

# HIV Infection Project

M442, Fall 2007

Due Friday November 9

## 1 Overview

Over the last fifteen or twenty years mathematical models have proven an invaluable resource in the understanding and treatment of a wide variety of diseases. One of the most studied (and most difficult) cases is that of *human immunodeficiency virus* (HIV). Though a number of drugs have been approved for the treatment of HIV infection—most notably the *reverse transcriptase inhibitors*<sup>1</sup>, AZT (zidovudine, approved in 1987, one of the first), DDC (zalcitabine), DDI (didanosine), D4T (stavudine), and the *protease inhibitors* indinavir, nelfinavir, ritonavir, saquinavir, and 141W94—none have proven generally effective for eradicating the virus. In fact, HIV evolves so rapidly, producing on average  $10^{10}$  viral particles (virions) per day, that effective treatment by any single drug is unlikely to be effective over a long period of time. For this reason, most current treatments rely on various combinations of drugs (surf, for example, the web site in reference [IAPAC]). Rather than establish optimal treatment strategies through clinical trial and error, researchers have relied in part (and when possible) on mathematical models that can suggest ranges of treatment with greater likelihoods of success.

In this project, we will study the role mathematical models have played in understanding the dynamics of HIV infection and develop models of our own.

## 2 Biological Aspects

The first step toward modeling a new phenomenon mathematically consists in learning as much as possible about the phenomenon. In this section, we will review a few concepts from biology, virology, and immunology that will be critical for the development of any reasonable model of HIV infection. Though I will try to give you what seem to be the most important points regarding HIV infection, you will need to consult at least a couple of other references.

A number of specialized terms arise, so I've included a (highly abbreviated) glossary in Section 4.

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<sup>1</sup>Hold your horses, definitions are on the way.

## 2.1 Viral Infection

A viral particle, also known as a *virion*, is essentially a renegade gene: a nucleic acid (DNA or RNA) enclosed in a protein shell (or *capsid*), about 15–25 nanometers ( $10^{-9}$  meters) in diameter. Unlike cells, virions are unable to reproduce independently and must rely on using living cells as hosts—which is why most biologists don’t consider virions to be alive in the strictest sense of the word. Built for this purpose, their protein shells act like Trojan horses, tricking unsuspecting cells into thinking they’re harmless, or even friendly. The typical MO of a virion is to attach itself to some particular type of cell (its host) and insert a copy of its DNA into that cell.<sup>2</sup> Then, when the host is stimulated to reproduce (also by the virus), it produces a multitude of copies of the virus. For example, it will be important for this project that the HIV virus creates roughly 1000 copies of itself from a single host T cell. This “budding” can take place slowly, sparing the host cell, or rapidly, destroying it.

A second type of virus, a *retrovirus*, carries a copy of its RNA (instead of its DNA) and must transcribe it into DNA before replicating. HIV is a retrovirus. This process of transcribing RNA into DNA is carried out by an enzyme called *reverse transcriptase* and adds a step to the virion’s reproductive process. On first glance, this extra step may sound like an inefficiency that would mitigate the potency of HIV, but instead it turns out to be one of its strengths. The problem with retroviruses in general is that errors are sometimes made in the transcription of RNA into DNA, leading to inexact, or mutated, copies of the virus. With billions of virions being created every day, new strains are constantly emerging, and drugs that work on one version may not work on another. (Survival of the fittest over extremely short time intervals.)

A family of drugs has been created to take advantage of this extra step by interfering with the HIV reverse transcriptase. For obvious reasons, these drugs are called *reverse transcriptase inhibitors* (see Section 1 for a short list).

## 2.2 Basic Immunology

The insidious thing about HIV is that it chooses as its host cells  $CD4^+$  T cells, the cells that control immune system response. (“CD4” denotes a protein marker on the surface of the T cell, one that thinks the HIV virus is friendly; “T” refers to the thymus, where T cells mature after migrating from the bone marrow, where they are created.) During the course of most infections, the immune system plays the role of police officers swooping in to stop an assault. In HIV infection, the police officers are the ones being assaulted.

