Mathematical Modeling of AIDS Progression: Limitations, Expectations, and Future Directions

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ABSTRACT

Mathematical models have been proposed as an aid to understanding the immune dynamics underlying adult immunodeficiency syndrome (AIDS). The best-known models, the Ho/Shaw models published in 1995, failed as predictive tools, largely because levels of circulating HIV RNA (as measured by viral load assays) are not well correlated with immune decline (as measured by CD4+ levels). An alternate model is proposed, based on the ratio of reduced to oxidized glutathione (GSH:GSSG) as an indicator of the shift to Th2-dominance within the immune system.

Introduction

More than 20 years into the AIDS era, it has become increasingly clear that the current single-virus causation model is lacking in predictive and explanatory power. Central to the clinical features of AIDS is a decline in cell-mediated immunity as measured by circulating CD4+ cells. This decline renders the patient susceptible to intracellular opportunistic infections such as *Pneumocystis* pneumonia and *Candida*, among many others. However, theoretical and clinical models of AIDS that assume that human immunodeficiency virus (HIV) has a central role in disease progression have not met expectations. A recent paper published on the correlation between viral load and CD4+ decline in unmedicated HIV-positive individuals[1] found that very little (typically 46%) of the observed variation in CD4+ decline could be accounted for by plasma viral load levels. In their conclusions, the authors state: "The results of our study challenge the concept that CD4 cell depletion in chronic HIV infection is mostly attributable to the direct effects of HIV replication. Future efforts to delineate the relative contribution of other mechanisms will be crucial to the understanding of HIV immunopathogenesis and to the ability to attenuate it."

It has been extremely difficult to construct a realistic theoretical model of immune suppression that is entirely mediated by HIV. Mathematical models have failed in their predictions, as will be discussed in more detail below. It is also becoming clear that the role of nutrition in typical AIDS-like immune dysfunction is an extremely important one.[2]

A Brief History of Mathematical Modeling of Infectious Disease

Deterministic mathematical modeling of infectious diseases on the molecular level is still a relatively new field. Although the field of epidemiology has a long and well-established history, it is only within the past several decades that mathematicians and immunologists have begun to work together to create models that attempt to predict the progression of disease in an individual. Diseases that have been modeled, with varying degrees of success, range from cancer to measles, and even to more recently discovered diseases such as severe acute respiratory syndrome (SARS).

Classical epidemiologic models use *variables* to describe the state of being of individuals within a population that has been exposed to an infectious disease. The standard categories, represented by the variables in the system, include susceptible and infectious individuals. Occasionally, recovered, immune, or exposed (but not infectious) individuals are also represented.[3] The number of variables to be incorporated depends on the particular disease being studied as well as on the desired complexity of the model. *Parameters* incorporated into the equation represent fundamental quantities such as birth rate, rate of transmission of infectious agent, death rate, and so forth, and are constants that can nonetheless be changed.

For a very simple example, consider the following: A model of susceptible (represented by S) and infected (I) individuals in a non-fatal disease framework can be constructed from a flow chart. Let b represent the birth rate, k the transmission rate from susceptible to infected (thus susceptibles are lost at a rate k, which is a net gain for those infected), and r the recovery rate (since no one infected will die). Assume that the time course of the disease in the population is short compared to the lifespan of the individuals, so that there is no need to consider death from natural causes. The dynamics of such as system can be shown to be:

$$\xrightarrow{b} S \xrightarrow{k} I \xrightarrow{r} S$$

The mathematical formulation of this system is simple:

If $\frac{dS}{dt}$ represents the rate of change of S with respect to time,

and $\frac{dI}{dt}$ the rate of change of I with respect to time, then:

$$\frac{dS}{dt} = bS - kIS$$

$$\frac{dI}{dt} = kIS - rI.$$

Of course, most models are far more complex, and it is possible to add a great degree of detail to the flow chart, which is then reflected in the equations. We can also add age and space variability by means of partial derivatives, or time-delay components such as a latent period by means of delay differential equations, if we need a more complex framework than simple change over time. In general, there is a trade-off between the desired accuracy of the model and its ease of analysis. This is because a system with dozens of variables may be realistic, but it is often intractable, even with numerical computer simulation methods.

