993).

similar to the blood circulation throughout the body in that it delivers a fluid that reaches the body's organs. The lymphatic circulation carries lymph, a clear fluid containing lymphocytes. Lymphocytes can travel to the tissues of the body either through the lymphatic vessels or through blood vessels.

After an immune response has mounted an attack against an antigen, most of the immune cells destroy themselves in the process of attacking an antigen or by a natural death process

such as apoptosis. However a certain threshold level of immune cells remain in the host, creating a memory of the pathogen that was attacked. This allows for the antigen to be destroyed more quickly the next time it enters the host (NIH 2003). These types of cells are often referred to as memory cells, or acquired immunity.

Acquired immunity can be created in several ways. An infection destroyed by the immune system, will cause the immune system to maintain a level of antigen specific lymphocytes that can attack any future infections of the same or similar antigen (NIH 2003). Acquired immunity can also be created through vaccines or serum injections. Vaccines introduce a small amount of altered antigen to the host, provoking a small infection in the body, thus an immune response resulting in memory cells. Serum that is antibody rich against a particular microbe can be extracted from one individual and injected into another to resist a particular infection. Usually these serums do not provide permanent protection against the antigen (NIH 2003). Another type of innate immunity is natural immunity, which is usually due to genetics, where the immune system naturally eliminates the antigen (NIH 2003). Variations in genetics can allow one host to combat an antigen more readily than another host.

2 BASIC VIRUS MODEL

2.1 Basic Virus Dynamics

One of the simplest models for virus dynamics within an individual was originally developed by Nowak and coworkers. The model considers the interaction between virus particles and the target cells that they infect. The number of uninfected target cells is denoted by x, the

number of free virus particles by v, and the number of infected cells by y (Nowak and Bangham 1996, Bonhoeffer et al. 1997, Nowak and May, 2000, De Boer and Perelson 1998).

$$\dot{x} = \lambda - dx - \beta xv \quad (1)$$
$$\dot{y} = \beta xv - ay \quad (2)$$
$$\dot{v} = ky - uv \quad (3)$$

This model was produced originally to model persistent viruses such as HIV and the human T cell leukemia virus (HTLV-1) (Nowak and Bangham 1996, De Boer and Perelson 1998), but has been since used for other viruses as well. For biological reasons, all of the parameters in the above model take positive values.

This set of ordinary differential equations assumes that the various populations are of large enough size that we can treat their numbers as continuously varying quantities and ignore stochastic effects. Depending on how one measures the system, the variables could represent cell counts or concentrations. Assuming that the system has constant volume, switching between the two interpretations would merely involve a scaling of the parameter values. It should be noted that the number/ concentration issue is rarely discussed in the papers of Nowak and co-workers.

Target cells are assumed to be produced at a constant rate of λ and are assumed to be lost to the system at rate dx. The death term, with constant per capita death rate d, corresponds to target cells having exponentially distributed life spans, with average lifespan equal to $1/d$. Biologically, this loss in target cells could be due to the natural shedding, or death, of the target cells. Later, in section 2.3, we discuss other assumptions for the modeling of the target cells.

The infection term, which depicts the rate at which target cells are lost because of infection, and the rate at which infected cells are produced due to infection by a virus particle, is assumed to be proportional to x and v: βxv. The parameter β depends on the rate at which virus particles encounter target cells and the probability that such an encounter will lead to the cell becoming infected. This mass action term assumes the system to be perfectly mixed, however, this is a weakness of the model since this model does not take into account the spatial structure of the system. It is assumed that the infection of an uninfected target cell by a virus particle is the only means by which new infected cells appear (for instance, there is no vertical transmission of infection: infected cells do not reproduce and give rise to infected offspring). It is assumed that infected cells die at a constant per capita rate of a, which implies that the lifespans of infected cells are exponentially distributed with an average lifespan equal to $1/a$.

Infected cells are assumed to produce virus particles, at a constant rate k per infected cell. Noting that infected cells live for an average of $1/a$ time units, this means that an infected cell will give rise to an average of k/a virus particles over its life. This quantity is termed the 'burst size', since, for some infections, virus is not produced continuously over an infected cell's lifetime, but rather released in a single event as the infected cell is killed by the virus (Bonhoeffer et al. 1997).

Virus particles are lost from the system, as a result of natural death, at a constant per capita rate of u. Virus particles, therefore, have an average lifespan of $1/u$ time units. In this model,

the loss of virus particles due to infection, which occurs at rate βxv, is assumed to be negligible compared to uv, so it is ignored. This may be a weakness of the model, since this assumption may or may not hold depending on what virus is being modeled.

2.2 Equilibriums and Stability Analysis for Basic Virus Dynamics

The nonlinear nature of the system means that we cannot expect to solve equations (1)-(3) explicitly. Analysis of the dynamics typically revolves around a discussion of the long-term behavior, in particular the equilibriums of the system and their local stability. Linear stability analysis (Strogatz 1994) can be used to describe the dynamics in the vicinity of an equilibrium.

2.2.1 Equilibriums

There are two equilibriums of this model. The first equilibrium describes an infection free state and has $(x^*, y^*, v^*) = \left(\frac{\lambda}{d}, 0, 0\right)$. A second, non-trivial, equilibrium of the system, when the virus is able to establish an infection, has

$$\begin{aligned}(x^*, y^*, v^*) &= \left(\frac{au}{\beta k}, \frac{\lambda}{a} - \frac{du}{\beta k}, \frac{\lambda k}{au} - \frac{d}{\beta}\right) \\ &= \left(\frac{au}{\beta k}, \left(\frac{\beta\lambda k}{adu} - 1\right)\frac{du}{\beta k}, \left(\frac{\beta\lambda k}{adu} - 1\right)\frac{d}{\beta}\right).\end{aligned} \tag{4}$$

Depending on parameter values, this equilibrium can have a positive number of infected cells and virus particles. Since this model is in many ways analogous to certain epidemiological models, this equilibrium is sometimes called the endemic equilibrium of the system.

2.2.2 Introduction of Infection near Infection Free Equilibrium

The local behavior near the equilibriums is determined by the basic reproductive number, R_0. This quantity describes the average number of secondary infected cells that result from the introduction of a single infected cell (or, equivalently, the average number of secondary virus particles that are produced as a result of introducing a single virus particle) (Anderson and May 1991). We can derive this value in an intuitive way, imagining what happens when the system is in the disease free state and a single infected cell or virus is introduced. To do this we linearize the system about the infection free equilibrium. Near this infection free state, x $\approx \frac{\lambda}{d}$. If the number of infected cells and virus particles is small, then *x* will remain close to this value for quite some time, since there are few infection events. During the initial part of the infection, or near this equilibrium, the transmission term has $\beta xv \approx \frac{\beta\lambda}{d}v$, so, each virus gives rise to new infections at the average rate of $\frac{\beta\lambda}{d}$. Virus particle live on average for $\frac{1}{u}$ time units, so one virus gives rise to $\frac{\beta\lambda}{du}$ infected cells. Infected cells create virus at rate k, and live for $\frac{1}{a}$ time units, so each infected cell produces $\frac{k}{a}$ viruses. Our initial virus gives rise to $\beta\lambda/du$ infected cells, and so the number of viruses resulting from our initial virus is $(\frac{\beta\lambda}{du}) \times (\frac{k}{a})$. Therefore, the basic reproductive number is given by $R_0 = \frac{\beta\lambda k}{adu}$. It is easy to see that the same expression would be obtained if one calculated the average number of infected cells that are produced as a result of the introduction of a single infected cell.

In terms of R_0, it is straightforward to show that expression (4), the endemic equilibrium, can be written as

$$(x^*, y^*, v^*) = \left(\frac{\lambda}{dR_0}, (R_0 - 1)\frac{du}{\beta k}, (R_0 - 1)\frac{d}{\beta} \right). \quad (5)$$

We notice that this equilibrium requires R_0 to be greater than or equal to one for it to be biologically feasible (otherwise infected cell and virus numbers are negative) and that this equilibrium coincides with the infection free equilibrium when R_0 is equal to one.

2.2.3 Linear Stability Analysis near Equilibriums

Linear stability analysis involves the evaluation of the Jacobian matrix of the system (in our case, this is the matrix of the partial derivatives of the functions appearing on the right hand sides of equations (1)-(3) with respect to the state variables, x, y and v) at the equilibrium. The (linear) stability properties can then be discussed in terms of the eigenvalues of the Jacobian matrix. If all eigenvalues have negative real part, then the equilibrium point is stable. We remark that this is a local analysis since the linearization is only a good approximation 'close' to the equilibrium point. Other techniques (such as Lyapunov functions) can be used to address more general stability questions, but we shall not pursue these here.

Linearizing the model gives the following Jacobian matrix

$$J = \begin{bmatrix} -d - \beta v & 0 & -\beta x \\ \beta v & -a & \beta x \\ 0 & k & -u \end{bmatrix}.$$

The eigenvalues are given as the roots of the characteristic equations $|J - \gamma I| = 0$, where I is the 3 x 3 identity matrix and $|\ |$ denotes the matrix determinant. Since we have a 3 x 3 system, this will give rise to cubic characteristic equations. We use the notation J1 to correspond to the Jacobian evaluated at the first equilibrium point, and J2 is the Jacobian evaluated at the second equilibrium point, and so forth. Notice that, since the standard representation for eigenvalues, λ, is a parameter of the virus model, we use γ to stand for the eigenvalues.

Looking at the infection free equilibrium, we have

$$J1 = \begin{bmatrix} -d & 0 & -\dfrac{\beta\lambda}{d} \\ 0 & -a & \dfrac{\beta\lambda}{d} \\ 0 & k & -u \end{bmatrix}.$$

We see that the eigenvalues are given by $\gamma_1 = -d$, and the two roots of the following quadratic

$$\gamma^2 + (a+u)\gamma - au(R_0 - 1) = 0.$$

Recalling that both roots of a quadratic have negative real part if and only if both the linear and constant coefficients are positive (May 1973, Murray 2002, Strogatz 1994; this result follows from the two dimensional form of the Routh-Hurwitz conditions- see Appendix A), we see that the infection free equilibrium is only stable when R_0 is less than one. If, on the other hand, R_0 is greater than one, the roots of the quadratic have positive real part and so the infection free equilibrium is unstable.

Linearizing the model for the endemic equilibrium gives us

$$J2 = \begin{bmatrix} -\frac{\beta\lambda ku}{a} & 0 & -auk \\ \frac{\lambda ku\beta - da}{a} & -a & auk \\ 0 & k & -u \end{bmatrix}.$$

We find that the eigenvalues satisfy the following cubic equation

$$\gamma^3 + \left(a + u + R_0\right)\gamma^2 + (a+u)R_0 d\gamma + dau\left(R_0 - 1\right) = 0.$$

The Routh-Hurwitz conditions applied to this polynomial show that the equilibrium is stable if and only if both R_0 is greater than one and

$$\left(a + u + R_0\right)(a+u)R_0 d > dau\left(R_0 - 1\right).$$

We multiply out the brackets on the left hand side, giving a collection of positive terms, one of which is $dauR_0$. Subtracting $dauR_0$ from both sides leaves an inequality in which the left hand side is positive and the right hand side is negative (and equals -*dau*). We see that this second condition always holds. Therefore, the endemic equilibrium is stable when $R_0 > 1$, and unstable when $R_0 < 1$.

Biologically, these stability properties tell us that infection can only take off when R_0 is greater than one, in which case the system will approach the endemic equilibrium. (Keep in mind that this analysis is a local stability analysis.)

2.3 Critical Discussion of Basic Virus Model

Since most virus dynamic models build upon Nowak's Basic Model, it is well worth closely examining its assumptions before continuing on to more complex models. This basic virus model is not very satisfactory in modeling acute virus dynamics. When $R_0 > 1$ the model

reaches an endemic equilibrium, which is a good description of a persistent viral infection. However, this model does not address acute virus behavior which has a peak in viral load which then decays toward zero after a short time, allowing the target cells to regenerate and return to equilibrium. Discussion of the target cell model, by Perelson, with this feature will be introduced in section 5.

2.3.1 Alternative Models for Demography of Uninfected Cells

The description used in the Basic Virus Model for the demography of the uninfected cells is given by $\dot{x} = \lambda - dx$, when infection is absent. A close look at the model for this demography reveals some strengths and weaknesses of the model.