Typically, when a foreign substance (antigen) is introduced into the body, the body elicits an immune response in an attempt to purge the thing as quickly as possible. The antigen is first encountered by *macrophages*, cells that scavenge, engulf, and examine foreign particles, then present their findings to the  $CD4^+$  T cells. These cells, often referred to as *helper T cells*, decide whether or not a response is necessary. If so (which is to say, if the antigen turns out to be a pathogen), they typically respond in three ways: 1. They build up a command

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<sup>2</sup>Most of the action at this level is determined by the shapes of things, how amino acids fit together to make proteins, and how proteins fit together with other proteins (like three-dimensional puzzle pieces). The protein on the capsid of a virus fits in this way with certain cells in the host’s body. In the case of HIV, the best fit is with T cells.

center by producing more of themselves; 2. They stimulate the production of  $CD8^+$  T cells (or *killer T cells*), who hunt down infected cells and put them out of their misery (this is called *cellular immune response*); and 3. They stimulate B cells to produce *antibodies*, which are specifically engineered to destroy the pathogen at hand (this is called *humoral immune response*).

If the immune response is successful, certain cells of each type retain memory of the pathogen, and if it ever has the audacity to come around again, they'll be ready. This, of course, is the idea behind vaccinations. In particular, currently proposed HIV vaccinations consist of benign cells covered with the same protein that covers HIV. When exposed to these cells, the body learns to respond to this particular protein without having to actually fight off the virus. Unfortunately, to date no such vaccine has proved generally effective.

## 2.3 HIV Infection

As mentioned above, one of HIV's favorite (though by no means only) target hosts are helper T cells. This leads to a direct battle during which not only is the immune system trying to destroy the virus, but simultaneously the virus is trying to destroy the immune system. The interactions here can be very subtle, and certainly are not entirely understood, but evidence over the last ten years or so indicates that the infection generally progresses on three time scales (see Figure 1, below): 1. During the first ten weeks or so after the virus is introduced into the body, the number of (uninfected) helper T cells falls dramatically, while the number of virions spikes, as does the number of infected T cells;<sup>3</sup> 2. Over a period ranging from 2 to as many as 20 years (and sometimes even longer) the number of virions and number of T cells roughly stabilize (small fluctuations persist), though with significantly fewer than the body's typical (uninfected)  $1000\text{ mm}^{-3}$  T cells (read: 1000 T cells per cubic millimeter); 3. In the final phase of the infection, a relatively short period of 1–3 years, the T cells once again crash, and the number of virions grows out of control. When the T-cell count falls below  $200\text{ mm}^{-3}$ , the person is officially classified as having AIDS (Acquired Immune Deficiency Syndrome).

## 2.4 HIV Treatment

Currently, there are two predominate types of drugs used in the treatment of HIV infection. As discussed in Section 2.1, the first of these (in order of appearance on the market) were reverse transcriptase inhibitors, which inhibit the ability of HIV to transcribe the RNA it carries into DNA appropriate for insertion into a host cell. The second type, and the ones we will consider in this project, are the more recent *protease inhibitors*.

*Protease* is one of HIV's enzymes, required to continue the process of HIV infection. It comes into play late in the HIV infection process, when a long chain of proteins that will ultimately become new virions has already been formed in the host. Before this chain can actually produce new copies of HIV, it must be cut into smaller pieces, each of which will produce a virion. This cutting is done by the enzyme protease. As the name indicates, protease inhibitors block (inhibit) protease from carrying out its job.

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<sup>3</sup>During this period the patient often exhibits symptoms similar to those of pneumonia, hence giving rise to HIV's original designation as the *gay pneumonia*.

