Revisiting Early Models of the Host-Pathogen Interactions in HIV Infection

The interactions between infectious pathogens and the immune system have been mathematically modeled for numerous diseases. Human immunodeficiency virus (HIV), the agent that causes AIDS, has been modeled most extensively. In 1986, three years after HIV was isolated, investigators produced the first mathematical description of its pathogenesis. Since that time modelers have steadily developed more sophisticated systems that have brought the field of mathematical modeling to the consciousness of virologists and immunologists. In this paper we review five of the first attempts of modeling HIV and the immune system. We consider these models in their own right as predictors for $CD4^+$ T-cell depletion and viral growth, as records of the immunovirological understanding of the day, and as forerunners of the current generation of models.

Keywords: *mathematical modeling; HIV; human immune system; numerical simulations*

INTRODUCTION

The clinical symptoms of acquired immune deficiency syndrome (AIDS) were first described in 1982; the etiologic agent, human immunodeficiency virus (HIV), was isolated in 1984. HIV has been responsible for the deaths of millions and the epidemic is still growing. Accordingly, investigators have extensively studied its pathogenesis in the hopes of finding targets for pharmaceuticals and immunotherapies for prevention and treatment. In this paper we will review some of the earliest mathematical models characterizing disease progression at the cellular level. We begin by briefly reviewing the natural relationship

Comments Theor. Biol.
2000, Vol. 5, No. 6, pp. 383-411
Reprints available directly from the publisher
Photocopying permitted by license only

© 2000 OPA (Overseas Publishers Association) N.V.
Published by license under the Gordon and Breach
Science Publishers imprint.
Printed in Malaysia

between math models and infectious disease, the human immune system, and HIV infection-this background is necessary to put the models into context.

We present five models from three groups who published in the 1980s. The first, by Leon Cooper, appeared in 1986 and is historically important because it is the first mathematical model that directly addressed the immune response to HIV [6]. Dolezal and Hraba produced the second model we consider [7],[8]. They developed their model based on the assumption that HIV produced a chemical that rendered immune system cells non-functional. The third model was developed by Perelson. He has contributed many HIV mathematical models over the past decade [9],[11],[12],[13],[14], but we discuss only his first [10],[15].

For each model, we discuss the key variables, assumptions, strengths, weaknesses, and concordance of simulations to clinical data. We selected the above three because of their diverse approaches and relative novelty, but it is important to note that there were several other HIV-immune models published in the late 1980s [16],[17],[18] as well the authors in [16] implemented a deterministic model that included a detailed immune response. In [17], Merril used a stochastic approach specifically designed for the early phase of HIV infection. McLean determined an important threshold condition regarding susceptibility to opportunist infections in [18]. Space limitations prevent us from detailing these other models, but the reader is urged to consult the references for further study.

MATHEMATICAL MODELING OF INFECTIOUS DISEASE

Infectious diseases and math modeling have a natural symbiosis at two distinct levels. At the population level math models have been used to characterize the transmission and spread of disease [1],[2],[3],[4],[42],[43],[47]. Often used in epidemiology, these models address questions such as: 1.) What proportion of a community will be infected? 2.) How fast will the epidemic progress? 3.) Will the epidemic reach a maximum and then steadily decrease? 4.) What proportion of the population needs to be immunized for the entire population to be protected from an epidemic? 5.) What is the best intervention to curtail the epidemic? Answers to these questions benefit society as a whole. Mathematically,

disease transmission is not considered as a predator-prey interaction [2], but is typically characterized in terms of human compartments – the classic susceptible-infected-recovered (SIR) categorization (c.f. [3],[4]). These models merit attention but are outside the scope of the present study.

The second inroad mathematical modeling has made in the study of infectious diseases is host-pathogen interactions at the cellular level. These models treat the individual human as populations of cells in conflict with the population of pathogens. They have been most extensively used to describe disease progression in HIV infection. Questions typically addressed include: 1.) What causes the hallmark depletion of $CD4^+$ T cells over the course of infection? 2.) What are the dynamics of viral population growth? 3.) What is the turnover rate of immune system cells and virus during infection? 4.) At what point in infection is therapy most effectively administered? 5.) What treatment regimens will minimize the likelihood of development of drug resistance? In answering these questions, investigators address disease dynamics at the individual level, rather than at the aggregate level. Due to the two distinct backgrounds necessary to develop such models, immunovirology and mathematics, these models most often arise out of the laboratories of biomathematicians (for a recent review of HIV-specific immune system models, see [5]).

IMMUNOLOGY

The human immune system is responsible for determining self from non-self. It is the body's distributed defense system that prevents infection and disease, aids the body in recovering from infection, and retains memory of past infections.

When a foreign substance (antigen/pathogen) is introduced into the body, the body elicits an immune response in an attempt to clear the substance as quickly as possible. In the tissues, pathogens are first scavenged and engulfed by macrophages, which then present digested pieces of the pathogen (antigens) to the CD4 positive T lymphocytes ($CD4^+$ T cells). "CD4" denotes a protein marker on the surface of the cell, and the "T" refers to thymus, the organ responsible for maturing these cells after they migrate from the bone marrow. These $CD4^+$ T cells, also referred to as helper T cells (which normally average 1000 per cubic mm of

blood), orchestrate the primary immune response. First, the helper T cells expand by division, and then undergo differentiation to one of two distinct subclasses-TH1 or TH2. The defining difference between the two is the profile of cytokines each secretes. Cytokines are the signaling chemicals between cells that regulate cellular activities.

Macrophages process and present antigens to $CD4^+$ T cells; they are a part of a larger class of cells known as antigen-presenting cells (APCs). The two other types of antigen-presenting cells are dendritic cells and B lymphocytes. Dendritic cells reside in bulbous organs called lymph nodes, through which lymph fluid and most of the immune system cells continually flow while surveying the body. Lymph nodes are the physical structures that facilitate the immune response. Their specialized architecture increases the likelihood that immune system cells will contact foreign antigen. B lymphocytes are described below.

Immune responses consist of a combination of cellular and humoral immune responses-the relative balance depends on the type of pathogen. A cellular immune response involves not only building up helper T cells, but also activation of additional macrophages and CD8 positive T lymphocytes ($CD8^+$ T cells). Macrophages become activated and participate not only as antigen presenters, but as phagocytes, destroying both pathogens and infected cells. $CD8^+$ T cells eliminate intracellular pathogens directly by destroying infected cells, or by suppressing viral replication through non-cytotoxic means. A particular class of $CD8^+$ T cells, called cytotoxic T cells (CTLs), carry out most of the infected-cell killing. CTLs are the most important line of offense against viral pathogens, including HIV [20, 21, 31]. Once given a target, CTLs seek out and destroy cells infected with intracellular pathogens. A second, less understood, class of $CD8^+$ T cells is called suppressor cells (for review see [22]). Their mechanism of action is believed to be inhibition of viral replication via the release of soluble chemical effectors [23, 26]. The clonal expansion of either type of $CD8^+$ T cells requires signals from $CD4^+$ T lymphocytes to be most effective.

In the humoral immune response (also known as the antibody response) the TH2 subclass of $CD4^+$ T cells signal another set of cells, called B lymphocytes (B cells). These are immune system cells that produce the chemical weapons called antibodies. Antibodies are engineered to bind to and mark specific pathogens as foreign, thereby facilitating their phagocytosis.

During and after the immune response, certain cells of each type retain knowledge of the attack; these specialized cells are known as memory cells. If the same pathogen (or a similar strain) invades the body a second time, a much quicker and more aggressive campaign can be launched and the antigen is eradicated more efficiently. This is the idea behind vaccines. A weaker version of the pathogen (or subunit of the pathogen) is introduced into the body eliciting a primary immune response; then, if the individual becomes infected with the more aggressive relative, the response is immediate and powerful, and the pathogen cannot establish infection. For a complete reference on Immunology, see [19].