If $\lambda = 0$, then there would be no trivial infection free equilibrium. There would be nothing to maintain an uninfected cell population, as there would only be exponential decay. Other possibilities for the model of uninfected cells would be having the production rate dependent on the number or concentration of uninfected cells in the host. This gives a model like $\dot{x} = \lambda x - dx = (\lambda - d)x$, which (in an infection free setting) has exponential growth ($\lambda > d$) or decay ($\lambda < d$) and no trivial infection free equilibrium, or If $\lambda = d$, then there is no change in the number of uninfected cells over time ($\dot{x} = 0$). A third option for the uninfected cells model is a logistic growth model $\dot{x} = rx(1 - \frac{x}{k})$. Although, this is a more realistic picture for the demography of the uninfected cells, the presence of quadratic terms complicates analysis.

Having the growth rate of the uninfected cells, λ, not dependent on the number or concentration of uninfected cells in the system makes sense if we assume some exterior force continually feeds new host cells into the system we are studying, rather than each host cell splitting and reproducing itself. This could be accurate depending on which host system is being modeled.

For instance, the HIV virus infects the immune system's $CD4^+$ T cells released by the thymus into the host's circulation. If the thymus is not included in the target region of the system, then it is appropriate to assume that the thymus acts as the exterior force providing target cells into the system. The rhinovirus, on the other hand, infects the epithelial lining of the nasal cavity. Epithelial cells are continually produced from the bottom layers of the skin. In both of these models the cells are being produced by some force.

2.3.2 Perfect Mixing Suggested by Mass Action Term

The rate at which target cells are infected by virus is βxv. This term is a mass action term and assumes a well mixed system. Depending on the host system, this may or may not be a good assumption, since it does not take the spatial structure of the host system into consideration. Since most infections take place in solid tissues Funk et al. (2005) study spatially structured models of virus and virus-immune dynamics where dispersal of virus and immune effector cells were constrained to occur locally.

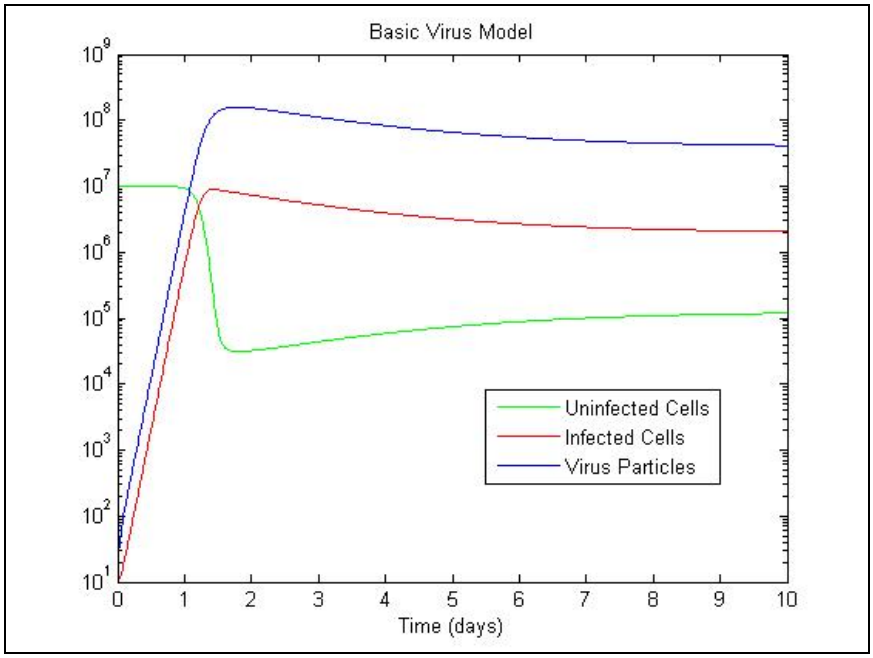
A brief discussion of suggested spatial models, in addition to the model proposed by Funk et al., can be found in Appendix B, however we hope to pursue more research of spatial models in the future.

2.4 Quasi Steady State Assumption

Biological considerations suggest that the dynamics of virus particles occur on a much faster timescale than that of target and infected cells. This separation of timescales means that a quasi steady state (QSS) assumption can be made (Murray 2002) since the number of free virus particles rapidly comes to a (quasi) equilibrium with respect to the number of infected cells. In simulations of the model (figure 2) we see that, following an initial transient period, the numbers of infected cells and virus particles are proportional to each other.

A rigorous deployment of the QSS assumption requires a careful analysis of the ODEs, separating slow and fast dynamics (c.f. Murray's discussion of a similar phenomenon in the well-known setting of Michaelis-Menten enzyme kinetics). A heuristic use of the QSS involves setting $\dot{v} = 0$, leading to an algebraic relationship, $v = \frac{ky}{u}$, between the levels of virus and infected cells. Notice that the QSS simplifies the governing equations: since one ODE is replaced by an algebraic equation, the dimension of the system reduces from three to two.

a)



b)

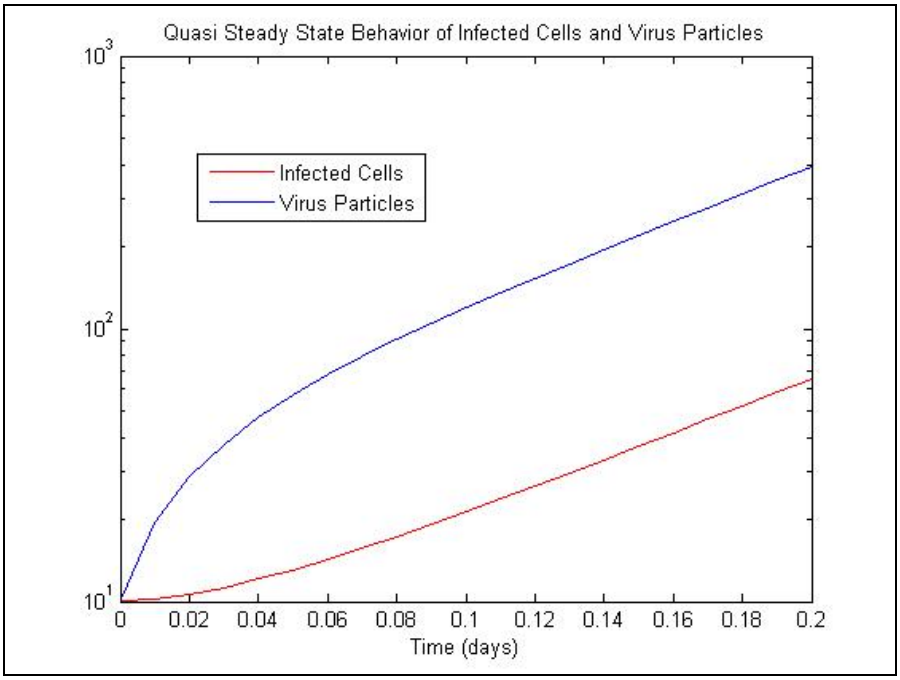


Figure 2 Virus count and infected cell count for Basic Virus Model

a) Virus count and infected cell count rapidly reach quasi steady state.
b) Initially the rates of growth for the virus and infected cells are different, but they quickly approach the same rates. Parameters: $\lambda=10^5$, d=0.1, $\beta=2^{-7}$, a=0.5, k=100, u=5.

Incorporating this proportion into equations (1) and (2) allows us to create a simpler model, written in terms of $\beta' = \frac{\beta k}{u}$ (Wodarz In Press). Usually this new parameter is written without the prime.

3 BASIC INFECTION MODEL

3.1 Basic Infection Dynamics

Applying the QSS assumption allows us to eliminate the free virus equation and rewrite the model in terms of x and y alone.

$$\dot{x} = \lambda - dx - \beta xy \quad (6)$$

$$\dot{y} = \beta xy - ay \quad (7)$$

This model is practically the same as the predator-prey like SIR (susceptible, infected, and recovered) population model with demography, where x would correspond to the susceptible subpopulation and y would correspond to the infected subpopulation (Anderson and May 1991). In this system, R_0 is defined as the number of secondary infections that are produced from one initial infected cell. Following a similar logic as before, we find that $R_0 = \frac{\beta\lambda}{ad}$.

3.2 Equilibriums for Basic Infection Dynamics

The first, infection free equilibrium, for this model is $(x^*, y^*) = \left(\frac{\lambda}{d}, 0\right)$. The second, endemic equilibrium is given by $(x^*, y^*) = \left(\frac{\lambda}{dR_0}, \frac{d}{\beta}(R_0 - 1)\right)$. Linearizing this model gives the following Jacobian matrix,

$$J = \begin{bmatrix} -d - \beta y & -\beta x \\ \beta y & \beta x - a \end{bmatrix}$$

When linearizing around the infection free equilibrium we have

$$J1 = \begin{bmatrix} -d & -\frac{\beta\lambda}{d} \\ 0 & \frac{\beta\lambda}{d} - a \end{bmatrix},$$

which is upper triangular. The eigenvalues are the diagonal entries, -*d* and $\frac{\beta\lambda}{d} - a = a(R_0 - 1)$. So, the infection free equilibrium is stable when R_0<1. The Jacobian for the endemic equilibrium is given by

$$J2 = \begin{bmatrix} -\frac{\lambda\beta}{a} & -a \\ \frac{\lambda\beta - ad}{a} & 0 \end{bmatrix}.$$

The characteristic equation for the endemic equilibrium, in terms of γ, is

$$a\gamma^2 + \lambda\beta\gamma + a\lambda\beta - da^2 = 0.$$

Using the quadratic formula, the eigenvalues of this equation are

$$\gamma_1, \gamma_2 = -\lambda\beta \pm \sqrt{(\lambda\beta)^2 - 4a^2\lambda\beta + 4da^3}\ .$$

The endemic equilibrium is stable when the real parts of γ_1, γ_2 are negative which occurs when $R_0>1$. Note that there is a bifurcation as R_0 passes through one, as witnessed by the changes in their stabilities.

For similar reasons as the basic virus model, the weakness of the basic infection model is that it is not a good model for acute viruses. Infection either decays immediately to zero, if $R_0<1$ or it reaches an endemic equilibrium. This is satisfactory for a persistent infection, however for an acute infection there needs to be a peak in infection load, with subsequent decay to zero, or near zero.

4 BASIC CTL MODEL

4.1 Basic CTL dynamics

The previous two models presented ignore the effect of an induced immune response, unless one considers the $-ay$ term in (2) or (7) to incorporate some basic immune response. A common suggested model incorporates healthy cells, infected cells, virus cells, and an immune response (Nowak and Bangham 1996). However, a model taking advantage of the steady state assumption for virus particles (Wodarz and Lloyd 2004), is given by

$$\dot{x} = \lambda - dx - \beta xy \tag{8}$$
$$\dot{y} = \beta xy - ay - pyz \tag{9}$$
$$\dot{z} = cyz - bz. \tag{10}$$

In this model, z symbolizes the CTL immune response cells. We can make other general assumptions about what type of immune cells z represents, but since there is a direct killing effect based on the load of immune cells, it is safe to assume these are CTLs, also referred to

as effector cells in other models. A flow diagram of the model is presented below to assist interpretation of the equations in (8), (9), and (10).

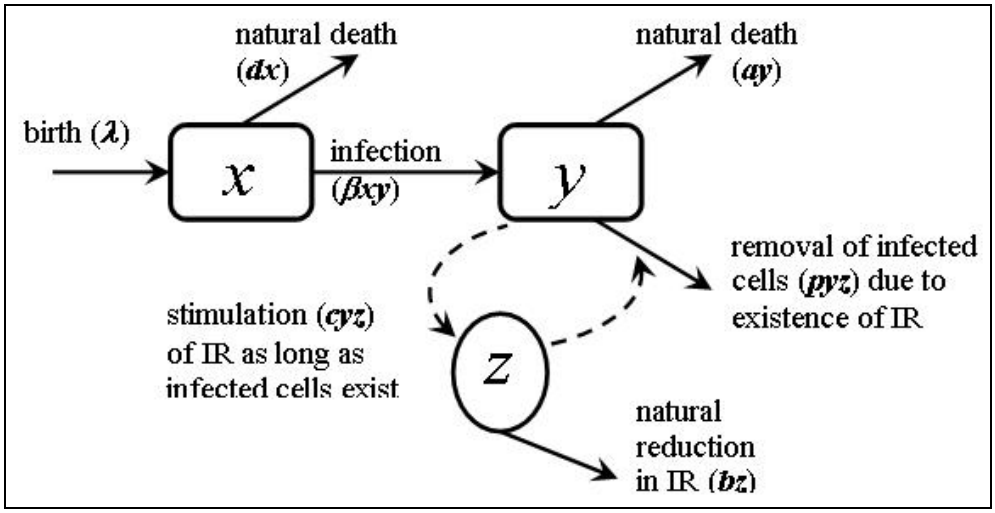


Figure 3 The Basic CTL Model with an Immune Response (IR)

x: uninfected target cells; **y**: infected cells; **z**: immune response.