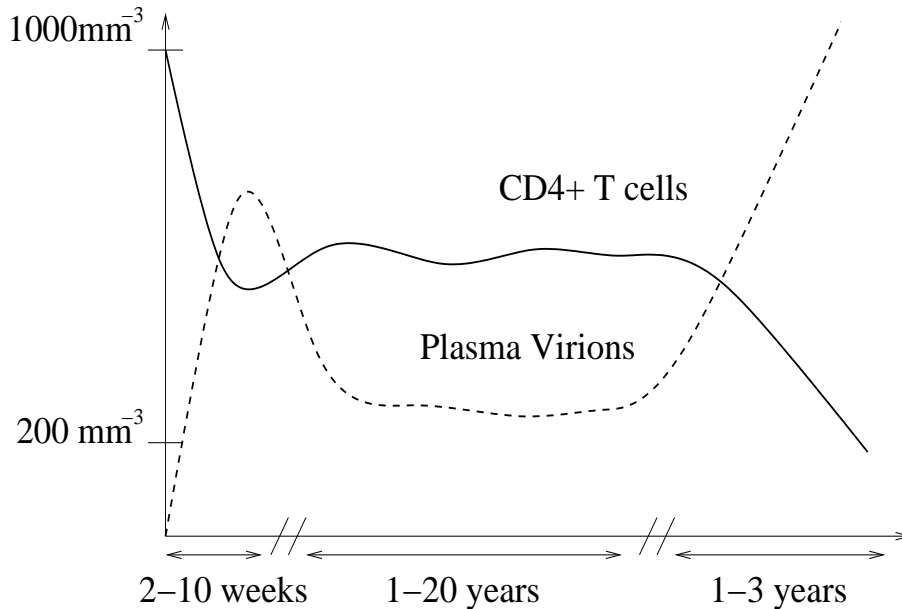


Figure 1: Time Course of HIV infection. The scale refers to the number of CD4+ T cells. In practice, the initial virion spike can be much too large to fit on this figure ( $\sim 10^6$ ).

### 3 Assignments

For this project, your group will carry out two analyses. First, you will study a very crude (though certainly useful) mathematical model of HIV proposed in 1995. Second, you will build on this model to develop one of your own. We will begin our study of HIV infection by considering a critical advance in its understanding, made in 1995 by David Ho and his collaborators. (David Ho was named *Time* magazine’s Man of the Year in 1996 for his contributions.)

1. Get a copy of the paper listed at the end of this assignment as reference [PNMLH], and also of the article beginning on p. 56 of the December 30, 1996 / January 6, 1997 copy of *Time* magazine. (These are both available, for example, in the Evans library, or more directly in (printable) electronic format from the E-journal option on the TAMU libraries web site.)
2. In reference [PNMLH], the authors have two HIV models, equations (1)–(2) for infection in steady state, and equations (3)–(4)–(5) for the infection under treatment with the protease inhibitor Ritonavir. In terms of the population dynamics we have discussed in class, describe the processes modeled by each term in these equations.
3. Use equations (1)–(2) and equations (3)–(4)–(5) to derive the authors’ expression in equation (6) for  $V(t)$ —total virion concentration.
4. The critical contribution these authors made in this paper was their determination of the virion production and clearance rate, which was previously considered much smaller (see, for example, the section entitled “A Team Effort” in *Time*’s article on

David Ho). We will repeat this calculation for Patient 107, whose viral RNA counts are given graphically in the bottom left plot of the first figure in [PNMLH]. In tabulated form, these values are given in Table 1 of this assignment.<sup>4</sup> You can mimic the log plot the authors use with MATLAB's command, *semilogy()*. Use these data points and MATLAB's *lsqcurvefit()* to determine values  $V_0$ ,  $c$ , and  $\delta$  for Patient 107. (Notice that each virion contains two copies of RNA, so you will need to divide the numbers in Table 1 by two if you want to work with virion count.) Your report should include a scatterplot of your data along with your best-fit curve.

5. Use your results from Part 4 to determine the number of virions produced per day in the entire body during the course of HIV infection. (Read the third column on p. 1583 of [PNMLH] carefully.) Explain the significance of this number. In particular, explain why this changed the way we view the dynamics of HIV infection.
6. Use your results from Part 4 to determine values for 1. the average life-span of a virion, 2. the average life-span of an infected T-cell (productively infected T-cell), and 3. the average viral generation time.
7. Use your results from Part 4 and the data from Table 1 of [PNMLH] to find an equilibrium point  $(T_e, T_e^*, V_e)$ .
8. When developing a mathematical model, researchers often try to find a balance between simplistic models that are easy to analyze and more complex models that might require very sophisticated mathematics to analyze. While the model employed in [PNMLH] is simple enough to analyze exactly, it fails to capture the full dynamics of HIV infection (as depicted qualitatively in Figure 1 of this assignment). Using the discussion of HIV progression given in class and in this assignment (as well as any outside material you find appropriate, such as the discussion in [PNMLH] and the article from *Time*),<sup>5</sup> develop your own model of HIV infection that captures the first two phases of infection shown in Figure 1 of this assignment. (Notice that you're only studying the progression of infection here, *not treatment*, so your starting point is (1)–(2), not (3)–(4)–(5).) Here's a good place to begin: Notice that in [PNMLH], equations (1)–(2), the number of uninfected T cells is assumed to remain constant. The idea there was that during the steady-state period of the infection (labeled as 1–20 years in Figure 1 of this assignment), T cells do remain constant. It's clear from Figure 1 of this assignment, however, that if you want to model the full dynamics of HIV infection, the number of uninfected T cells will have to become a variable that changes with time. Begin by adding a third equation to (1)–(2) in which uninfected T cells are created by the body and killed by the virus. Then think about how this new variable should appear in the other equations. Since we don't have any additional data, estimating new parameters is difficult, so you will want to only introduce terms you consider absolutely necessary.