From the above discussion, it is clear that $CD4^+$ T cells act as a link between the various divisions of the immune system. They recruit and activate macrophages, direct the humoral branch by stimulating B cells to produce antibody, and are necessary for a proper cell-mediated response. Each $CD4^+$ T cell has a given specificity to only one antigenic sequence; there are many specificities a given cell could have, on the order of 10^8 [19]. It is estimated that on average $2x10^{12}$ $CD4^+$ T lymphocytes are present in an individual at any given time [24], meaning the frequency of cells specific for a given antigenic sequence is relatively low: $2x10^{12} \div 10^8 = 20{,}000$ cells. Therefore, $CD4^+$ T cells specific for a given antigen need to undergo rapid proliferation, called clonal expansion, to provide protection if that antigen is encountered. It is clear that $CD4^+$ T cells are critically important elements of immune responses. If a pathogen successfully diminishes $CD4^+$ T cell levels, it can seriously compromise host immunity. That is precisely what HIV infection does.

HUMAN IMMUNODEFICIENCY VIRUS

HIV is a single-stranded retrovirus; it consists of two copies of the RNA genome and necessary enzymes packaged within an icosahedral capsid, surrounded by an envelope coat. As with other viruses, HIV can only reproduce using the machinery of its host target cells. The primary target cells of HIV are macrophages and $CD4^+$ T cells [20]. Cellular entry by HIV requires the CD4 receptor and one of several coreceptors.

Each infection of a target cell can be one of three types: productive, latent, or abortive [25],[48],[49]. Productive or active infection occurs

when the virion enters the cell, successfully hijacks the cellular machinery, and produces new virions (progeny) at a steady rate. These cells live approximately two days after being infected [13]. Latent infection occurs when the virion has entered an unactivated cell (one that has not received stimulatory signals). HIV can replicate only in activated cells, so a latently-infected cell is not currently producing viral progeny. Instead, a latently-infected cell may at a later time, given certain stimulation, begin producing progeny. In contrast, an abortively-infected cell is one in which the virion has entered the cell, but due to some defect in the infection process, can never produce virus. Abortive infection is, in fact, the most common type of cellular infection [25],[48],[49]. Productively-infected cells account for only about 2% of cellular infections [19].

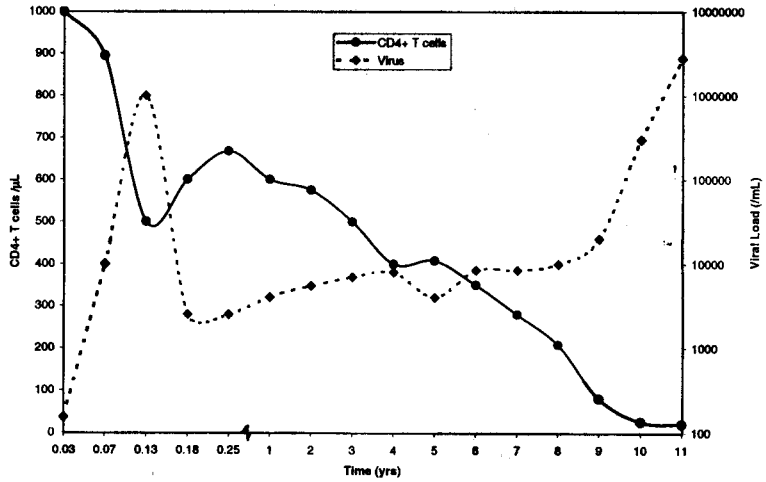

FIGURE 1 Clinical data representing $CD4^+$ T cell levels and viral load during the pathogenesis of HIV [28]. $CD4^+$ T cell levels (solid line) and viral load (dashed line) are shown throughout the long disease course, including expanded detail during the first four months of infection known as primary infection. Note the characteristic steady depletion of the $CD4^+$ T cells and the burst of viremia during late-stage disease

The clinical picture of HIV is similar in a majority of cases [19]. Progression generally consists of three stages: primary, asymptomatic, and AIDS. Primary infection occurs in the first several months and is char-

acterized by a transient burst of viral replication and a sudden temporary decrease in $CD4^+$ T cells, as seen in Figure 1 [28]. Asymptomatic infection lasts for 6–10 years (depending on treatment) during which time the population of $CD4^+$ T cells in the blood decreases slowly but steadily (Figure 1). AIDS is defined as end-stage HIV infection when $CD4^+$ T cell counts in the blood drop below 200 cells/μL; it lasts 1–3 years.

It is suspected that the cytopathology due to productive cellular infection cannot by itself explain the decrease in $CD4^+$ T-cell level from the blood. So what can explain this phenomenon? Several hypotheses have been suggested, but none has indisputable experimental support [27]:

1. **toxic viral gene products**: Viral gene products such as structural proteins and enzymes may be directly toxic to $CD4^+$ T cells, causing their death and eventual depletion of the population.
2. **syncytium formation**: Syncytium are large amalgamations of $CD4^+$ T cells and other immune cells that effectively destroy many cells at once while producing large amounts of virus.
3. **direct virus killing of cells by productive infection**: Productive infection kills target cells by disrupting normal cellular functions or bursting the cell.
4. **apoptosis**: $CD4^+$ T-cell receptor stimulation by HIV without viral penetration may cause affected cells to die by programmed cell death (apoptosis).
5. **autoimmunity**: Infection with HIV may cause the immune system to destroy its own healthy $CD4^+$ T cells, a condition of autoimmunity.
6. **cytokine expression**: Cytokine and chemokine dysregulation may contribute to $CD4^+$ T-cell depletion by selecting for the wrong dominant immune response.
7. **altered circulation patterns**: $CD4^+$ T cells may be progressively lost from the blood compartment due to enhanced homing to the lymph system, a state of altered lymphocyte circulation.
8. **disruption of the lymphoid architecture**: Infection with HIV can alter the architecture of the lymph node, thus weakening the immune response and making $CD4^+$ T cells more susceptible to infection.

The models we will consider in the following sections are based on assumptions of how $CD4^+$ T cells are lost from the blood. Even though they were formulated in the late 1980s, the assumptions used correspond to one or more of the hypotheses listed above.

We view these historical models with an eye toward understanding $CD4^+$ T-cell depletion and viral load levels over the course of disease. The models can be used to predict $CD4^+$ T cell and virus population levels that can then be compared to clinical data. These comparisons are helpful in determining the value a given model has in explaining disease mechanisms. While no model adequately explains all the features of the clinical data, each has its virtues.

COOPER MODEL-1986

In 1986, the physicist Leon Cooper published the first mathematical model of the immune system response to what he termed "immune system retroviruses", HIV being the relevant example [6]. Two years later he reported detailed conclusions and analyses of the equations [29]. He presented three systems with progressive complexity. The first described an immune response to a "normal", non-retrovirus; the second characterized the response to an infection with retrovirus only; and the third analyzed an infection with both normal replicating virus and retrovirus, termed a "mixed infection". Here we focus on the latter two models.

Cooper structured his models in the context of immune-pathogen heterotrimeric and heterodimeric complexes. He assumed the binding of HIV virions to B lymphocytes specific to HIV was the required first step in the adaptive immune response. These heterodimeric complexes then would bind further to $CD4^+$ T lymphocytes specific to HIV. The formation of the heterotrimeric complex was proposed to activate the $CD4^+$ T cells and induce their clonal expansion. Today it is generally accepted that antigen presenting cells (APCs), namely dendritic cells, macrophages and B cells, process and present digested pieces of virion peptides, called antigens, to the $CD4^+$ T cells [19]. $CD4^+$ T cells that specifically recognize these antigenic sequences then bind to the APC, forming a transient heterodimeric complex that function similarly to the heterotrimeric complex of the Cooper model.