Compared to the earlier model (6 and 7), the description of the target and infected cells is identical, except for (9) has an added term of *–pyz*. A simple model of how the immune system might respond to an infection is described by (10). The interaction between y and z is predator-prey like, similar to the interaction of x and y. The expansion of immune cells, described by the term *cyz*, is updated according to the density of the infected cells present in the host. So, if no infection exists, y = 0, then there is no stimulation of the immune response. As long as $y > \frac{b}{c}$ the immune response is increasing due to the infection, whereas when the infection falls below this level, $y < \frac{b}{c}$, the host withdraws its immune response, driving the predator-prey dynamics of the infection and the immune response.

Research shows that the induction of the immune response determined by the density of infection in the host is not a realistic approach (Murali-Krishna et al. 1998, Ahmed and Gray, 1996, Badovinac et al. 2002). Alternate immune response models, including immune cell proliferation, are discussed in section 6.

4.2 Equilibriums of the CTL dynamics

During initial stages of infection within the host, near the infection free equilibrium, the average number of secondary infected cells resulting from the introduction of a single infected cell is given by $R_0 = \frac{\beta\lambda}{ad}$. This is the same expression for R_0 as in the Basic Infection Model. Since R_0 is defined in terms of the infection free equilibrium, when no immune response has yet been induced, the immune system parameters do not appear in the expression for R_0.

4.2.1 Equilibriums and Linear Stability Analysis

This model has the following three equilibriums:

1. Infection Free: $(x^*, y^*, z^*) = \left(\frac{\lambda}{d}, 0, 0\right)$

2. No Immune Response: $(x^*, y^*, z^*) = \left(\frac{\lambda}{dR_0}, \frac{d}{\beta}(R_0 - 1), 0\right)$

3. Endemic Equilibrium: $$(x^*, y^*, z^*) = \left(\frac{\lambda c}{(dc + \beta b)}, \frac{b}{c}, \frac{\beta \lambda c}{(pdc + p\beta b)} - \frac{a}{p} \right)$$

$$= \left(\frac{\lambda c}{(dc + \beta b)}, \frac{b}{c}, \frac{1}{p} \frac{cad(R_0 - 1) - \beta ab}{(dc + \beta b)} \right)$$

Linear stability is again determined by the eigenvalues of the characteristic equations $|J - \gamma I| = 0$. The Jacobian matrices evaluated at the equilibrium points are:

$$J1 = \begin{bmatrix} -d & -\frac{\beta\lambda}{d} & 0 \\ 0 & \frac{\beta\lambda}{d} - a & 0 \\ 0 & 0 & -b \end{bmatrix},$$

$$J2 = \begin{bmatrix} \frac{-\lambda\beta}{a} & -a & 0 \\ \frac{\lambda\beta - da}{a} & 0 & \frac{p(da - \lambda\beta)}{a\beta} \\ 0 & 0 & \frac{c\lambda\beta - cda - ba\beta}{a\beta} \end{bmatrix},$$

$$J3 = \begin{bmatrix} \frac{-cd - \beta b}{c} & \frac{-c\lambda\beta}{cd + \beta b} & 0 \\ \frac{\beta b}{c} & 0 & -\frac{pb}{c} \\ 0 & \frac{-c(-c\lambda\beta + cda + ba\beta)}{(cd + \beta b)p} & 0 \end{bmatrix}.$$

For the first and second equilibrium point, determining the eigenvalues is straightforward. Since J1 is an upper triangular matrix, we know the eigenvalues are the entries of the diagonals of the matrix. These are $\gamma_1 = -d,\ \gamma_2 = \frac{\lambda\beta}{d} - a = a(R_0 - 1),\ \gamma_3 = -b$.

This equilibrium is stable for $R_0 < 1$, and unstable for $R_0 > 1$. Hence, introduction of a low number of infected cells, and a low immune response will result in the infection dying out.

The characteristic equation for the second equilibrium, with no immune response, is

$$\left(\frac{c\lambda\beta - cda - ba\beta}{a\beta} - \gamma\right)\left(\gamma^2 + \frac{\lambda\beta}{a}\gamma + (\lambda\beta - da)\right) = 0,$$

which implies

$$\left(\frac{c\lambda\beta - cda - ba\beta}{a\beta} - \gamma\right) = 0 \text{ or } \left(a\gamma^2 + \lambda\beta\gamma + (a\lambda\beta - da^2)\right) = 0.$$

Note that the quadratic factor above is equivalent simply the characteristic equation of the endemic equilibrium of the basic infection model given by (6) and (7). The eigenvalues for this model with the immune system are

$$\gamma_1, \gamma_2 = -\lambda\beta \pm \sqrt{(\lambda\beta)^2 - 4a^2\lambda\beta + 4da^3} \text{ and } \gamma_3 = \frac{c\lambda\beta - cda - ba\beta}{a\beta}.$$

For the second equilibrium point to be locally stable, the real parts of γ_1, γ_2 and γ_3 must be all negative. From the calculations given in 3.2 we know γ_1, γ_2 have negative real parts when $R_0 > 1$. γ_3 is negative when $cad(R_0 - 1) < ba\beta$.

The characteristic equation for the third equilibrium point is not easy to factor, so the Routh-Hurwitz stability criterion is used (see Appendix A). From this theorem, we determine that

the third fixed point is stable when $cad(R_0 - 1) > ba\beta$. So we can see that there are two transcritical bifurcations: between the first and second equilibrium points at $R_0 = 1$, and the second and third fixed points at $cad(R_0 - 1) = ba\beta$.

The graphs below show the dynamics of the CTL model when the parameters are chosen such that the third equilibrium is stable ($cad(R_0 - 1) > ba\beta$).

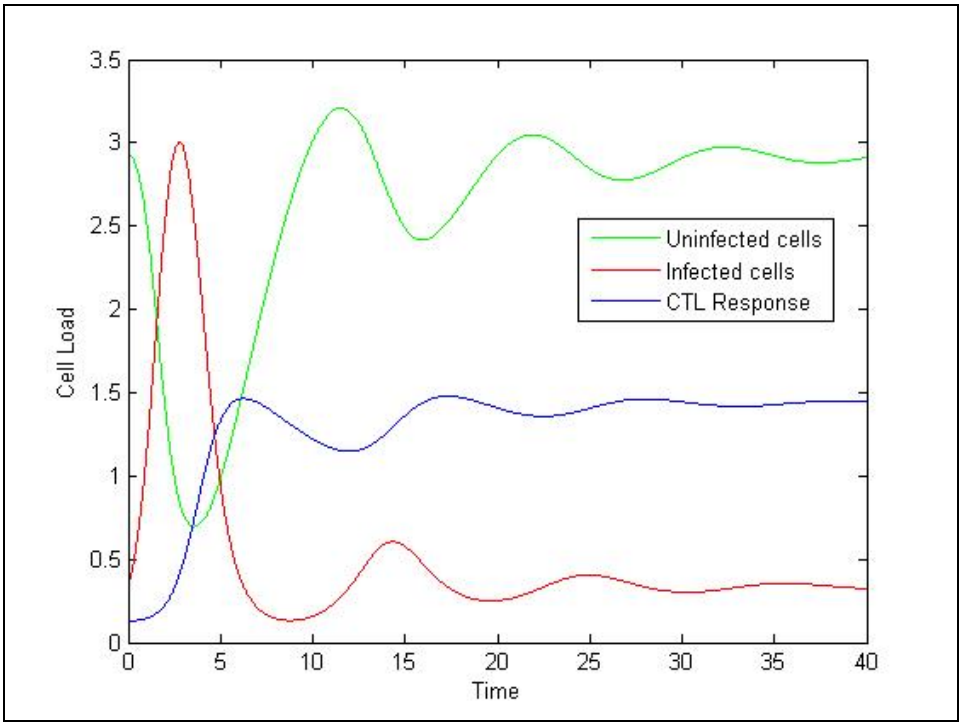


Figure 4 Basic CTL Model Dynamics when the Endemic Equilibrium is Stable

Although the uninfected cells recover, the infection never totally decays to zero. These dynamics are appropriate for persistent viruses, but not acute viruses. Parameters are: λ=1, d=0.175, β=0.5, a=0.02, p=1,c=0.3, b=0.1.

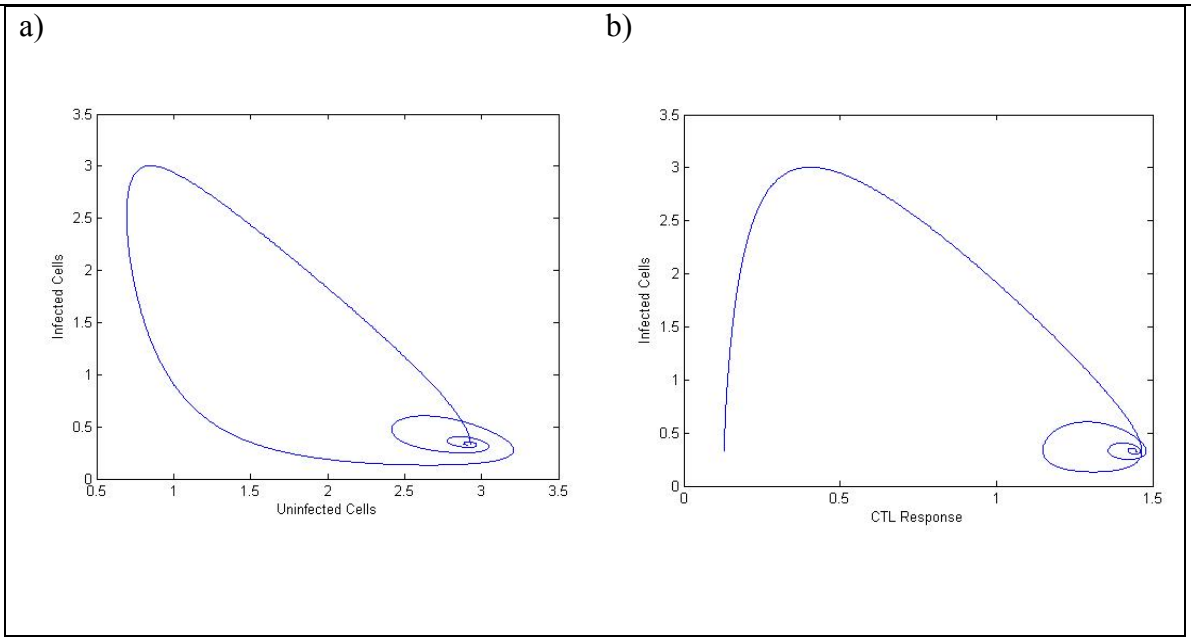


Figure 5 Phase Portraits of the Basic CTL Model

a) Phase portrait of uninfected cells against infected cells (counter clockwise), which shows a stable spiral. b) Phase portrait of infected cells against CTL Response (clockwise), which is also a stable spiral. Parameters are the same as the previous figure.

The predator-prey-predator behavior of the CTL model over time is displayed in figure 4. In this simulation, we see that the endemic equilibrium is reached. For the phase portrait of uninfected cells versus infected cells, in part a) of figure 5, the stable spiral "travels" inward in a counter clockwise direction, similar to a predator-prey type oscillation towards equilibrium. The phase portrait of the infected cells against the CTL response, in part b) of figure 5, is a stable spiral is in a clockwise direction. Again, we see predator-prey type behaviors for the immune response reacting to the density of the infection. Initially the immune response is at low levels, but when the infection takes off (since $R_0 > 1$), the immune response kicks in slowly until it reaches a threshold that can kill off the infected cells. Once this level is reached, the number of infected cells fall, and the immune response decreases as well.