The utility of such models lies not only in their predictive values but in the fact that we can use such theoretical frameworks to simulate treatment and vaccination programs, as well as to determine important parameters such as the basic reproductive ratio (*R0*), which tells us the number of secondary infections expected to be produced by a single primary infection. In general, an epidemic will occur if $R0 > 1$; thus, when theoretically simulating control strategies such as vaccination, we seek to drive this parameter below 1 with a minimum of cost. Murray provides an excellent overview of epidemiologic modeling.[3]

Mathematical modeling of infectious diseases on the cellular or molecular level operates on a principle similar to that applying to large-scale epidemic modeling. Within an individual, there are cells infected by the pathogen as well as cells susceptible to the pathogen, and often there are other variables incorporated to represent various components of the immune response.[4] Depending on the level of biological understanding we have of a disease, very accurate models can be constructed and used to aid in predicting things such as appropriate drug dosages, threshold values for various parameters that affect progression to disease, and more.

Ever since the discovery of HIV as the primary virus associated with AIDS, and its assumption as the etiologic agent of AIDS, mathematical models have been constructed to determine rates of progression to AIDS,[5-10] optimal drug regimens,[11-17] and the effects of the intracellular latency period on progression to disease.[18-20] However, the vast majority of these models have been lacking in predictive value, owing to a lack of complete understanding of the disease mechanism, as well as the fundamental nature of the immune system. It is thus imperative that an alternative framework is needed wherein non-HIV-mediated mechanisms of immune dysfunction are considered, either as cofactors of HIV or alternate mechanisms.

Limitations of HIV Models

The best-known mathematical models of HIV infection are surely the 1995 Ho/Shaw models.[9,21] Based on these extremely simple sets of differential equations, together with some patient data (which was not published together with the models themselves), a whole new era of HIV treatment began. The new approach, called "Hit Hard, Hit Early," was based on the assumption that HIV was in fact replicating furiously from the initial stages of infection, rather than being inactive and relatively latent as had been previously thought. The treatment strategy was simple: begin aggressive treatment with antiretrovirals from the first moment a patient was suspected to be infected, and the drugs and the immune system would be able to "nip the virus in the bud," effectively controlling it—and possibly eradicating it.

However, this strategy has been an abysmal failure in terms of eradication, and many doctors no longer advocate early treatment because of the serious adverse effects of many antiretroviral drugs. There is also scant if any evidence that early treatment has any beneficial effect.

Fundamental flaws led to the failure of the Ho/Shaw models. The biological assumptions and conclusions have been heavily criticized, largely in several papers published in 1998.[22-4] It was shown that drug treatment may have caused a transient increase in the levels of CD4+ cells measured in the peripheral blood, but closer inspection revealed that treatment was not causing a net increase in CD4+ cells, but rather their redistribution throughout the body—which is not an immunological advantage.[24] Possible reasons for this redistribution are discussed below.

The equations in the Ho/Shaw models were either oversimplified, or so poorly explained that they appeared oversimplified. As Mark Craddock has shown, when constructed and interpreted correctly, the equations predict the onset of AIDS within 30 to 60 days of initial infection by HIV.[25]

Another problem, with both the Ho/Shaw and the majority of recent mathematical models, is the use of plasma HIV RNA ("viral load") as the primary measure for the health status of HIV-infected individuals. Owing to the quantitative nature of mathematical modeling, patient status must, of necessity, be measured via so-called surrogate markers. In the case of HIV, the most commonly used surrogate markers are either CD4+ levels or viral load. It is generally assumed that the drop in CD4+ cells is concomitant with a steady increase in plasma viral load. Therefore, if a theoretical model used to simulate a particular treatment strategy shows a dramatic (theoretical) lowering of viral load, this strategy is assumed to be an excellent candidate for therapeutic success.

The foremost problem with this approach is the commonly held assumption that viral load either represents infectious virus units, or at the very least is an accurate estimator of true infectious virus units. However, viral load does not measure intact, infectious virus, but rather uses the polymerase chain reaction (PCR) to amplify viral fragments. This amplification is then used to estimate how much infectious virus actually exists. While this strategy is workable in theory, the problem is that not only does viral load overestimate infectious virus by an average factor of 60,000:1,[26] but this estimation is not consistently linear. Thus, there is no way to make an accurate estimate of infectious virus. What is referred to as the "efficiency" of PCR must be perfect, or else any estimates obtained using PCR will be wrong.