We first examine the model of HIV infection alone. In his system of equations, Cooper includes the concentration of only those $CD4^+$ T cells specific to HIV antigen, labeled $T(t)$. This subset has been shown to comprise a very small portion of the total $CD4^+$ T population, even at late stages of disease [30]. The other state variables also denote concen-

trations and include HIV, $V(t)$; HIV-specific B cells, $B(t)$; HIV-infected, HIV-specific T cells, $T^*(t)$; HIV:HIV-specific B-cell complex, $C_{BV}(t)$; HIV-specific T-cell:HIV:HIV-specific B-cell complex, $C_{BVT}(t)$; and HIV-specific, infected T-cell:HIV:HIV-specific B-cell complex, $C_{BVT^*}(t)$. The corresponding nonlinear, ordinary differential equations describing their rates of change are as follows:

$$\frac{dB}{dt} = \varepsilon_B + \gamma_3 C_{BVT} - \gamma_1 BV - \lambda_3 B \tag{1.1}$$

$$\frac{dT}{dt} = \epsilon_T + \gamma_4 C_{BVT} - \gamma_2 C_{BV} T - \gamma_4' TV - \lambda_4 T \tag{1.2}$$

$$\frac{dT^*}{dt} = \gamma_4' TV - \gamma_2' C_{BV} T^* - \lambda_4' T^* \tag{1.3}$$

$$\frac{dV}{dt} = \gamma_6' C_{BVT^*} - I(V, B, t) - \lambda_6 Y \tag{1.4}$$

$$\frac{dC_{BV}}{dt} = \gamma_1 BV - C_{BV}(\gamma_2 T + \gamma_2' T^*) - \lambda_1 C_{BV} \tag{1.5}$$

$$\frac{dC_{BVT}}{dt} = \gamma_2 C_{BV} T - \lambda_2 C_{BVT} \tag{1.6}$$

$$\frac{dC_{BVT}^*}{dt} = \gamma_2' C_{BV} T^* - \lambda_2' C_{BVT^*} \tag{1.7}$$

Description of the Cooper Equations

Equation 1.1 characterizes the changes in the HIV-specific B-cell population. ε_B is the constant influx of B cells from a precursor population that is independent of antigen activation. The $\gamma_3 C_{BVT}$ term is the clonal expansion of B cells based on the presence of HIV. B cells can encounter virus and be lost either the formation of C_{BV} at rate γ_1, or die naturally at rate λ_3.

Equation 1.2 represents the changes in the uninfected HIV-specific T-cell population. Like the B-cell population, T cells have a constant source of input, ε_T, that arises from the thymus. The clonal expansion of T cells is analogous to that of B cells and occurs at rate γ_4. T

cells can be lost to the formation of the C_{BVT} complex, infection, and natural death.

Equation 1.3 represents the rate of change in the infected T cell compartment, T^*. Infected T cells are formed only when a T cell encounters a virion, at rate γ_4, and T^* are lost when they form a complex or by death.

Equation 1.4 is an important equation because it shows that the virus population has only one source. According to this model, virus is only produced upon formation of the C_{BVT*} complex. We will return to this point later, when we critique the model. Virus is removed by an immune response that is dependent on time, B cells, and virus: $I(V,B,t)$, as well as lost to natural decay.

Equation 1.5 represents the rate of change of the HIV-specific B-cell:HIV complex compartment, C_{BV}. The only source term for this population is the mass-action interaction between a virion and an HIV-specific B cell, occurring at rate γ_1. Complexes in this population are lost in three ways: to the B-cell:HIV:uninfected T-cell complex, C_{BVT}; to the B-cell:HIV:infected T-cell complex, C_{BVT*}; and to natural death.

Equation 1.6 represents the rate of change of the B-cell:HIV:uninfected T-cell complex population, C_{BVT}. These are formed at a rate that is equivalent to the first loss term in equation (1.5). The loss term represents natural death.

Equation 1.7 characterizes the changes in the B-cell:HIV:infected T-cell complex, C_{BVT*}. It is formed when the B-cell:HIV complex encounters an HIV-specific infected T cell, and can be lost to natural death.

Among the several hypotheses that attempt to explain why HIV causes progressive $CD4^+$ T-cell depletion, Cooper's model assumes that direct killing by productive infection is responsible (hypothesis 3, HIV section) since the only occurrence of HIV-dependent death of $CD4^+$ T cells is by infection (equation (1.3)). This very mechanistic system, (1.1)-(1.7), uses the law of mass-action for most of the nonlinear interactions. Specific events are required for virus replication, T-cell expansion, and cellular infection. In general, the more mechanistic a model is, the more explanatory it can become. More mechanistic also means they

are more susceptible to obsolescence by advances in scientific understanding. Thirteen years and several billion research dollars later, this model can be viewed with an updated understanding of HIV pathogenesis. There are several items that should be re-evaluated.

First, this model cannot account for the observed $CD4^+$ T cell depletion or virus population growth in its entirety. As mentioned in the section on HIV biology, HIV can infect all $CD4^+$ T cells and macrophages. But the only occurrence of HIV replication in the model is in the formation of the infected heterotrimeric complex. The overarching problem with this idea is that only $CD4^+$ T-cell subsets specific for HIV are included, and thus much less viral replication is possible. Therefore its viral load predictions will be substantially smaller than what is observed clinically. Many studies have shown that a good predictor for clinical disease course is viral load [36],[37],[38],[39],[40], a value this model cannot accurately monitor or predict. Furthermore, the only $CD4^+$ T cell loss that will be predicted with this model is from the small subset of HIV-specific cells. The eradication of the entire subset would not be enough to account for the observed clinical picture.

Second, this model does not include the most important mechanisms of T-cell activation, that of antigen presentation by dendritic cells and macrophages. As discussed above, it is known that antigen presentation to $CD4^+$ T lymphocytes occurs by dendritic cells, macrophages, and B cells, collectively referred to as professional antigen presenting cells [19]. The subset of APCs included in the Cooper model is the small number of B cells that are specific to HIV. Dendritic cells and macrophages together play a more important a role in the activation of $CD4^+$ T cells in HIV infection, because they have no inherent limiting specificity for HIV or any other antigen [20]. That means all dendritic cells and macrophages have the ability to activate $CD4^+$ T cells by presenting HIV peptides. Since the model does not include the more important methods of antigen presentation and $CD4^+$T-cell activation, it will not account for all the *in vivo* cell activation.

Third, the system incorrectly models the immune response as independent of $CD4^+$ T lymphocytes. In the model, the immune response is based on viral concentration, B cells, and time. Current immunological understanding underscores the role of $CD4^+$ T cells in the development and control of the immune response, and is supported by clinical observations [19]. Even in the 1980s it was known that HIV infecteds with low $CD4^+$ T-cell counts have an impaired ability to mount an immune

response against opportunist pathogens [19],[20]. Thus, a more accurate immune response function would depend heavily on $CD4^+$ T cells.

Forth and finally, this model does not address the important issue of latent cellular infection. Researchers recently observed that latent infection is at least as common as productive infection [50],[51]. Latently-infected cells are by definition not producing progeny, but have the capacity to if activated at a later time.

The above four concerns are those we believe have the largest negative impact on the accuracy of the model. In addition, Cooper emphasizes the role of HIV-specific B cells and downplays the role of $CD4^+$ T cells. Today, investigators realize just the opposite is true: humoral immunity in HIV is less important than originally thought [21],[22],[23].

The third model Cooper developed, and the second one we examine, is that of a mixed infection with HIV and another "normal" non-retrovirus. He asserts this model is more reflective of reality because it is likely the immune system in an HIV-infected individual is activated by more than just HIV. This additional activation is most likely needed because insufficient activation was included in the first model. The revision necessitates the addition of seven new equations (not shown), augmented with Equations (1.1–1.3) and (1.5–1.7). A major change with the addition of these equations is the expansion of the HIV target cell population to include $CD4^+$ T cells that are specific to normal virus. Now HIV can replicate in a larger number of cells and, as Cooper pointed out, this more closely resembles the real situation.

One of the insightful features of Cooper's models is the distinction that HIV can only replicate in $CD4^+$ T cells that are activated. The mixed infection model could be generalized to account for all of the $CD4^+$ T cells that are activated by any antigen, be it normal virus infection, allergen stimulation, or bacterial infection. This rather easy extension would go a long way to solving the first of the above four problems: viral population growth. The three other issues remain unresolved in Cooper's mixed infection model.

The numerical analyses given in [6],[29] emphasize the importance of the initial concentrations of HIV-specific B cells and virus, B_0 and V_0, on the maximum concentration of virus, V_{max}. While there is controversy as to whether or not the size of the viral inoculum, V_0, is important to disease progression it has recently been suggested that the level of virus in the body after primary HIV infection (~ 6 months after infec-

tion) has prognostic value, the so-called "setpoint" hypothesis [52]. Unfortunately Cooper does not publish time plots of viral load or $CD4^+$ T-cell counts, or even the parameter values he used in the simulations. Due to the large number of parameters, some that have no biological meaning, we do not to attempt to obtain predictions from the Cooper model. We therefore cannot compare model simulations to the clinical data in Figure 1. This is an obvious weakness of the model, since it cannot be verified whether it produces the routinely-observed $CD4^+$ T-cell and viral population dynamics.