4.2.3 Application in an Acute Virus Settings

Wodarz & Lloyd (2004) used this model to describe the dynamics of wild type and resistant viral strains during the treatment of an acute virus infection. As previously mentioned, the model is not appropriate for acute infections, due to the predator-prey dynamics, even though the infected cell counts come to low levels, the model does not allow them to die out.

The Basic CTL Model is a continuous model, therefore numbers in each cell population (uninfected, infected, CTL response) are treated as continuously varying quantities. If the model treated the numbers in each population as individual cells, as in a stochastic model, then it would take into account discrete events. When the infection decays to a certain level, close to zero, the stochastic model would allow the infection to completely die out (the stochastic model would not allow a fraction of an infected cell to remain alive, therefore it could not produce more infection), whereas the continuous model allows the infection to increase again.

Wodarz & Lloyd artificially force the model to work for an acute virus by setting b to a low value, and stating a low infection threshold beyond which they ignore the dynamics and assume the infection has died out. They do so, assuming that if stochastic effects were taken into account, the infection would be cleared. For instance, in figure 4, they would have considered the acute infection to be resolved at around time = 9.

Another way to address the weakness of this model in an acute setting is to improve the immune response. The immune response in (10) assumes that all CTLs generated are active

in killing off infection, whereas in reality it can take the immune system a while to kill off an infection. Additional immune response models will be reviewed and suggested to address this weakness. In the meantime, we entertain a special case of this CTL model that may be more accurate for modeling acute infections.

4.3 A Special Case of the Basic CTL model

We introduce this special case, broadening the interpretation of z to include CTL cells and T-memory cells. Using equations (8), (9), and (10), we set b=0, assuming that any natural death of the CTLs are insignificant due to any production of CTLs. Equilibrium 3, the endemic equilibrium from the Basic CTL model, reduces to

3. CTL Memory: $$(x^*, y^*, z^*) = (\frac{\lambda}{d}, 0, z^*)$$

This equilibrium is neutrally stable, and depends not only on parameters of the model, but also on initial conditions. During infection T-memory cells are generated and continue to remain in the system after the infection has been cleared. They are maintained at a certain level, which allows the host to fight off future infections of the same, or similar type.

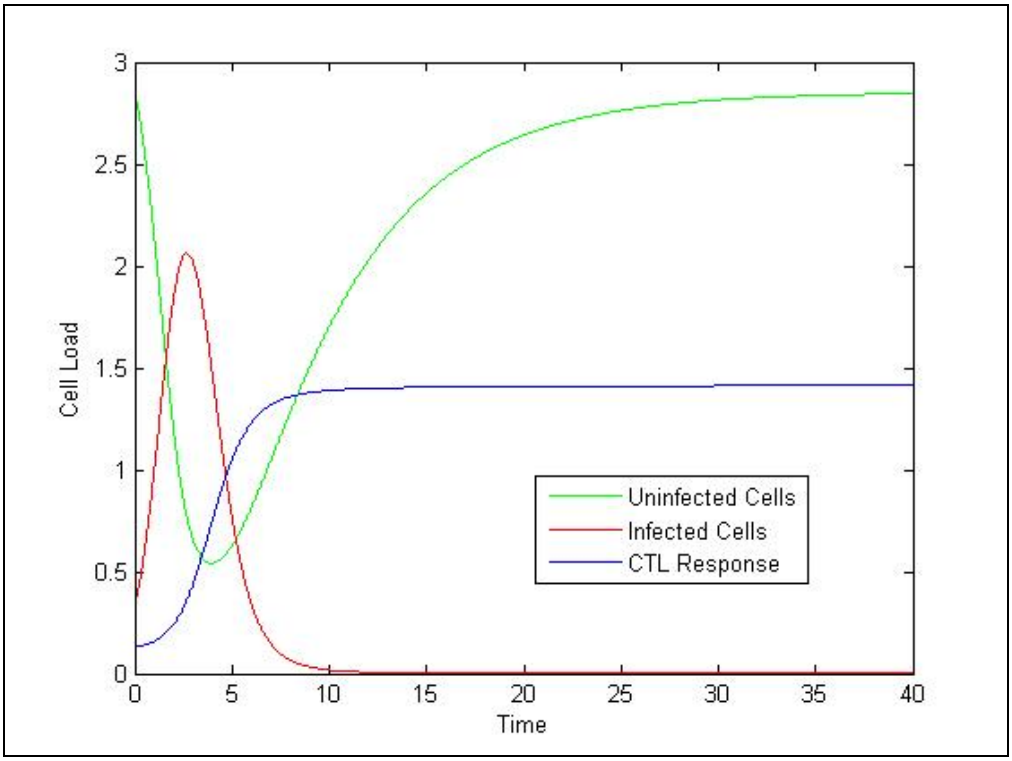


Figure 6 Basic CTL Model Dynamics with *b*=0.

Parameters are: λ=.5, d=0.175, β=.5, a=0.02, p=1, c=0.3, b=0.

The benefit of the $b = 0$ case is that the infection dies out quickly and there are no prolonged predator-prey dynamics. By our assumptions, we suggest that the equilibrium reached by the immune response accounts for an increased level of effector and memory T-cells specific to the virus strain that the CTL response has cleared. Because of this, the system has acquired lifetime immunity against this virus. This representation is not adequate for modeling viruses for which a host cannot build up a lifetime immunity. One characteristic of this model is that when a second infection is introduced into the same system after clearing the first infection, the CTL response increases in a similar fashion as it did for the first infection, as can be seen

in figure 7. The secondary viral infection, with the same transmission rates, quickly dies out, due to the acquired resistance to this viral strain.

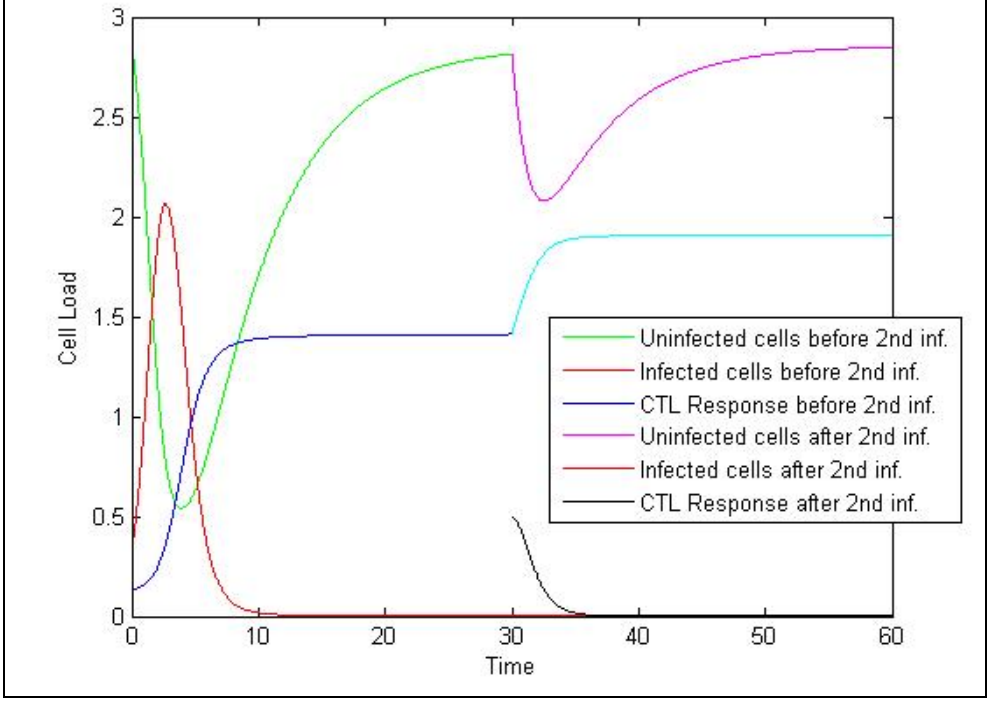


Figure 7 . Re-Introduction of Infection in Basic CTL Model with *b*=0.

A second infection is introduced at a level of y_0=.05 at time = 30, which is the reason for the discontinuity. Parameters are the same as the previous figure.

The weakness of this special case is that the equilibrium level for the immune response will continue to increase, with no limit, as the infection is reintroduced into the model. Another variation of the Basic Virus Model that accounts for acute viruses, that may address some of these weaknesses, is called the Target Cell Limited Model, was introduced by De Boer and Perelson in 1998.

5 TARGET CELL LIMITED MODEL

5.1 Target Cell Limited Dynamics

Recall that in the Basic CTL Model (Figure 4) we chose parameters that enabled the infection to reach an endemic equilibrium. We can attribute this to ongoing input of uninfected target cells into the system, which enables the virus to continue replication. However, the Baccam et al. version of the Target Cell Limited Model, prevents production of uninfected cells, or target cells, therefore once the population of uninfected cells is eliminated, any new free virus in the system cannot be established. Eventually this leads to the infection being cleared due to a natural death of the virus cells.

$$\dot{x} = -\beta xv \tag{11}$$
$$\dot{y} = \beta xv - a\, y \tag{12}$$
$$\dot{v} = ky - uv\,. \tag{13}$$

This model was discussed in the context of modeling the influenza A virus, assuming that the limited availability of the epithelial nasal target cells limits the virus infection, rather than the immune response. This model assumes that since acute infections occur over such a shorter scale than the timescale of the demographics for the target cells that natural birth/death rates are small enough to be ignored.

A schematic diagram is given in figure 8 to describe the Target Cell Limited dynamics. Notice that unlike the diagram for the Basic Virus Model (figure 2a), the uninfected target cells have neither a birth nor a death term.

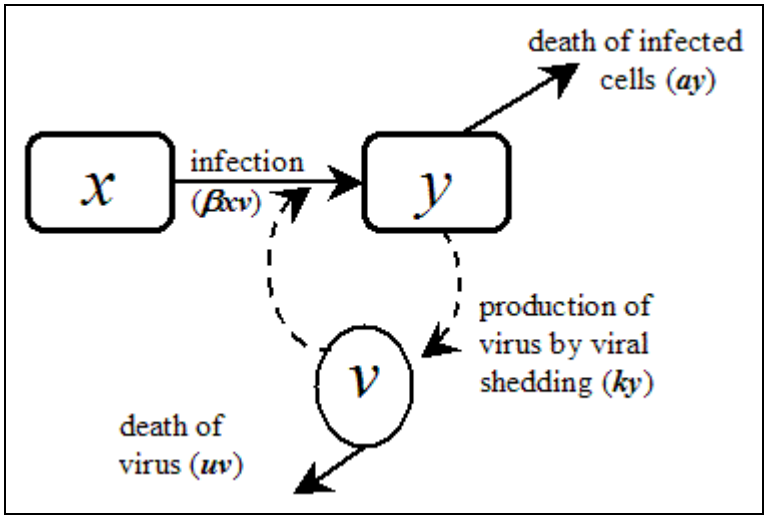


Figure 8 Target Cell Limited Model

x: uninfected target cells; ***y*:** infected cells; ***v*:** virus count

The target cell limited approach is a good model for a short term infection, but the main weakness is that any immune response that could have developed is being ignored. Also, the model has no non-trivial equilibrium; even if there is no infection, the healthy cells will all die out eventually. This is not realistic. Also, the average number of secondary infections produced by one infected cell is given by, $R_0 = \frac{k\beta}{ua} x_0$ (Baccam et al. in press). This value depends on the initial availability of target cells, since there is no infection free equilibrium.

Building on the QSS assumptions, from section 2.4, the Target Cell Limited model can be rewritten as

$$\dot{x} = -\beta xy \tag{14}$$

$$\dot{y} = \beta xy - a\,y, \tag{15}$$

simplifying the dynamics. If uninfected target cells exist in the system, then infection can become established. However, once the uninfected cell population is depleted, the infection will die out, as shown in figure 9.