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<sup>4</sup>These values were taken directly from the plot, and so are certainly only an estimation of the exact values, which the authors do not provide in the article.

<sup>5</sup>Another useful resource along these lines is the *Science* magazine special HIV issue, volume 280, June 19, 1998, also available in the Evans library.

9. Specify the units (not dimensions) of each parameter that arose in your model from Part 8. Use your results from Part 4, your equilibrium point from Part 7, and anything else you can think of to get a value for each parameter in your model.
10. Find all equilibrium points for your model and determine whether or not each is stable. (**Hint.** You have one equilibrium point from Part 7, and it should be stable, albeit perhaps not by much.)
11. Using your model from Part 8 and your parameter values from Part 9, create two stacked plots, both with uninfected T cells as a function of time on top and virions as a function of time on bottom. For the first plot, take a time interval only long enough to capture the first stage of infection. For the second plot, take a time interval that begins after the first phase of infection and is long enough to capture the second stage of infection. As initial values, you should take  $1000 \text{ mm}^{-3}$  uninfected T cells,  $0 \text{ mm}^{-3}$  infected T cells, and  $.001 \text{ mm}^{-3}$  virions.
12. According to your model, determine the total number of T-cells created in the body per day while it is in phase 2 of infection.
13. The most difficult dynamic to capture in the progression of HIV infection is the eventual collapse of T cells and the spike of virions in the final period of infection. One possible explanation for the first aspect of this behavior is that T cell production increases during infection to fight the virus, but cannot continue indefinitely at this new level and eventually gives out. Incorporate this idea by changing your model so that all T cell production stops 10 years after infection. Compute the length of time between T cell failure and the onset of AIDS (when the T cell count drops below 200), and create a stacked plot beginning at 10 years after infection and ending at the onset of AIDS. (While T cell production fatigue can explain the eventual collapse of the immune system, it does not necessarily account for the final spike of virions. A possible explanation for this final spike of virions is that HIV virions can also replicate in cells other than T cells. We will not try to model this effect.)
14. Incorporate medication by Ritonavir into your model from Part 13 in the following way: combine your model with (3)–(4)–(5) in [PNMLH] to create a full model (with four equations) of HIV infection and treatment. Assume that once medication has been given, T cell production returns to full capacity, and that all virions created after medication—whether created in T cells or elsewhere—are non-infectious. Taking T cell collapse at 10 years, and supposing medication is administered at the onset of AIDS, determine the time required until the T cell count returns to  $900 \text{ mm}^{-3}$  (90%).

## 4 Glossary

- **B cell:** Any lymphocyte not matured in the thymus. (cf. T cells.)

Times (in days)	RNA counts ( $\times 10^5$ )
1/12	2.37
1/6	2.74
1/4	2.21
1/2	2.37
3/4	1.78
1	2.05
5/4	1.78
3/2	1.15
7/4	1.33
2	1.43
3	.60
4	.49
5	.32
6	.16
7	.11

Table 1: Table of RNA counts and time for Patient 107 in [PNMLH].

- **Amino Acids:** Any of a large group of organic acids containing a carboxyl group, COOH and an amino group NH<sub>2</sub>. These guys link together to form proteins. The human body requires 20 to form its proteins: 1. alanine, 2. phenylalanine, 3. arginine, 4. asparagine, 5. aspartic acid (sold commercially as Nutrasweet), 6. cysteine, 7. glycine, 8. glutamine, 9. glutamic acid (in MSG), 10. histidine, 11. leucine, 12. isoleucine, 13. lysine, 14. methionine, 15. proline, 16. serine, 17. threonine, 18. tryptophan (in, for example, turkey; remember those Thanksgiving naps?), 19. tyrosine, and 20. valine.
- **Antibodies:** Oh, come on, I know you remember this much. But just in case: Protein molecules shaped to fit foreign proteins that are typically carried on the surface of invading germs (antigens).
- **Antigen:** A protein, toxin, or other substance of high molecular weight, to which the body reacts by producing antibodies.
- **Budding:** The process through which a multitude of virions emerge from their host cell, killing it.
- **Capsid:** The protein shell enwrapping a virion.
- **CD4<sup>+</sup> T cells** (helper T cells): Leukocytes that serve as the command center for the immune system, and one of HIV's favorite host targets.
- **CD8<sup>+</sup>T cells** (killer T cells): Leukocytes that, in the event of *cellular immune response*, attack and kill friendly cells that have been infected.
- **Cellular Immune Response:** The immune system response to a pathogen in which killer T cells are created.