Cooper does mention that among the many parameters in the model, some of the more numerically-sensitive ones are the decay rates of the complexes, C_{BVT}, C_{BV}, and C_{BVT*}. The sensitivity of the Cooper equations to the decay of C_{BV} has no biological meaning because, as mentioned above, the current understanding of antigen presentation conflicts with the idea of whole virions participating in the formation of complexes. The sensitivity to the decay of C_{BVT} and C_{BVT*}, however, may reflect the biologically important binding time of the $CD4^+$ T cells to the B cells. Longer binding time may result in greater clonal proliferation, as depicted in the equations.

With the first immune-system model of HIV infection, Cooper primed the field for biologically mechanistic models. In retrospect, some features he chose to emphasize were less important than others: B cells are less important to $CD4^+$ T-cell proliferation than other APCs, the immune response is critically dependent on $CD4^+$ T cells, and antigen processing is now more well-understood. However, he incorporated aspects of HIV infection that investigators speculated were important at the time. His cause-and-effect, mechanistic style of modeling distinguishes the Cooper model from the first model presented in the next section.

DOLEZAL AND HRABA MODEL-1988

Early in 1988, Dolezal and Hraba published a mathematical model of the immune system infected with HIV [7]. Subsequent analyses and extensions of their initial model soon followed [8],[32],[33],[34],[35]. One of the striking characteristics of their model was that it was originally developed in 1980 to describe B-cell immunological tolerance to human serum albumin in chickens. Dolezal and Hraba adapted their

model to explore HIV infection in humans. The evident adaptability of this model foreshadows one of its weaknesses: the general nature of its substituent parts.

The model is rather simple, consisting of two compartments of $CD4^+$ T lymphocytes: precursors, *P(t)*, and fully-functioning mature cells, *M(t)*. HIV antigen, *A(t)*, is assumed to cause immunological tolerance, or inactivation, of the $CD4^+$ T cells that it comes into contact with. This tolerance effectively means loss from the system, so that affected cells leave their respective compartments permanently. Dolezal and Hraba used three equations to capture this behavior:

$$\frac{dP}{dt} = \tau_P P_0 - \tau_P P - c_P AP \tag{2.1}$$

$$\frac{dM}{dt} = \tau_P P - c_M AM - \lambda_M M \tag{2.2}$$

$$A(t) = A_0 \exp(\delta t) \tag{2.3}$$

Description of the Dolezal and Hraba Equations

Equation 2.1 represents the rate of change of the precursor cell compartment, *P*. The only source term for this population is the constant influx at rate τ_P. The value of the constant, P_0, is found from steady state considerations in the uninfected case [7]. Precursors are converted into mature lymphocytes at rate τ_P, and are effectively lost to anergy by contact with HIV tolerizing antigen at rate c_P. Natural death is not included for the precursor population.

Equation 2.2 characterizes the changes of the mature $CD4^+$ T-cell population, *M*. Mature cells are converted from precursors at rate τ_P. They are lost in two ways: to anergy via contact with HIV tolerizing antigen at rate c_M, and to natural death at rate λ_M.

Equation 2.3 is a description of HIV antigen levels, A. The model simplistically assumes antigen grows exponentially without bound at rate δ.

Dolezal and Hraba utilize a different hypothesis than Cooper to explain $CD4^+$ T-cell depletion. In this model tolerizing antigen (toxic viral gene products produced by HIV) renders $CD4^+$ T cells nonfunc-

tional, effectively removing them from the population. This corresponds to hypothesis 1 given in the section on HIV biology: toxic viral gene products. While an interesting idea, too many simplifying assumptions are made in its implementation making it difficult to draw useful conclusions. Several considerations lead us to this assessment.

First, this model does not represent antigen levels realistically. Although HIV is a replicating pathogen, unbounded exponential growth is not observed over the time course of infection until very late stage disease (see Figure 1). This assumption also means that once antigen is formed, it is not removed either by an immune response or by a natural half-life. As mentioned above, HIV is an obligate intracellular pathogen and requires target cells to effectively produce gene products and replicate. This is overlooked in the model since HIV growth is independent of $CD4^+$ T-cell levels. It has also been noted that HIV has a natural life-span in the blood on the order of hours to a few days, which would also function to decrease effective antigen loads [13],[41]. Whereas the Cooper model underestimates the amount of viral replication possible, the Dolezal and Hraba model vastly overestimates it.

Second, this model neglects expansion of the mature $CD4^+$ T-cell pool by clonal division. When presented with antigen, $CD4^+$ T cells are stimulated and undergo rapid division and these newly formed cells then orchestrate the immune response. The absence of clonal expansion coupled with unbounded viral growth makes this model a worst-case scenario for infection: the body does not defend itself and merely succumbs to exponential viral growth.

This worst-case theme is apparent in the numerical solutions of equations (2.1–2.3). As the model is constructed, rapid $CD4^+$ T-cell depletion results anywhere from 900 to 1500 days. Normally $CD4^+$ T-cell loss is slower (see Figure 1). The authors recognized this, and attempted to account for this discrepancy by hypothesizing variations in viral growth rates as a result of an immune response. They use the piecewise function, $\delta(t)$, to capture this time-dependent growth rate:

$$\delta(t) = \begin{cases} \delta_1 : & t < t_1 \\ 0 : & t_1 \leq t < t_2 \\ \delta_1 : & t \geq t_2 \end{cases}$$

That is, the immune response holds viral replication in check beginning at time t_1, but is eventually overwhelmed at time t_2, when viral replication begins anew. This artificial manipulation is basically data

fitting. A better way to implement an immune response is to construct a more biologically mechanistic model, which Dolezal and Hraba do in their follow-up paper [8].

In this subsequent work, they include a population of cytotoxic lymphocytes (CTLs) which directly reduces the amount of antigen in the system. Equations (2.1) – (2.2) combined with the two new equations depicting antigen, *A(t)*, and CTL levels, *C(t)*, comprising the revised system:

$$\frac{dA}{dt} = A(\delta - \gamma C)\exp(\delta t) \tag{2.4}$$

$$\frac{dC}{dt} = (\varepsilon I_C + \alpha C)A\left(\frac{M}{M_0}\right)^{v} - \lambda_C C \tag{2.5}$$

Description of the Revised Dolezal and Hraba Equations

Equation 2.4 represents the new rate of change of the HIV antigen compartment, *A*. Antigen replicates with a maximal rate constant, δ. This rate constant decreases as the cytotoxic T lymphocyte population, *C*, grows, representing replication limitation from an immune response.

Equation 2.5 characterizes the changes in the cytotoxic T lymphocyte population. A constant source, εI_C, is augmented by clonal proliferation, αC. This rate is modified by antigen levels: the higher the antigen levels, the greater growth. The factor $(M(t)/M_0)^{\nu}$ depicts the importance of $CD4^+$ T cells on CTL proliferation. It is known that $CD4^+$ T cells are required for successful CTL proliferation [19]. The authors hypothesize that a decreased number of $CD4^+$ T cells will negatively impact the ability of CTLs to proliferate. The power ν quantifies the intensity of this effect.

The numerical solutions of the revised model are more satisfying; the CTLs visibly hold viral replication in check without artificially manipulating viral growth rates (Figure 2A and Figure 2B). Adding the mechanistic feature provides more insight into which parameters of the model are important. For example, by varying ν from $\nu = 1$ to $\nu = 2$, the qualitative behavior changes drastically from a stable nonzero $CD4^+$T-cell level to complete depletion of the $CD4^+$ T-cell population [8]. Clearly in

this model the power ν, corresponding to the intensity of the $CD4^+$ T-cell helper effect on $CD8^+$ T-cell proliferation, is critically important to the system.