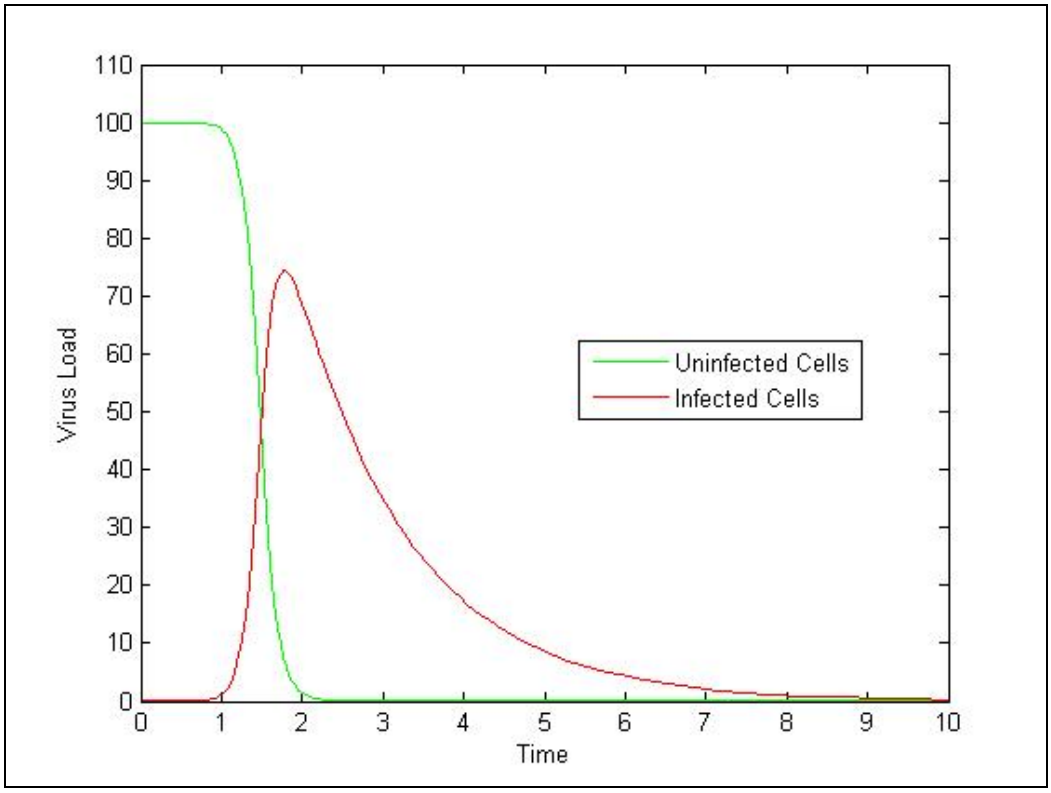


Figure 9 Target Cell Limited Model Dynamics

Notice how the load of the uninfected cell decays to zero, which artificially causes the infection to clear. Parameters are: $\beta = .1$, $a = 0.7$.

6 CTL Proliferation Models

As discussed in section 1.2, the immune system responds to certain foreign antigens with specific antibodies and T-cells to fight off that antigen. All previous models have lacked

detail on how the immune system actually produces this response. Many current CTL models are analogous to simple predator-prey models, where the rate of expansion and contraction of the immune cells is updated according to the current density of the virus present in the host (Bell 1970, Nowak and Bangham 1996, De Boer and Perelson 1995, De Boer et al. 2001). Research has clarified the dynamics of the immune response in acute and persistent viruses (De Boer et al.. 2001, Antia et al.. 2002). For acute viruses, there are three main stages of immune response: expansion, contraction, and memory (Murali-Krishna et al. 1998, Ahmed and Gray 1996).

Due to the peptides expressed on the surface of an infected cell, there is an expansion in CD8 cells that may vary depending on the peptides. After the infection has been controlled, the CD8 cells decline through apoptosis as well as converting to memory cells. Whereas the basic CTL model displays a predator-prey behavior between the immune response and the pathogen, models have been suggested that distinguish the stages of the immune response into the following subpopulations: the expansion stage is composed of naïve CTL cells, which do not necessarily kill the virus, rather they divide, escalating the load of effector cells; the contraction stage is when the immune system has halted division of CTL cells, so the CTL load begins to decrease, while some of the effector cells turn into long term T memory cells (Antia et al. 2003, De Boer et al. 2001). These models provide the basic expansion-contraction dynamics of the CTL immune response. We will discuss what Antia et al. dub the ‘strict program’, which forces the immune response to commit to a program based on the initial introduction of the pathogen, rather than continually updating the response by tracking the pathogen.

6.1 Strict Program Model

The Basic CTL Model is deficient in its predator-prey dynamics in that the immune system loosens its grip as virus (infected cells) decline, which is when the grip should strengthen, eliminating the pathogen. It has been shown (Badovinac et al. 2002) that the immune response continues at the same programmed rate whether the antigen is completely removed or not. This suggests the immune response is dependent on the initial invasion of the pathogen, rather than on its following behavior. So, the density dependent Basic CTL Model does not behave in accordance with these results. Also, as mentioned in section 1.2, there are pathogens like the malarial parasite and HIV that subvert the immune response, so the immune system doesn't have the chance to update the response based on the changes in the pathogen (Gooding 1992, Antia et al. 2003). CTL proliferation models allow programmed divisions of CTLs, which carry out the same behavior independent of the pathogen density. This allows a stronger effector cell load when the virus infection is at low levels, enabling clearance of the virus (Wodarz and Thompson 2005). The proliferation model described by Anita et al. (2003) accommodates for both of these weaknesses of the Basic CTL Model.

The strict program model defined by Antia et al. (2003) considers the following variables: z_0 represents naïve immune cells that have undergone 0 divisions, z_1 denotes recruited immune cells, z_n ($n > 1$) represents the division stage of the recruited cells, E represents all of the effector cells, and M to represent the memory cells, and y, the pathogen.

$$\dot{y} = ry(1-\frac{y}{c}) - hyE \tag{16}$$

$$\dot{z}_0 = -bz_0 y \tag{17}$$

$$\dot{z}_1 = bz_0 y - k_1 z_1 - d_1 z_1 \tag{18}$$

$$\dot{z}_n = 2k_{n-1} z_{n-1} - k_n z_n - d_n z_n, \; n = 2,3,4... \tag{19}$$

$$E = \sum_{n=1}^{a+b-1} z_n \tag{20}$$

$$M = \sum_{n=a+b}^{\infty} z_n, \tag{21}$$

where the cells are effector cells when $n<(a+b)$ and after that, they are memory cells. The proliferation phase occurs during the first a divisions and the production rate of the effector cells exceeds the death rate ($k_n > d_n$). The contraction phase takes place during the subsequent b divisions and the death rate exceeds the production rate ($d_n > k_n$). During the memory phase, the production rate equals the death rate ($d_n = k_n$). In this model, a logistic type growth term is used for the pathogen since they assume that the numbers of infected cells initially increase at rate r, gradually slowing as a carrying capacity c is reached. They suggest this carrying capacity could be imposed by a target cell limitation. Also, infected cells are killed at a rate proportional to the product of pathogen density and effector cell density, which accounts for the $-hyE$ term. Antia et al. side step modeling the target cell and pathogen dynamics, so we introduce a model in section 6.2.1 that includes a target cell description. They also extend this model to multiple viral infections, but no analysis is provided.

6.2 Alternative Proliferation Models

The model proposed by Antia et al. (2003) is a good step towards understanding the complicated phases of the immune response, including effector and memory cells. However,

the model does not provide a source of uninfected target cells. Several other CTL proliferation models, not covered here, include Jones and Perelson (2005) and Wodarz and Thompson (2005).

6.2.1 CTL Program Incorporating Uninfected Cells

Presented below are variations of Antia's expansion-contraction immune model, incorporating uninfected target cells into the model. We also allow the naïve CTL cells to be driven by the pathogen, which provides an expansion phase that Antia et al. did not, forcing a limited number of naïve immune cells. We suggest that as long as the infection has not been cleared, that CTL cells could still be generated to recruit effector cells. We use the Basic CTL model, or effector cell model, in conjunction with the expansion-contraction type immune model, where x is the uninfected target cell population, y represents the infected cells, and z_n represents the divisions of the CTL cells, some of which become effector cells (n=2,3,..m-1) and others that become memory cells (n=m,m+1,…. ∞).

$$\dot{x} = \lambda - dx - \beta xy \tag{22}$$

$$y = \beta xy - ay - pyE \tag{23}$$

$$\dot{z}_0 = -bz_0 y \tag{24}$$

$$\dot{z}_1 = bz_0 y - k_1 z_1 - d_1 z_1 \tag{25}$$

$$\dot{z}_n = 2k_{n-1} z_{n-1} - k_n z_n - d_n z_n, \quad n = 2,3,4...,m-1 \tag{26}$$

$$\dot{z}_n = 2k_{n-1} z_{n-1}, \qquad n = m, m+1...\infty \tag{27}$$

$$E = \sum_{n=1}^{m-1} z_n \tag{28}$$

$$M = \sum_{n=m}^{\infty} z_n, \tag{29}$$

As well, the target cell limited population can be used, where λ=d=0, but we do not analyze this as we are interested in the effects of the immune response where target cells are naturally

produced. For an acute infection, we would hope to see that the stages of immune response: naïve, expansion, contraction and memory will bring the infection completely to zero, however this is not the case, as seen in figure 10. Note how the infection does not clear, rather it reaches an equilibrium. The nice aspect of this model is that the memory CTLs reach an equilibrium, however this has been seen before in the Basic CTL Model, when we assume that z represents effector and memory cells (figure 4 and figure 6).

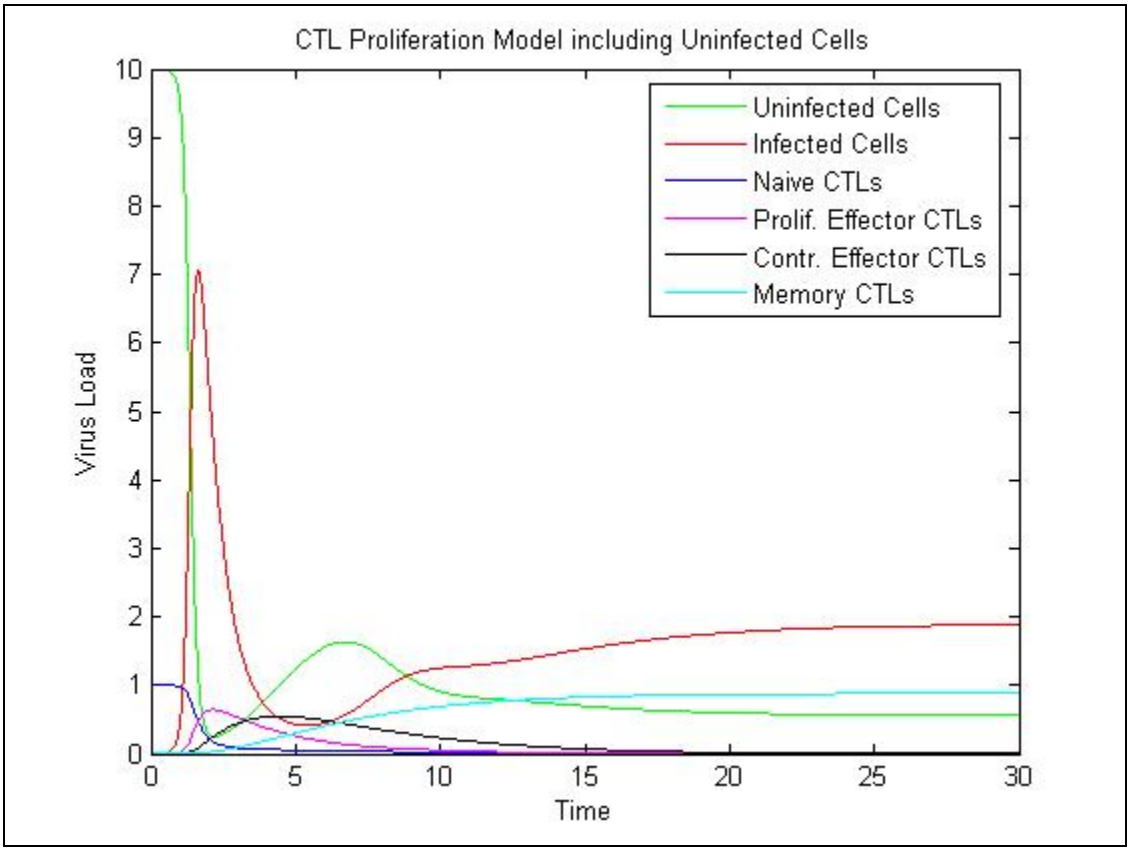


Figure 10 CTL Proliferation Model Dynamics

Using equations (22)-(29) for n=1,2 and m=3. Parameters are: $\lambda = 1$, $d = 0.1$, $\beta = .9$, $a = 0.5$, $p = 1$, $b = 0.3$, $k_1 = 0.3$, $d_1 = 0.2$, $k_2 = 0.1$, $d_2 = 0.2$, $k_3 = 0.3$, $d_3 = 0.3$.