- **Chromosome:** A chain of genes. Each human cell (except red blood cells) contains 46 chromosomes (22 nearly identical pairs, plus two sex-determining), each composed of hundreds or thousands of genes linked end to end. Each series of chromosomes contains the entirety of the person's DNA.
- **DNA** (deoxyribonucleic acid): A nucleic acid bound in double helical chains by hydrogen bonds between bases, forming the basic material of genes. The chains consist of four molecules: adenine, thymine, guanine, and cytosine, commonly referred to as A, T, G, and C. They fit together A–T and G–C, but no other way.
- **Enzyme:** A protein that acts as an organic catalyst to speed up specific chemical reactions.
- **Gene:** A collection of DNA (or, more specifically, a segment along the strand of DNA) whose purpose in life is to create exactly one type of protein. (Recently, this notion has come under suspicion, as there appear to be something like 30,000 genes in the human genome and roughly 100,000 types of proteins at work in the human body. (Though these numbers are debated.) An accessible account of this debate appears in [C].)
- **Humoral Immune Response:** The immune system response to a pathogen in which B cells are stimulated to create antibodies appropriate for killing the pathogen.
- **Leukocyte** (white blood cell): Any of the small, colorless nucleated cells in the blood, lymph, and tissues, which are important in the body's defenses against infection. T cells and B cells are both leukocytes.
- **Lymphocyte** (white blood cell in lymphatic tissue): A particular type of leukocyte formed in lymphatic tissue.
- **Macrophages:** Any of the various phagocytic cells in lymphatic tissue, bone marrow, etc.; these guys serve as lookouts for the immune system and, on occasion, as hit men.
- **mRNA** (messenger RNA): A version of RNA that serves as a copy of a DNA strand and can be used to transport that copy outside of the nucleus where it can be copied by ribosomes.
- **Pathogen:** Any agent, especially a microorganism, able to cause disease.
- **Phagocyte:** Any cell, especially a leukocyte, that ingests and destroys other cells, microorganisms, or other foreign matter in the blood and tissues.
- **Protease:** An enzyme carried by HIV that cuts the long line of proteins HIV forms in its hosts into individual proteins that make new HIV particles.
- **Protease Inhibitor:** Any drug that inhibits the ability of protease to do its job, and thus limits the reproductive capacity of HIV.

- **Protein:** Any of a large class of nitrogenous substances consisting of a complex union of amino acids and containing carbon, hydrogen, nitrogen, oxygen, frequently sulfur, and sometimes phosphorous, iron, iodine, or other elements. A particular protein is distinguished by the specific sequence of amino acids that appears in its structure.
- **Reverse Transcriptase:** The enzyme that transcribes RNA into DNA. (Called “reverse” because, as you may recall, the typical cellular process is for DNA to be copied onto RNA.)
- **Reverse Transcriptase Inhibitor:** Any drug that inhibits the enzyme reverse transcriptase from transcribing RNA into DNA.
- **RNA** (ribonucleic acid): a nucleic acid essential to all cells, playing a key role in the copy and transfer of DNA; see also RNA-polymerase and mRNA.
- **RNA-polymerase:** A version of RNA that “unzips” the double helix of a DNA molecule and copies the DNA into mRNA.
- **Virus:** Escaped genes or fragments of genetic code, virus particles (or virions) typically consist of DNA or, less commonly, RNA surrounded by a protein shell called a capsid. The shell works like a Trojan horse, tricking cells into thinking it’s friendly.

## References

- [ARA] The AIDS Research Alliance of America website: <http://www.aidsresearch.org>.
- [C] B. Commoner, *Unraveling the DNA Myth: the spurious foundation of genetic engineering*, Harper’s **304** (2002), no. 1821, 39–47.
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