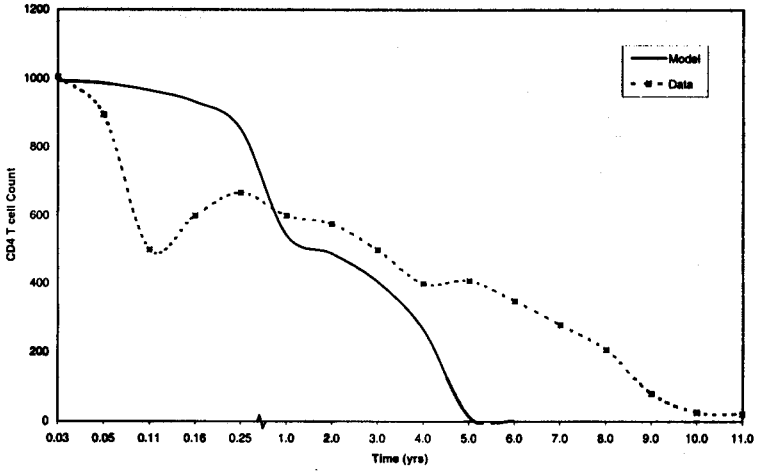

FIGURE 2A Dolezal and Hraba's model predictions for $CD4^+$ T cell levels [8] compared with clinical data [28]. The model simulations (solid line) are qualitatively similar to clinical data (dashed line), but lack the initial drop associated with primary infection and predict complete $CD4^+$ T cell eradication about five years early. Parameter values are as listed in [8]

Although it was not presented in their work [8], we analyze the steady-states of the system, fixing $\nu = 2$. If we assume precursors cannot be tolerized by the antigen, that is $c_P=0$, by brief manipulation we conclude there are three states the system can attain. The first is an uninfected steady-state where $A=C=0$. When the system is at this state, local stability analysis yields three real, negative eigenvalues and one real, positive eigenvalue, δ. This means the state is always locally unstable and the "degree" of instability is governed by the viral growth rate δ. The other two steady-states for the system both include nonzero, finite antigen and $CD4^+$T cell levels-one is stable and one is unstable. The steady-state values of P and M are functions of the steady-state level of antigen, A. In fact, the choice of c_M, the viral infectivity rate of mature $CD4^+$ T cells is critically important in determining the state of the sys-

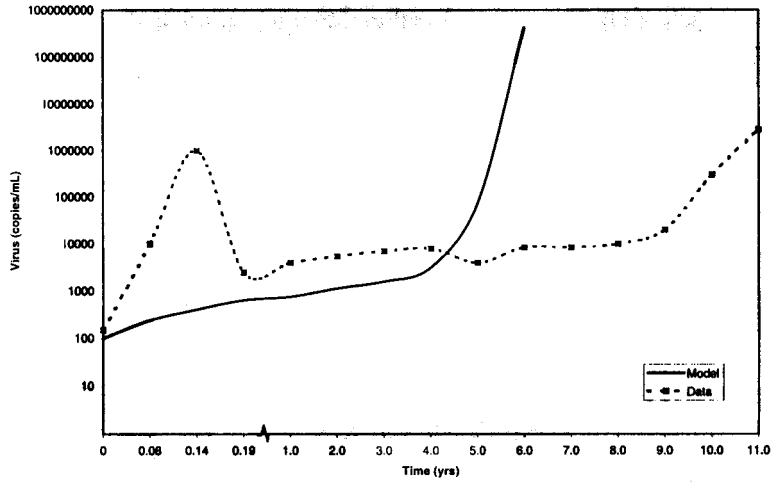

FIGURE 2B Dolezal and Hraba's model predictions for viral load [8] compared with clinical data [28]. The model simulations (solid line) are a simplified version of the clinical data (dashed line). The model predicts exponential growth by year five, approximately five years early and thus exaggerates this growth. Furthermore the model lacks the initial burst of viremia associated with primary infection. Parameter values are as listed in [8]

tem. This is readily seen in the bifurcation diagram where c_M is plotted versus A (Figure 2C). A saddle-node bifurcation is seen to occur at $c=c_{crit}$ (there is a similar saddle-node bifurcation for the parameter δ as well). For values of $c_M < c_{crit}$, the system assumes the stable, infected steady-state. For values of $c_M > c_{crit}$, the system has no steady-state and antigen increases without bound. This behavior shown in Figure 2B occurs when $c_M=1$. Why did Dolezal and Hraba choose $c_M=1$? There is no obvious reason for doing so. In fact, it is reasonable to argue that every encounter of a $CD4^+$ T cell with the antigen would not result in the $CD4^+$ T cell becoming anergic. In such a case, c_M would be less than one.

When we use values of the parameters given in [8], no steady-state is achieved: antigen load increases to infinity and $CD4^+$ T cells decrease to zero. We interpret this as the "crash to AIDS" state. Maintaining $\nu = 2$, we compare the model's prediction of the time course of infection to clinical data for $CD4^+$ T-cell counts (Figure 2A) and viral load (Figure 2B). There are two key similarities in the qualitative behavior

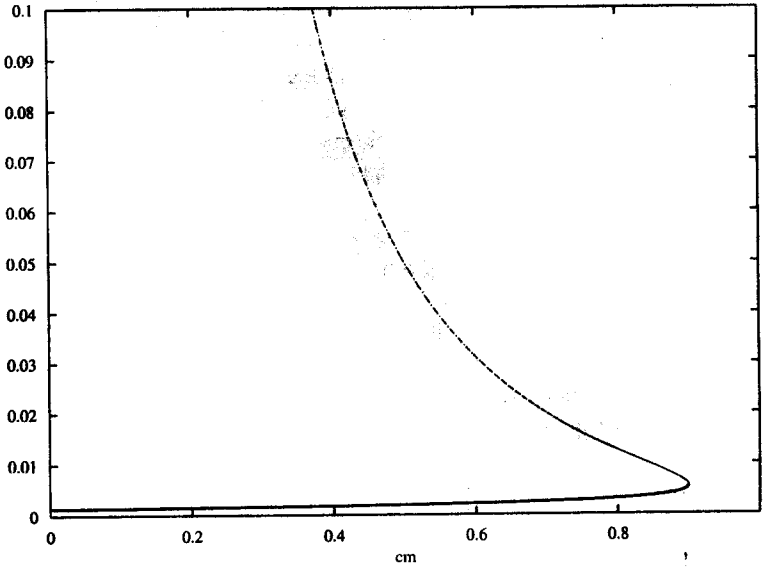

FIGURE 2C Bifurcation diagram of c_M versus the steady-state valne of A, steady-state value of A in the Dolezal and Hraba model. Stable steady states are denoted by solid lines and unstable steady states by dashed lines. Note the drastic change in the behavior of the system as c_M increases past a critical value ($c_{crit} \approx 0.9$). This change is a saddle-node bifurcation. Other parameter values are as in Figure 2A and Figure 2B

between the model predictions and the clinical data. There is an initial decrease of $CD4^+T$ cells followed by a transient leveling off before complete depletion, and viral load correspondingly grows exponentially in end-stage disease. However, there are two key differences between the model predictions and the clinical data. First, the time to depletion in the model is almost half of what is observed clinically. Second, the initial burst of viremia in primary infection is absent from the model predictions.

Although the qualitative behavior of the model is similar to the clinical picture, we have concerns with Dolezal and Hraba's estimation of parameter values. Unfortunately the values of the parameters are listed without units, literature references, or any biological rationalization. Current modelers are expected to justify parameter estimates with scien-

tific reasoning or references, as demonstrated by particularly successful works [41],[53],[54],[55],[56]. In fact, the field is evolving toward detailed sensitivity analysis of parameter estimations [45].

Another concern is the frequent changing of the value of ϵ, the CTL maturation rate, from 0.05 to 0.3 and many values in between. These changes take place while varying other parameters, making it difficult to discern the causes of the changes observed in successive simulations. Moreover, one would suspect that the CTL maturation rate is relatively constant *in vivo*.

In years subsequent, Dolezal and Hraba continued to explore and extend their base model, making minor revisions [32],[33],[34],[35]. As one of the first models of HIV immunobiology, their model predicts some of the key behaviors of the clinical picture. Mathematically it is a clean and simple description. Biologically, the critical reader recognizes a very simple model with a lack of justification for parameter values. This leads us to question which, if any, parameter values were chosen solely for fitting the data? This is not a concern in the next model; biological justification is given for most of the parameters used in the numerical simulations.

PERELSON MODEL-1989

The third and final model we consider was published by Perelson in 1989 [15]. In depth mathematical analysis followed several years later [10]. Perelson discussed two versions of his model. The first is very general and includes many aspects of HIV immuno-biology. He described this model as having many unknown parameters and several functions of unknown form. As a result, numerical analyses cannot be performed. The second model is a simplified version of the general one incorporating only obtainable parameters and no unknown functional forms. Because it represents the limits of HIV-immune system understanding in the late 1980s, it is the latter model that we consider in depth.