7 WILD/RESISTANT INFECTIONS AND TREATMENT DYNAMICS

7.1 Two Strain Wild-type and Drug Resistant Infections

As mentioned in section 1.1, understanding drug resistant viral infections is at the forefront of research due to viral infections like HIV-1, which rapidly produces resistant strains and causes life-long infection, and the heightening resistance to Influenza-A drugs. Wodarz and Lloyd (2004) use the Dietz (1979) model in a within host setting. This model is typically used in an epidemiological setting to describe the co-circulation of two strains of infection in a population prohibiting superinfection. In the model presented here, Wodarz and Lloyd assumed that the host individual was simultaneously infected with two viral strains (keep in mind the target cell cannot be simultaneously infected) using the following model:

$$\dot{x} = \lambda - dx - \beta_w x y_w - \beta_r x y_r \tag{30}$$

$$\dot{y}_w = \beta_w x y_w - a y_w - p y_w z \tag{31}$$

$$\dot{y}_r = \beta_r x y_r - a y_r - p y_r z \tag{32}$$

$$\dot{z} = c(y_w + y_r)z - bz. \tag{33}$$

The transmission parameter for the wild-type infection and drug resistant infections are represented by β_w and β_r respectively. It is clear in (30) that competition exists between wild-type and drug resistant viruses to establish infection in the target cells. For this model, $R_0^w = \frac{\beta_w \lambda}{ad}$ and $R_0^r = \frac{\beta_r \lambda}{ad}$, therefore the transmission parameters, β_w and β_r, will drive the competition between the two strains in the initial stages of infection, determining early on which strain will win out. A common ecological concept is that two species occupying the same niche cannot exist; therefore, two strains infecting the same target cell pool can not

coexist over time. Eventually one will out overtake the other. Naturally, we expect the wild-strain to be stable therefore with a higher transmission rate than the drug resistant strain, $\beta_w > \beta_r$. However when treatment is given, this influences the transmission rates of the viruses. In the Wodarz and Lloyd study, it is assumed that β_w post treatment is much smaller than its value pre-treatment. And, it is also assumed that post treatment, $\beta_r >> \beta_w$. The phenomena that the resistant strain is less fit in the absence of treatment due to being out competed by the wild type strain is termed "cost of resistance".

Co-infection of wild-type virus and drug resistant virus within a host allowed them to study the effect of a drug treatment on the dynamics of two acute strains of rhinovirus, so they set *b* to be very small. As mentioned above in section 4.2, Wodarz and Lloyd force a low infection threshold beyond which they assume the infection has died out, ignoring any predatory-prey type dynamics that would apply to chronic virus dynamics. Maintaining this threshold, they simulate applying treatment to the wild-type viral infection to see how it will affect the dynamics of the drug resistant viral infection.

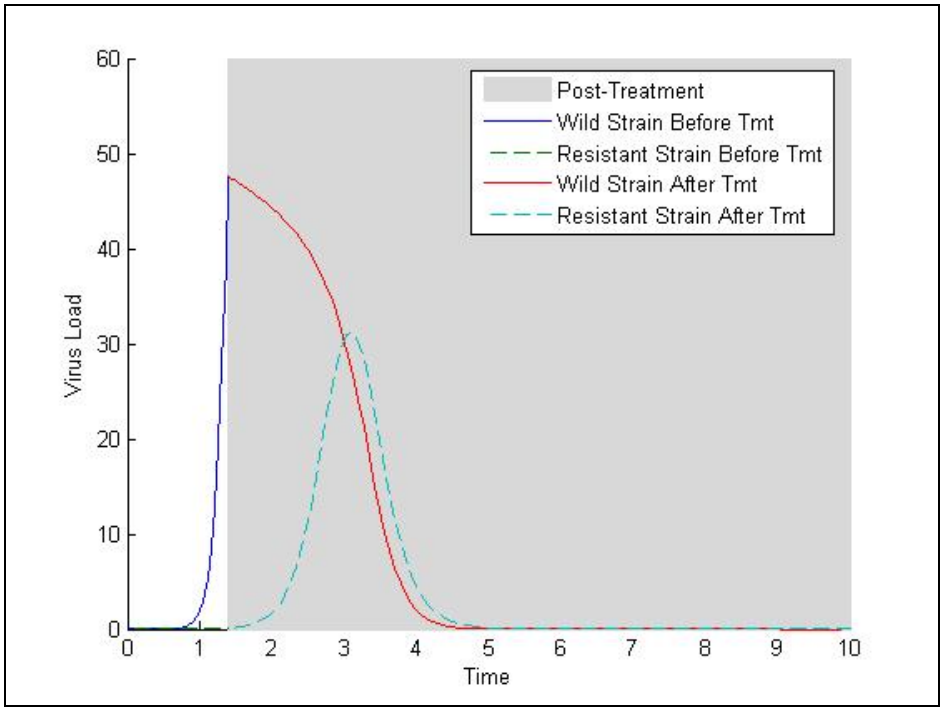
7.2 Treatment of Wild-type Drug Resistant Infections

7.2.1 Treatment Dynamics in an Acute Setting of the Basic CTL Model

Suggestions of Wodarz and Lloyd (2004) are that the application of drug to a co-infected host individual will dampen, or clear, the wild-type infection, whereas the drug will not have an effect on the resistant strain. The figures presented by Wodarz and Lloyd (2004) do not plot the CTL response, but they state that once the drug clears out the wild infection, the

immune response is what eliminates the wild strain. In order to demonstrate the drug being activated, the drug effect is turned on by decreasing the β_w value. The drugs, like Tamiflu and Relenza, prevent replication of the virus, which translates to prevention of future infection of target cells. Since this simulation is done in an acute setting, it is assumed that the treatment effect will remain consistent throughout the simulation. However, a more realistic model would incorporate the fact that drugs are metabolized by the body, therefore after reaching maximum effect, drug levels will decrease over time.

a)



b)

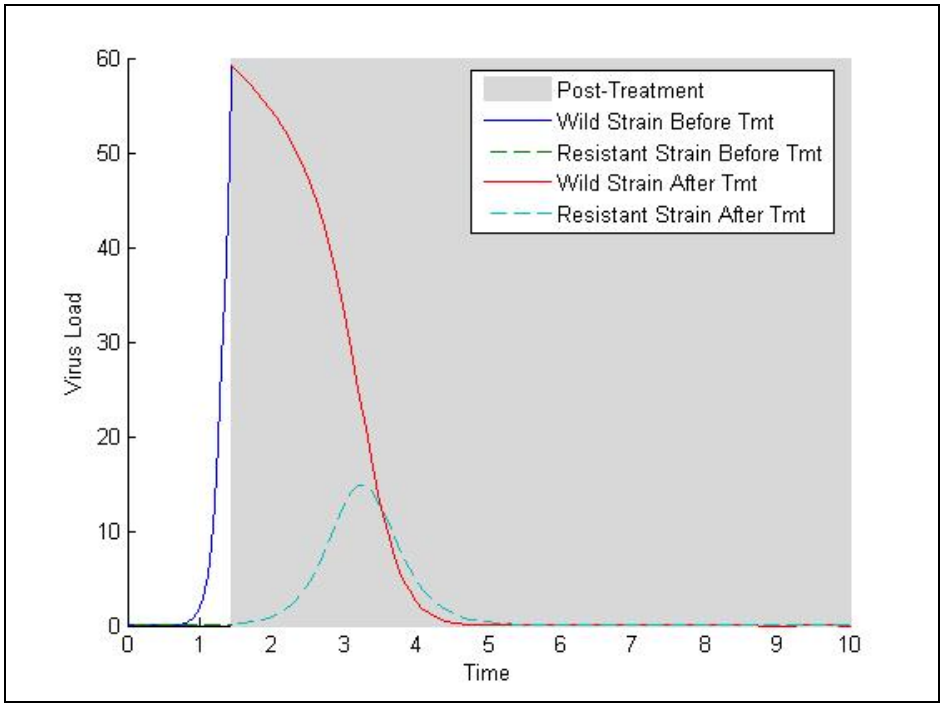


Figure 11 Treatment Effect on the Basic CTL Model

with Wild and Resistant Strains Coexisting. The treatment in a) is at time 1.40 while the treatment in b) is at time 1.45. Parameters are: $\lambda = 10$, $\beta_w = 0.1$, $\beta_r = 0.09$, $d = 0.1$, $a = 0.2$, $p = 1$, $c = 0.05$, $b = 0.01$, after treatment $\beta_w = 0.0021$.

Modeling the treatment effect on a host with acute wild and resistant viruses coexisting prior to treatment, figure 11 shows how the treatment administration time can affect the peak load of the resistant virus. These simulations suggest that applying treatment later could potentially decrease the possible peak load of the resistant virus. Wodarz & Lloyd suggest whether the resistant virus can grow or not during treatment depends on the level of CTL response that has developed before treatment is started.

7.2.2 Treatment Dynamics in Target Cell Limited Model

Applying the same simulation to the Target Cell Limited Model is of interest due to the claims that infection of HIV-1 virus is a target cell limited infection (De Boer and Perelson 1998). As well, the target cell limited model allows us to describe acute virus dynamics, such as influenza A or rhinovirus (Baacam et al. 2006). We point out that in this target cell limited model we apply the QSS assumption between virus particles and infected cells, and we remind that there is no immune response built into this model:

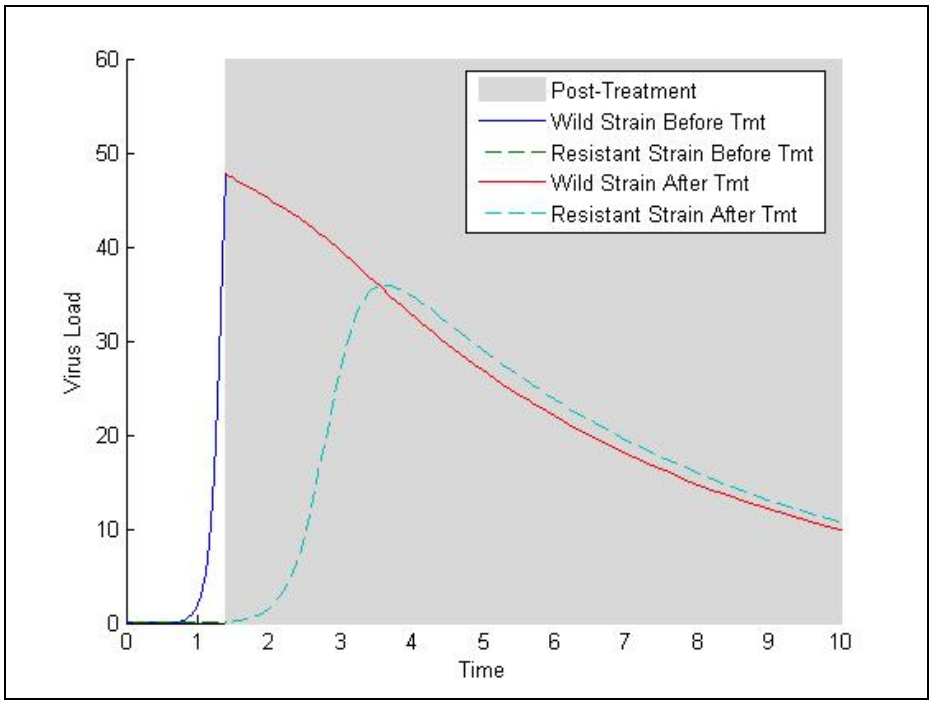
$$\dot{x} = -\beta_w x y_w - \beta_r x y_r \tag{34}$$

$$\dot{y}_w = \beta_w x y_w - a y_w \tag{35}$$

$$\dot{y}_r = \beta_r x y_r - a y_r \tag{36}$$

Since the results of this model (figure 11) favor the results from Wodarz and Lloyd's (2004) model, this leads us to speculate whether one can attribute the decreasing peak load of resistant virus solely to the CTL response. The simulation presented in figure 12 leads one to believe it is merely a lack of target cells that limits the resistant virus from replicating. This conclusion negates the results stated by Wodarz and Lloyd that the reduction in resistant peak load is merely due to the immune response.

a)



b)