One of the weaknesses of both the Cooper and Dolezal/Hraba models is that they neglect the latently-infected $CD4^+$ T-cell reservoir; this deficiency is absent from the Perelson model. He defines three populations of $CD4^+$ T cells: uninfected, $T(t)$; latently infected, $T_L(t)$; and actively infected, $T_A(t)$. The concentrations of cells in each of these subpopula-

tions, and the concentration of virus, *V(t)*, comprises the state variables. Perelson assumes that uninfected $CD4^+$ T cells must progress first through the latent stage before becoming actively infected, as depicted in the equations:

$$\frac{dT}{dt} = s + rT\left(1 - \frac{T + T_L}{T_{\max}}\right) - k_1 VT - \mu_T T \tag{3.1}$$

$$\frac{dT_L}{dt} = k_1 VT - \mu_T T_L - k_2 T_L \tag{3.2}$$

$$\frac{dT_A}{dt} = k_2 T_L - \mu_b T_A \tag{3.3}$$

$$\frac{dV}{dt} = N\mu_b T_A - k_1 VT - \mu_V V \tag{3.4}$$

Description of the Perelson Equations

Equation 3.1 characterizes the changes of the uninfected $CD4^+$ T-cell compartment, T. Perelson assumes uninfected $CD4^+$ T cells have a constant thymic source, s, and undergo logistic proliferation with intrinsic rate constant, r. Uninfected cells can be lost to latent infection via the mass action term k_1VT, or to natural death at rate μ_T.

Equation 3.2 quantifies the rate of change in the latently-infected $CD4^+$ T-cell population, T_L. These cells are produced from uninfected $CD4^+$ T cells with rate, k_1VT. They can be lost to natural death at a rate μ_T or converted to actively-infected cells at rate k_2.

Equation 3.3 is a description of the rate of change of actively-infected $CD4^+$ T cells. T_A. Their source is latently-infected cells with rate k_2, and they die with rate μ_b.

Equation 3.4 depicts the rate of change in the virus population, V. The only source term for virus is from actively-infected cells at rate $N\mu_b$, where N denotes the average number of virions produced by an actively-infected $CD4^+$ T cell. Virus is lost two ways: to natural decay at rate μ_V, and via infection of new T cells, k_1VT, as in equation (3.1).

Perelson assumes the only way a $CD4^+$ T cell can be lost due to HIV is by cellular infection. This means that in the model, he attempted to explain $CD4^+$ T-cell depletion by hypothesis 3 described in the section on HIV biology: direct virus killing of cells by productive infection. In the more general model described in his paper, Perelson incorporates two additional hypotheses to explain $CD4^+$ T-cell depletion: hypotheses 2 and 5, corresponding to syncytia formation and autoimmune responses, respectively. We now elucidate the salient features of the simple model.

As discussed before, macrophages are an important reservoir of HIV. Since this model does not include virus that is produced by macrophages, it underestimates the production and concentration of HIV. Perelson et al. does, however, include the macrophage component in his generalized model, and later work [15],[46].

Perelson's simple model assumes $CD4^+$ T-cell proliferation is independent of viral load. In the short term, activation and proliferation of $CD4^+$ T cells generally increases with increasing antigen levels during an immune response. This deficiency is parallel with the lack of immune response in this model. In fact, the only limitation on number of free virus in the Perelson model is the number of $CD4^+$ T cells available to infect. *In vivo*, $CD8^+$ T cells have been shown to greatly limit viral growth, not only by direct killing of virally infected cells by CTLs, but also inhibition of viral replication by suppressor cells [22]. The immune response is important to HIV-host interactions, especially early in infection, and should not be overlooked.

Despite these limitations, the design of the Perelson model is praiseworthy for two reasons. First, the latently-infected $CD4^+$ T-cell population is included in the system. Cellular infection is not a simple binary, yes/no response and Perelson incorporated this. Second Perelson used the literature to estimate parameter values. Unlike the Dolezal and Hraba model, Perelson gives scientific references for most of the parameter values he used. He also did not artificially vary parameter estimates to get more realistic pictures. The result is a more believable, and biological, description of the pathogenic processes.

One parameter value that Perelson et al. does analyze in depth is N, the average number of virions produced by an actively-infected $CD4^+$ T cell. N is a transcritical bifurcation parameter; the behavior of the system changes drastically when the value of N changes from less than Ncrit (=601) to greater than Ncrit [15]. This is important because the

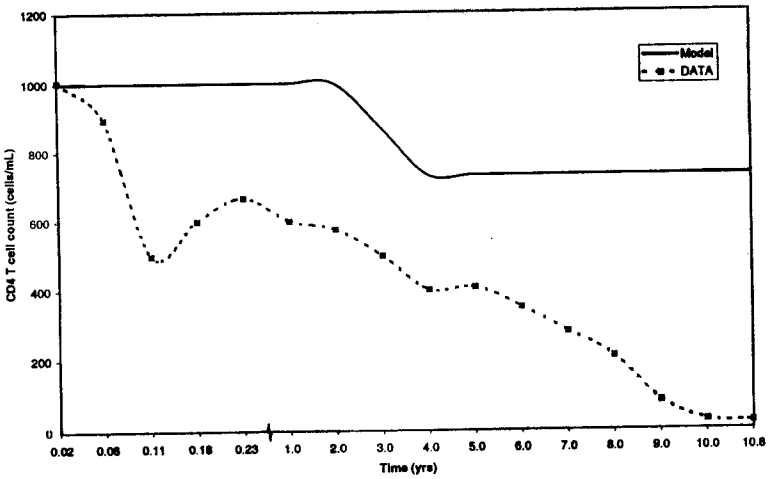

FIGURE 3A Perelson et al. model predictions for $CD4^+$ T cell levels compared to clinical data. The model simulations (solid line) contrast with the clinical data (dashed line). Most noteworthy is the steady-state level of $CD4^+$ T cells attained by year four. Complete $CD4^+$ T cell depletion during AIDS is not predicted in this model. Parameter values are as listed in [15]

value of N is rather difficult to obtain experimentally; estimates in the literature are currently in the range 100–1000 [57]. As the model is constructed the value of N is of critical importance: if $N<Nrcit$, the virus population will be eradicated from the body; if $N>Ncrit$, the body progresses to an infected steady-state with a sizeable viral population.

Using reasonable parameter values and $N=800$, Perelson et al. perform numerical simulations, in particular, a time plot of viral load and $CD4^+$ T cell count is given. Comparing these results to the clinical data (Figure 3A and Figure 3B), we find that the model does not depict well the *in vivo* case. In particular, the model predicts the attainment of a steady-state level of $CD4^+$ T cells after about 4 years of infection. Thus, this model could capture the primary and asymptomatic states of disease progression, but does not capture the crash to AIDS. This is the most serious flaw of the model, since $CD4^+$ T-cell depletion is the hallmark of infection with HIV. The viral load predictions are also discordant with the clinical data.

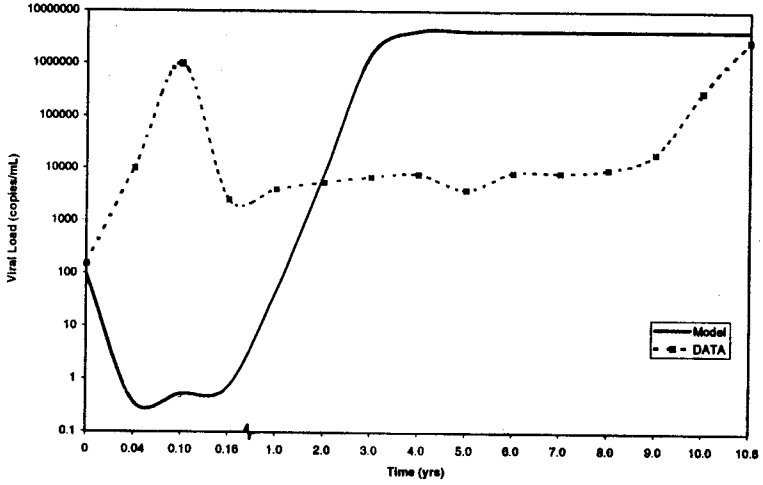

FIGURE 3B Perelson et al. model predictions for viral load compared to clinical data. The model simulations (solid line) deviate significantly from the clinical data (dashed line). During primary infection, the model predicts a significant decrease in viral load, reciprocal to what actually happens. Furthermore, a viral steady state is attained by year three. The crash to AIDS that is apparent in the clinical data is absent from the model predictions. Parameter values are as listed in [15]

CONCLUSIONS

Mathematical modeling is a useful tool for describing the dynamics of complex, nonlinear systems. The human immune system infected with HIV represents one such dynamical system. The immune system is composed of highly complex interactions between cells as well as between cells and virus. There is a delicate balance of viral replication, $CD4^+T$ cell turnover, and the CTL response during HIV disease progression. The particular elements of these interactions emphasized in a given model depend on the views of the investigator and the hypotheses being tested. However, mathematical descriptions are only as good as the current biological understanding: as the knowledge base expands, newer and more accurate models can be constructed. Here we have examined some of the earliest models of the HIV-infected immune system-models by Cooper[6],[29], Dolezal and Hraba [7], and Perelson

[15]-as predictors for clinical observations, as records of the biological understanding of the late 1980s, and as the predecessors of the current suite of models.

Cooper structured his model around heterotrimeric complexes that represented what is now understood to be antigen presentation. He biased his model toward B lymphocytes, both in the antigen presentation to $CD4^+$ T cells and in the immune response. Since then, scientists have determined that the role of B cells is less important than other immune system cells in HIV infection dynamics. Although Cooper emphasized the wrong features in his model, he did produce a detailed, mechanistic model that is more explanatory than phenomenological in its descriptions.

Dolezal and Hraba's first model attempted to describe $CD4^+$ T-cell depletion with three equations. Viral growth was assumed to be unbounded and exponential, and no immune response was included. Not surprisingly, this first model has limited value. Their second model was an improvement over the first by including a CTL immune response and thus limitations on viral growth. Moreover, numerical predictions with this model qualitatively match clinical data, although no parameter estimation discussion is given.

Perelson's model included a latently-infected $CD4^+$ T cell population and parameter estimates based on experimental data. The numerical predictions deviate significantly from the clinical data-most notably complete $CD4^+$ T cell depletion is not predicted. Improvements to his model, such as the addition of macrophage and infected macrophage populations, helped to improve the concordance between the model predictions and clinical data [15],[46].

Examining early models allows us to contrast current biological understanding with what was understood in the late 1980s. This retrospection helps keep current investigation in context. Indeed, early models such as these set the stage for the expansion of immune system modeling. Numerous models of HIV-immune interactions have since been developed. Current hot topics include pharmaceutical and immunotherapies as well as the dynamics of the latently-infected cell population in patients undergoing treatment with highly active anti-retroviral therapy (HAART). Certainly many aspects of treatment are not well-understood, and it is conceivable that ten years from now today's models will be evaluated against the current biological understanding to

assess how science has progressed, and how modeling has assisted that progression.

Acknowledgements

We thank Dr. Rosemary Rochford for helpful discussions. This work was supported by grant #R01HL62119 from NIH.

DOUGLAS J. COVERT and DENISE KIRSCHNER*

Department of Microbiology and Immunology
University of Michigan Medical School Ann Arbor,
MI 48109–0620

References

1. Ross and Hudson, (1915). Proceedings of the Royal Society of London 92 and 93, 1915–1917.
2. Kermack, W. O. and McKendrick, A. G. (1927). A Contribution to the Mathematical Theory of Epidemics. Proceedings of the Royal Society of London CXV, 700–721.
3. Hethcote, H. W. and Yorke, J. A. (1984). Gonorrhea Transmission Dynamics and Control. Springer-Verlag, New York.
4. Anderson, R. M. and May, R. M. (1992). Infectious Diseases of Humans: Dynamics and Control. Oxford University Press, New York.
5. Perelson, A. S. and Nelson, P. W. (1999). Mathematical Analysis of HIV-1 Dynamics in Vivo. Society for Industrial and Applied Mathematics Review 41, 3–44.
6. Cooper, L.N. (1986). Theory of an immune system retrovirus. PNAS 83, 9159–9163.
7. Dolezal, J. and Hraba, T. (1988). Application of Mathematical Model of Immunological Tolerance to HIV Infection. Folia Biologica 34, 336–341.
8. Hraba, T. and Dolezal, J. (1989). Mathematical Model of CD4+ Lymphocyte Depletion in HIV Infection. Folia Biologica 35, 156–163.
9. Nelson, G. W. and Perelson, A. S. (1992). A Mechanism of Immune Escape by Slow-Replicating HIV Strains. Journal of Acquired Immune Deficiency Syndromes 5, 82–93.
10. Perelson, A. S., Kirschner, D. E., and DeBoer, R. (1993). Dynamics of HIV infection of CD4+ T cells. Mathematical Biosciences 114, 81–125.
11. Perelson, A. S. (1994). Two Theoretical Problems in Immunology: AIDS and epitopes. In, Compiexity: Metaphors, Models and Reality (Cowen, G., Pines, D., and Meltzer, D., Eds.). Addison-Wesley, Reading.
12. Essunger, P. and Perelson, A. S. (1994). Modeling HIV infection of CD4+ T-cell subpopulations. Journal of Theoretical Biology 170, 367–391.
13. Perelson, A. S., Essunger, P., Cao, Y., Vesanen, M., Hurley, A., Saksela, M., Markowitz, M., Ho. D. D. (1997) Decay characteristics of HIV-1-infected compartments during combination therapy. Nature 387, 188–191.

* Corresponding Author.

14. De Boer, R. J. and Perelson, A. S. (1998). Target Cell Limited and Immune Control Models of HIV Infection: A Comparision. Journal of Theoretical Biology 190, 201–214.
15. Perelson, A. S. (1989). Modeling the Interaction of the Immune System with HIV. In, Mathematical and Statistical Approaches to AIDS Epidemiology (C. Castillo-Chavez, Ed.). Springer-Verlag, New York, pp. 350–370.
16. Reibnegger, G., Fuchs, D., Hausen, A., Werner, E. R., Dierich, M. P. and Wachter, H. (1987). Theoretical implications of cellular immune reactions against helper lymphocytes infected by an immune system retrovirus. PNAS 84, 7270–7274.
17. Merrill, S. J. (1988). AIDS: Background and the Dynamics of the Decline of Immunocompetence. In, Theoretical Immunology (A. S. Perelson, Ed.). Addison-Wesley, New York, pp. 59–75.
18. McLean, A. R. (1988). HIV infection from an ecological viewpoint. In, Theoretical Immunology (A. S. Perelson, Ed.). Addison-Wesley, New York, pp. 77–84.
19. Janeway, C. A. and Travers, P. (1997). Immunobiology: The Immune System in Health and Disease. Garland, New York.
20. Levy, J. (1998). HIV and the Pathogenesis of AIDS. ASM Press, Washington, D.C.
21. Harrer, T., Harrer, E., Kalams, S. A. Elbeik T., Staprans, S. I., Feinberg, M. B., Cao, Y.. Ho, D. D., Yilma, T., Caliendo, A. M., Johnson, R. P., Buchbinder, S. P. and Walker, B. D. (1996). Strong cytotoxic T cell and weak neutralizing antibody responses in a subset of persons with stable nonprogressing HIV type 1 infection. AIDS Research & Human Retroviruses 12, 585–592.
22. Yang, O.O. and Walker, B. D. (1997). CD8+ Cells in Human Immunodeficiency Virus Type I Pathogenesis: Cytolytic and Noncytolytic Inhibition of Viral Replication. Advances in Immunology 66, 273–311.
23. Stranford, S. A., Skurnick, J., Louria, D., Osmond, D., Chang, S. Y., Sninsky, J., Ferrari, G., Weinhold, K., Lindquist, C. and Levy, J. A. (1999). Lack of infection in HIV-exposed individuals is associtated with a strong CD8+ cell noncytotoxic anti-HIV response. PNAS 96, 1030–1035.
24. Westermann, J. and Pabst, R. (1992). Distribution of lymphocyte subsets and natural killer cells in the human body. Journal of Clinical Investigation 70, 539–544.
25. Chun, T. W., Carruth, L., Finzi, D., Shen, X., DiGiuseppe, J. A., Taylor, H., Hermankova, M., Chadwick, K., Margolick, J., Quinn, T. C., Kuo, Y. H., Brookmeyer, R., Zeiger, M. A., Barditch-Crovo, P. and Siliciano, R. F. (1997). Quantification of latent tissue reservoirs and total body viral load in HIV-1 infection. Nature 387, 183–188.
26. Greenberg, M. L., Lacey, S. F., Chen, C., Bolognesi, D. P. and Weinhold, K. J. (1997). Noncytolytic CD8 T cell-mediated suppression of HIV replication. Springer Seminars in Immunopathology 18, 355–369.
27. Ahsan, N., Langhoff, E. (1998). Immunopathogenesis of Human Immunodeficiency Virus. Seminars in Nephrology 18, 422–435.
28. Fauci, A. S., Pantaleo, G., Stanley, S. and Weissman, D. (1996). Immunopathogenic Mechanisms of HIV Infection. Annals of Internal Medicine 124, 654–663.
29. Intrator, N., Deocampo, G. P. and Cooper, L. N. (1988). Analysis of Immune System Retrovirus Equations. In, Theoretical Immunology (A. S. Perelson, Ed.). Addison-Wesley, New York, 85–100.
30. Caruso, A., Licenziati, S., Canaris, A. D., Corulli, M., De Francesco, M. A., Cantalamessa, A., Fallacara, F., Fiorentini, S., Balsari, A. and Turano, A. (1997). T Cells From Individuals in Advanced Stages of HIV-1 Infection Do Not Proliferate bu Express Activation Antigens in Response to HIV-1-Specific Antigens. Journal of Acquired Immune Deficiency Syndromes and Human Retrovirology 15, 61–69.