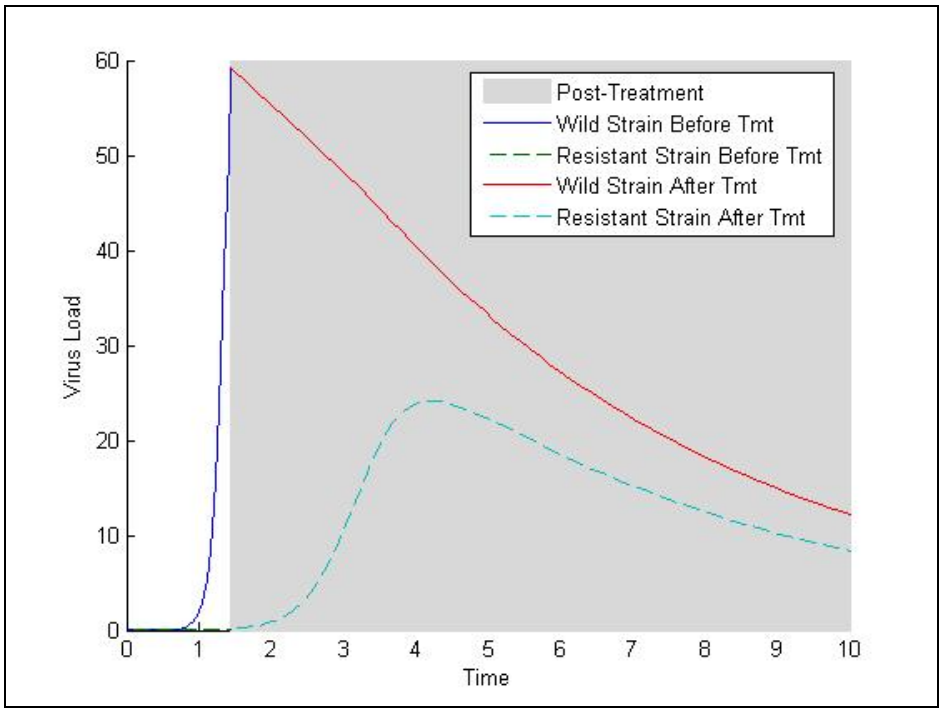


Figure 12 Treatment Effect on Target Cell Limited Model

(33) – (36) with Wild and Resistant Strains.
The treatment in a) is at time 1.40 while the treatment in b) is at time 1.45.
Parameters are: $\lambda = 10$, $\beta_w = 0.1$, $\beta_r = 0.09$, $d = 0.1$, $a = 0.2$, after treatment $\beta_w = 0.0021$.

7.2.3 Treatment Dynamics in CTL Proliferation Model

Utilizing the CTL Proliferation model from equations (22) – (29) we have recreated the wild-type drug resistant co-infection to test the effect of treatment administration at different times.

$$\dot{x} = \lambda - dx - \beta_w x y_w - \beta_r x y_r \tag{37}$$

$$\dot{y}_w = \beta_w x y_w - a y_w - p y_w E \tag{38}$$

$$\dot{y}_r = \beta_r x y_r - a y_r - p y_r z E \tag{39}$$

$$\dot{z}_0 = -b z_0 (y_r + y_w) \tag{40}$$

$$\dot{z}_1 = b z_0 - k_1 z_1 - d_1 z_1 \tag{41}$$

$$\dot{z}_n = 2k_{n-1} z_{n-1} - k_n z_n - d_n z_n, \; n = 2,3,4...,m-1 \tag{42}$$

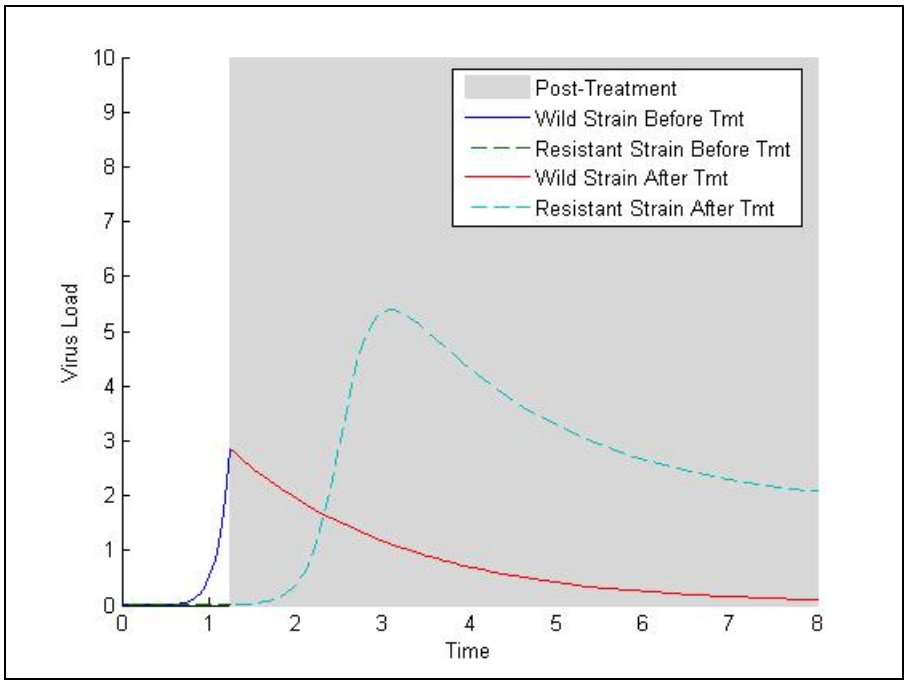
$$\dot{z}_n = 2k_{n-1} z_{n-1}, \qquad n = m, m+1....\infty \tag{43}$$

$$E = \sum_{n=1}^{m-1} z_n \tag{44}$$

$$M = \sum_{n=m}^{\infty} z_n, \tag{45}$$

As before, we are assuming the drug has no effect on the resistant virus. The effect is similar to that in the Basic CTL Model in that the later treatment is applied, the lower the peak load will be for the resistant strain infection (figure 13). As in the Basic CTL Model, we assume here that the lower peak load for resistant strain infection is due to the fact that the immune response has had a chance to establish. It is difficult to tell from this plot, however, the drug resistant infection is not cleared (although, the treatment effectively clears the wild-type infection). In fact, the drug resistant infection reaches an equilibrium, similar to how the infection in figure 10 reaches an equilibrium. The other two models, Basic CTL Model shown in figure 11 and the Target Cell Limited Model shown in figure12, have dynamics such that both infections are cleared (recall that in the Basic CTL Model Wodarz and Lloyd (2004) specify a cut off at t = 9 for acute infections, due to assumptions that stochastic

a)



b)

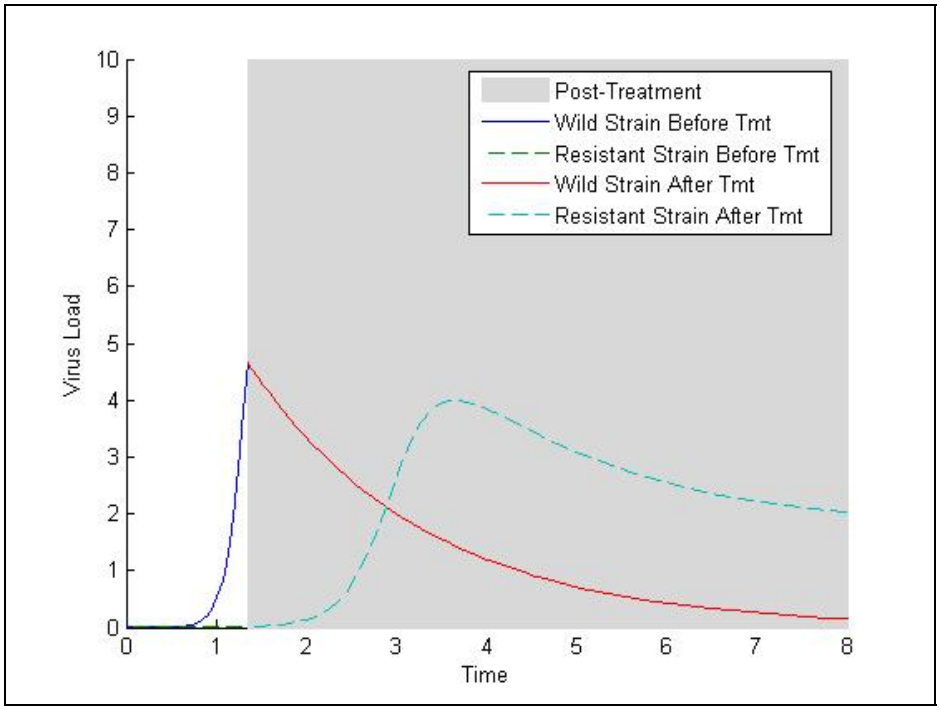


Figure 13 Treatment Effect on CTL Proliferation Model

(37) – (45) with Wild and Resistant Strains. The treatment in a) is at time 1.25 while the treatment in b) is at time 1.35. Parameters are same as in figure 10.

effects would enable the infection to clear). This leads us to note that the dynamics of the CTL Proliferation Model presented in (22) – (29) is not a realistic model for acute infection, unless one were to create a similar cut off based on a low infection load, as done in the Basic CTL Model. However, we must keep in mind that Antia et al. (2003) did not provide a description for including the uninfected target cells. They only assume the infection has logistic demographics with a carrying capacity imposed by target cell limitation and the rate the viral infection is killed off is proportional to the product of pathogen density and effector cell density.

7.2.4 Peak Loads of Resistant Virus: A Model Comparison

For a given model, we would like to determine how treatment administration time influences the maximum level of resistant virus in a host individual, also known as the "peak load" of the resistant virus. A peak load curve is created by measuring the peak load of the virus given a certain treatment administration time. Plotting these against the treatment administration time gives us the peak load curve.

A comparison of the peak load curves of resistant virus in figure 14 shows very similar results. With the chosen parameter settings, the target cell limited model slightly outperforms the CTL model, inducing the lower resistant peak load, when treatment is applied very early in the infections. However, the later treatment is applied, it seems that the CTL model outperforms the Target Cell Limited Model. The question here is not necessarily which model outperforms which, but rather, to determine which model most accurately reflects the dynamics in a realistic setting. Wodarz and Lloyd state that although the exact mechanisms

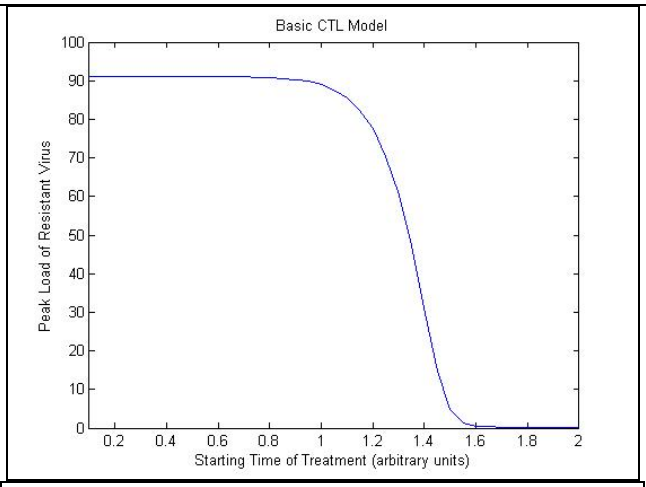
by which acute immune responses are induced are not known, that the results of their simulation should not depend on the details of how this immune response is induced, but rather depend on the well-documented assumptions that immunity rises in response to wild-type infections. Given that such similar results appear using a model deficient of an immune response (target cell limited model) leads us to assume that the effect of the target cells running out is near equivalent to the effect of having the resistant virus infection die out due to a CTL response.

The CTL Proliferation model in figure 14c has a similar shaped peak load curve for resistant virus, although you can see some fluctuation in the decline of the curve. Since this proliferation model was using the Basic CTL equations for uninfected target cells and the two strains, we see the predator-prey type dynamics between the uninfected cells and the resistant strain. One thing that we cannot see in this graph is that the immune response no longer has the predator-prey dynamic due to the CTL proliferation model used for the immune response.