31. Ogg, G. S., Jin, X., Bonhoeffer, S., Dunbar, P.R., Nowak, M. A., Monard, S., Segal, J. P., Cao. Y., Rowland-Jones, S. L., Cerundolo, V., Hurley, A., Markowitz, M., Ho, D. D., Nixon, D. F., McMichael, A. J. (1998). Quantitation of HIV-1-specific cytotoxic T lymphocytes and plasma load of viral RNA. Science 279, 2103–2106.
32. Hraba, T. and Dolezal, J. (1989). Applications of the Mathematical Model of Immunological Tolerance to Immunoglobulin Suppression and AIDS. Acta Applicandae Mathematicae 14, 135–142.
33. Hraba, T., Dolezal, J. and Celikovsky, S. (1990). Model-Based Analysis of $CD4^+$ Lymphocyte Dynamics In HIV Infected Individuals. Immunobiology 181, 108–118.
34. Dolezal, J., Hraba, T. and Celikovsky, S. (1991). Influence of the Cell Interaction Parameters on the Stimulated $CD4^+$ Lymphocyte Depletion in HIV Infection. Folia Biologica (Praha) 37, 42–51.
35. Dolezal, J. and Hraba, T. (1991). Model-Based Analysis of $CD4^+$ Lymphocyte Dynamics in HIV-Infected Individuals II: Evaluation of the Model Based on Clinical Observations. Immunobiology 182, 178–187.
36. Ioannidis, J. P., Goedert, J. J., McQueen, P. G., Enger, C. and Kaslow, R.A. (1999). Comparison of viral load and human leukocyte antigen statistical and neural network predictive models for the rate of HIV-1 disease progression across two cohorts of homosexual men. Journal of Acquired Immune Deficiency Syndromes and Human Retrovirology 20, 129–136.
37. Ragni, M. V. (1998). Progression of HIV in haemophilia. Haemophilia 4, 601–609.
38. O'Shea, S., Newell, M. L., Dunn, D. T., Garcia-Rodriguez, M. C., Bates, I., Mullen, J., Rostron. T., Corbett, K., Aiyer, S., Butler, K., Smith, R. and Banatvala, J. E. (1998). Maternal viral load, CD4 cell count and vertical transmission of HIV-1. Journal of Medical Virology 54, 113–117.
39. Lange, J. M. (1996). Changing Therapy in HIV. AIDS 10 Suppl 1, S27-S30.
40. Mellors, J. W., Rinaldo, C. R.Jr., Gupta, P., White, R. M., Todd, J. A. and Kingsley, L. A. (1996). Prognosis in HIV-1 infection predicted by the quantity of virus in plasma. Science 272, 1167–1170.
41. Ho, D. D., Neumann, A. U., Perelson, A. S., Chen, W., Leonard, J. M. and Markowitz, M. (1995). Rapid turnover of plasma virions and CD4 lymphocytes in HIV infection. Nature 373, 123–126.
42. Blower, S. M., McLean, A. R., Porco, T. C., Small, P. M., Hopewell, P. C., Sanchez, M. A. and Moss A.R. (1995). The intrinsic transmission dynamics of tuberculosis epidemics. Nature Medicine 1, 815–821.
43. Castillo-Chavez, C. and Feng, Z. (1997). To treat or not to treat: the case of tuberculosis. Journal of Mathematical Biology 35, 629–656.
44. Le Pont, F. and Blower, S. M. (1991). The Supply and Demand of Sexual Behavior: Implications for Heterosexual HIV Epidemics. Journal of Acquired Immune Deficiency Syndromes 4, 987–999.
45. Blower, S. M. and Dowlatabadi, H. (1994). Sensitivity and Uncertainty Analysis of Complex Models of Disease Transmission: an HIV Model, as an Example. International Statistical Review 62, 229–243.
46. Kirschner, D. and Perelson, A. S. (1995). A model for the immune response to HIV: AZT treatment studies. In, Mathematical Population Dynamics: Analysis of Heterogeneity. Vol One: Theory of Epidemics (Arino, O., Axelrod, D., Kimmel, M. and Langlais, M., Eds.). Wuerz Publishing Ltd., Canada, pp 295–310.
47. Hethcote, H. W. and VanArk, J. W. (1992). Modeling HIV Transmission and AIDS in the USA. Springer-Verlag, Berlin.
48. Wang, L., Chen, J. Y., Gelman, B., Konig, R. and Cloyd, M. W. (1999). A Novel Mechanism of CD4 Lymphocyte Depletion Involves Effects of HIV on Resting

Lymphocytes: Induction of Lymph Node Homing and Apoptosis Upon Secondary Signaling Through Homing Receptors. Journal of Immunology 162, 268–276.
49. Wang, L., Robb, C. W. and Cloyd, M. W. (1997). HIV induces homing of resting T lymphocytes to lymph nodes. Virology 228, 141–150.
50. Zhang, L., Ramratnam, B., Tenner-Racz, K., He, Y., Vesanen, M., Lewin, S., Talal, A., Racz, P., Perelson, A. S., Korber, B. T., Markowitz, M. and Ho, D. D. (1999). Quantifying Residual HIV-1 Replication in Patients Receiving Combination Antiretroviral Therapy. New England Journal of Medicine 340, 1605–1613.
51. Furtado, M. R., Callaway, D. S., Phair, J. P., Kunstman, K. J., Stanton, J. L., Macken, C. A., Perelson, A. S. and Wolinsky, S. M. (1999). Persistence of HIV-1 Transcription in Peripheral-Blood Mononuclear Cells in Patients Receiving Potent Antiretroviral Therapy. New England Journal of Medicine 340, 1614–1622.
52. Ho, D. D. (1996). Viral Counts in HIV Infection. Science 272, 1124–1125.
53. Kirschner, D. and Webb, G. F. (1997). Understanding Drug Resistance in the Monotherapy Treatment of HIV Infection. Bulletin of Mathematical Biology 59, 763–785.
54. Kirschner, D., Mehr, R. and Perelson, A. S. (1998). The Role of the Thymus in Pediatric HIV-1 Infection. Journal of AIDS and Human Retrovirology 18, 95–108.
55. Nowak, M. A. and Bangham, C. R. M. (1996). Population Dynamics of Immune Responses to Persistent Viruses. Science 272, 74–79.
56. Wei, X., Ghosh, S. K., Taylor, M. E., Johnson, V. A., Emini, E.A. Deutsch, P. Lifson, J. D., Bonhoeffer, S., Nowak, M. A., Hahn, B. H., Saag, M. S. and Shaw, G.M. Viral Dynamics in HIV-1 Infection. Nature 373, 117–122.
57. Haase, A. T., Henry, K., Zupancic, M., Sedgewick, G., Faust, R. A., Melroe, H., Cavert, W., Gebhard, K., Staskus, K., Zhang, Z. Q., Dailey, P. J., Balfour, H. H. Jr., Erice, A. and Perelson A. S. (1996). Quantitative image analysis of HIV-1 infection in lymphoid tissue. Science 274, 985–989.