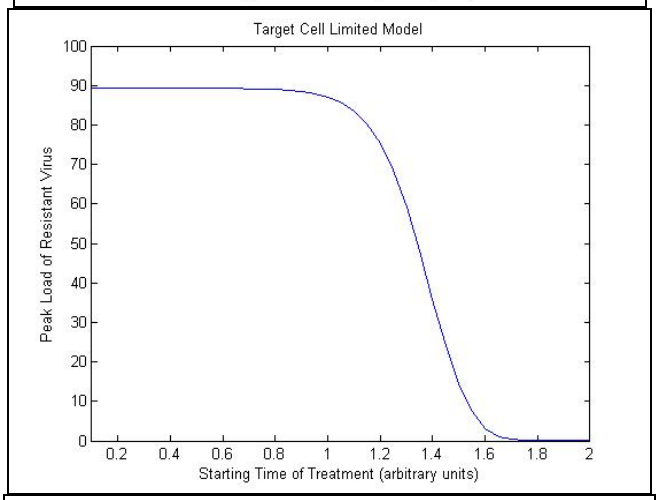
As mentioned previously in section four, the Basic CTL model was developed for chronic infections, and the weaknesses of using this model for acute infections are the predator-prey type dynamics. For the chosen parameters, if the simulation was allowed to run past time 7, analogous to lifting the infection threshold cutoff for the acute virus setting, one would see that the peak load begins to fluctuate for the CTL model. This is due to the fact that the resistant virus is displaying predator-prey type behavior and the later peaks of the resistant virus are higher than the initial peak. This can lead us to a conclusion that in a chronic virus

setting a different treatment regimen must be considered (such as combined treatment regimes for HIV-1 infections). One message is consistent between the models, the later the treatment is administered for the wild virus, the lower the resistant peak load. In determining treatment administration time, one must not only consider the peak load of the drug resistant infection, but also the severity of the adverse reactions of each type of infection. If there is knowledge that an individual is already co-infected, and the adverse reactions of the resistant strain are more severe than the wild-type, then it would be beneficial, from a quality of life perspective for that individual, to treat the wild-type after the immune system has had a

a)



b)



c)

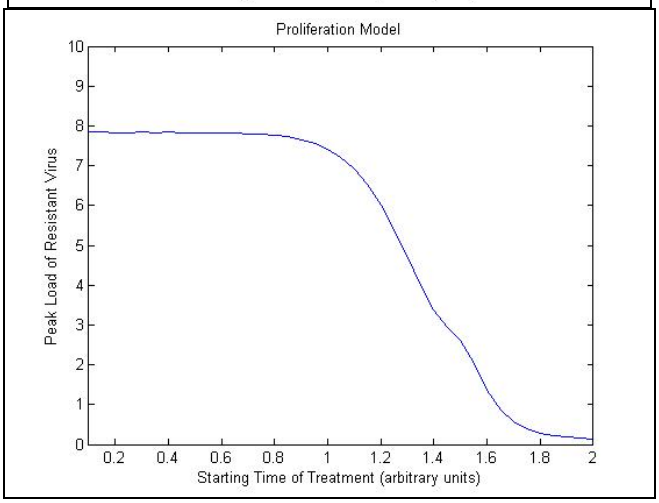


Figure 14 Load of Resistant Virus as a Function of Time at Which Treatment is Started

a) Basic CTL Model. Parameters are the same as in Figure 10.
b) Target Cell Limited Model. Parameters are the same as in Figure 11.
c) CTL Proliferation Model. Parameters are the same as in Figure 12. The difference in peak load as compared to a) and b) is due to differences in parameter values. The shape of the curves should be noted.

chance to establish a CTL response, which will assist in lowering the peak load of the resistant infection, and thus lowering the chance for severe adverse reactions. However, there is a conflict between the individual gaining the benefit of the treatment, and the population level issue resistance to the virus treatment growing within the population. If treatment is available for a weak virus, and it can be administered such that it decreases the adverse events of a co-infection of both weak and resistant viruses, then some would argue that this should not be abandoned due to the potential that the population can grow resistant to the treatment. There must be a balance such that the treatment is beneficial, yet it is used wisely such that the population does not quickly become resistant to the drug. Different selection processes exist based on age and other demographics. The determination of which individuals to treat would likely depend on what type of virus is spreading in the population and what demographics the virus affects.

8 DISCUSSION

Having reviewed the development of the virus dynamics models existing in the literature, it is evident that, although there are several good models for persistent viruses, a strong model has yet to be produced for modeling acute virus dynamics. A special case is given of the acute virus model suggested by Wodarz and Lloyd (2004) in (8) – (10), which is itself a variation of the acclaimed basic infection model developed to model HIV infection (Nowak and Bangham 1996, De Boer and Perelson 1998). The Wodarz and Lloyd model is a weak model of acute virus infections in that the infection never decays to zero, therefore they

ignore the virus dynamics produced by this model after infection has reached a very low level. They must do this for an acute infection since the predator-prey type dynamics are not relevant for an acute infection. A special case of the model that we presented assumes that the immune response contains effector cells and memory cells, and that the production of memory cells and the natural death of the immune cells balance out, allowing us to set b=0 in (10). The benefit of this case is that the infection dies out quickly and there are no prolonged predator-prey dynamics. Because the immune response is maintained at the level that clears the pathogen, the host has acquired lifetime immunity against the acute virus. Also, as shown, if a second infection of the same virus strain, is introduced, the infection will be cleared quickly, but an additional immune response is induced, increasing the load of immune cells. An improvement to this model would be to separate the effector cells and memory cells into two separate equations, similar to allowing $b = 0$. This is left for future research.

The immune system is very complex, with many different types of cells that play a part. Recent experimental results show that an immune response can be induced by a brief exposure to a pathogen, and the response will undergo sustained proliferation of effector cells, followed by production and a maintained level of memory cells (Badovinac et al. 2002). Advances have been made in modeling the phases that the immune response goes through, such as the Strict Programmed CTL Model (Antia et al. 2003), and other similar models. We suggested another proliferation model that incorporates uninfected target cells into the model. Our model takes advantage of the QSS assumption of virus and infected cells.

Despite the new ideas, much work remains in determining the local stability analysis and the detailed behavior of the model.

Drug resistance is a very relevant problem that deserves much attention. The treatment dynamics studied in an individual co-infected with wild and resistant virus strains were originally presented by (Wodarz and Lloyd 2004) for an acute virus setting. Their conclusion was that the drop in peak resistant virus load due to later treatment administration was solely due to the fact that the immune response had been induced to a level that would clear the resistant virus infection. A comparison of this simulation was presented using the Target Cell Limited Model, and our conclusion is that the decrease in resistant virus load could not only be attributed to an increase in the immune response, since there is no immune response in the target cell limited model. In this case, the resistant virus did not have enough target cells to infect, so it died out. Out of the two models, we believe that the Wodarz and Lloyd model is the most realistic model between the two for acute infections (in particular when b, in (10) is close to or equal to zero), since it does not ignore the immune response component of the virus dynamics.

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10 APPENDICES

10.1 Routh-Hurwitz Stability Criterion

As summarized by May (1973), If A is an *m* x *m* matrix, the equation for the eigenvalues γ is an m^{th} order polynomial equation

$$P(\gamma) = \gamma^m + a_1\gamma^{m-1} + a_2\gamma^{m-2} + \cdots + a_m = 0\,,$$

where the coefficients $a_1, a_2, \ldots, a_m$, are all real. It is assumed that $a_m \neq 0$ since otherwise $\gamma=0$ would be a solution, and the polynomial can be factored to give a polynomial of order *m*-1, with the equivalent $a_m \neq 0$. To guarantee local stability, conditions are required on the coefficients $a_1, a_2, \ldots, a_m$ such that the roots of $P(\gamma)$ have a negative real part. This holds true when the following Routh-Hurwitz conditions hold (shown up to m=5).

$$D_1 = a_1 > 0,$$

$$D_2 = \begin{vmatrix} a_1 & a_3 \\ 1 & a_2 \end{vmatrix} > 0,$$

$$D_3 = \begin{vmatrix} a_1 & a_3 & a_5 \\ 1 & a_2 & a_4 \\ 0 & a_1 & a_3 \end{vmatrix} > 0,$$

$$D_4 = \begin{vmatrix} a_1 & a_3 & a_5 & a_7 \\ 1 & a_2 & a_4 & a_6 \\ 0 & a_1 & a_3 & a_5 \\ 0 & 1 & a_2 & a_4 \end{vmatrix} > 0,$$

$$D_5 = \begin{vmatrix} a_1 & a_3 & a_5 & a_7 & a_9 \\ 1 & a_2 & a_4 & a_6 & a_8 \\ 0 & a_1 & a_3 & a_5 & a_7 \\ 0 & 1 & a_2 & a_4 & a_6 \\ 0 & 0 & a_1 & a_3 & a_5 \end{vmatrix} > 0.$$

This also equates to the following (Murray 2002):

m=2	$a_1>0;\ a_2>0$
m=3	$a_1>0;\ a_3>0;\ a_1a_2 - a_3>0$
m=4	$a_1>0;\ a_3>0;\ a_4>0;$ $a_1a_2a_3 > a_3^2 + a_1^2 a_4$
m=5	$a_n>0$ [n=1,2,3,4,5] $a_1a_2a_3 > a_3^2 + a_1^2 a_4$ $(a_1a_4-a_5)(a_1a_2a_3 - a_3^2 - a_1^2 a_4) > a_5(a_1a_2-a_3)^2 + a_1a_5^2$

10.2 Discussion of Spatial Virus-Immune Models

We suggest a model that accounts for the ability for the host to be infected with multiple different types of virus strains, acute and/or chronic. It also separates the immune response into pathogen specific responses.

$$\dot{x} = \lambda - dx - \sum_i \beta_i x y_i$$
$$\dot{y}_i = \beta_i x y_i - a_i y_i - p_i y_i z_i$$
$$\dot{z}_i = c_i y_i z_i - b_i z_i .$$

If one wants to model acute virus infections, then set $b_i=0$. Otherwise, for chronic infections, let $b_i>0$. This model allows for one host region to be modeled, assuming that we would only be interested in one type of target cell at a time. If one wants to expand and study the multiple types of infection in multiple target regions, then a model like the following can be used:

$$\dot{x}_j = \lambda_j - d_j x_j - \sum_i \beta_i x_j y_i$$
$$\dot{y}_i = \sum_j \beta_i x_j y_i - a_i y_i - p_i y_i z_i$$
$$\dot{z}_i = c_i y_i z_i - b_i z_i .$$

This might be interesting to consider in a case where a patient has multiple infections. If we wanted to breakdown target regions for one pathogen, into multiple target regions, then this would be possible. For instance, we may want to study how a strain of rhinovirus (i=1) affects the nasal epithelial cells (j=1) as well as the respiratory epithelial cells (j=2). Rather than treating those two target regions as the same system of target cells, we are able to separate the dynamics. This may allow us to track the progress of the infection throughout the body.

The spatially explicit virus-immune dynamics model used by Funk et al. (2005) is

$$\dot{x}_{i,j} = \lambda - dx_{i,j} - \beta x_{i,j} y_{i,j}$$
$$\dot{y}_{i,j} = \beta x_{i,j} y_{i,j} - a y_{i,j} - k y_{i,j} z_{i,j}$$
$$\dot{v}_{i,j} = k y_{i,j} - c x_{i,j} - \frac{m_v}{8} \sum_{i_0=i-1}^{i+1} \sum_{j_0=j-1}^{j+1} \left[v_{i,j} - v_{i_o j_0} \right]$$
$$\dot{z}_i = c y_{i,j} z_{i,j} - b z_{i,j} - \frac{m_z}{8} \sum_{i_0=i-1}^{i+1} \sum_{j_0=j-1}^{j+1} \left[z_{i,j} - z_{i_o j_0} \right],$$

where x represents uninfected target cells, y represents infected cells, *v* represents virus, and z represents immune system response. In a homogeneous environment, the parameters remained the same from site to site, whereas with t heterogeneous environment, the parameters were allowed to change from site to site. They assumed that the target cells and infected cells were sessile.

They found the stability properties for almost all biological plausible dispersal schemes hold. When the space was assumed to be homogenous, the models differed at the peak of the infection, which risks systematic errors in estimates of parameters for acute infection dynamics. When space was assumed to be heterogeneous, spatial coupling changed the equilibrium properties of the uncoupled populations and reduced the chance of dynamic elimination of the infection. Among other results, the most noticeable property of the spatially extended model was that it reached equilibrium much faster than the Nowak and Bangham (1996) model. They also note that a gap exists in data describing infection dynamics in solid tissues.