In determining viral load, referred to as the "target" DNA, a quantitative competitive PCR (QC-PCR) is often used. The target, the unknown, is amplified together with a known quantity of control DNA. Crucial to this technique is that the ratio of target to control remains exactly the same at each step because even a tiny difference in the ratio will be enormously magnified using PCR, which amplifies HIV DNA over 35-45 cycles.[26]

This troublesome aspect of the use of viral load as the primary surrogate marker for clinical health has been borne out in Piatak et al.[26] Half of all patients in the study who had detectable viral loads had no evidence of virus by culture. The lack of correlation between viral load and infectious viral particles, as measured by co-culture, has also been noted in other publications.[27,28]

Furthermore, the lack of correlation between viral load and CD4+ levels has been seen most recently in a groundbreaking study published in the *Journal of the American Medical Association,*[1] which showed that changes in viral load were only able to explain 4% of changes in the CD4+ count in the patients observed. Specifically, the study found that the coefficient of determination between viral load and CD4+ decline was only 4%. Mathematically, this means that viral load is not able to explain 96% of the variation in CD4+ levels, and that other mechanisms must therefore be responsible for most of the observed CD4+ decline. The implications of this are profound: If viral load is correlated neither with infectious virus nor with CD4+ levels, this

means of measurement constitutes insufficient evidence for making clinical decisions.

Patient status is often estimated by CD4+ levels in the peripheral blood, and while such measurements are a better health indicator than viral-load values, they are at best imperfect indicators, owing to their variability and to the fact that they cannot account for CD4+ cells residing in lymph tissue or bone marrow.

There have been many more mathematically sophisticated models of HIV infection, which have met with varying degrees of success. One of the most widely cited is that of Perelson, Kirschner, and deBoer,[8] which studies the interactions between healthy T-cells, actively infected T-cells, latently infected T-cells, and free virus in the bloodstream. Many treatment models have been based on the Perelson model. Its utility lies in its ability to predict an "infected steady state," meaning that the patient is HIV-positive, but not ill. Though it has some realistic basis, the model fails to take into account crucial immune system parameters. One such consideration is the existence of two principal subsets of CD4+ cells: the Th1 (responsible for cell-mediated immunity) and Th2 (responsible for humoral immunity) subsets.

There are two principal uses of such mathematical models. First, by examining the effects that changes in parameters have on the outcome of the system over time, we can determine which parameters are most important in disease progression, and further, we can determine critical "threshold values" for these parameters, which are often crucial in the attempt to control disease. Second, treatment strategies can theoretically be simulated, allowing the design of clinical trials to be streamlined.

There are, however, two major potential obstacles to the success of such treatment models. First, accurate parameter estimation is critical, and since different patients have drastically different rates of progression to AIDS, this suggests that parameter estimation may be impossible for a model that is meant to be applied to an individual patient. The second potential problem, which is more easily solved, is the fact that very few published mathematical models of HIV infection and treatment take into account drug toxicity, which has emerged as a major problem with anti-HIV treatment schemes.[29]

A few studies have partially addressed the toxicity problem by using optimal control theory to formulate theoretical treatment strategies.[11,13,15] Toxicity is built into the models in the form of an objective to be minimized. However, toxicities are assumed to be nonspecific. For example, bone marrow toxicity, which is clinically extremely relevant, is not mentioned explicitly. Moreover, the models remain beset by the original problems of parameter estimation and model structure.

The alternate strategy proposed here for the theoretical estimation of health and progression to AIDS in at-risk individuals also has the problem that parameter estimation is highly individual. It does, however, address the problem of using viral load as a stand-in for patient health, and takes into account the particular type of decline in CD4+ cells that is specific to AIDS patients.

Importance of the Th1/Th2 Balance in Immune Function

Until the immune system is fully understood, all models of AIDS progression will lack realism and usefulness. An alternate strategy is needed if we wish to achieve any theoretical success. It is proposed that, rather than attempting to model an infection whose dynamics remain poorly understood, we seek to consider other modes of immune dysregulation that have been demonstrated to occur primarily in those diagnosed HIV-positive.

With the exception of one major paper by Root-Bernstein,[30] all immunological models of HIV infection have failed to consider either cofactors for HIV infection, or other possible viral or nonviral mechanisms of immune dysfunction. For this reason, it is impossible, with current tools, to predict either immune dysfunction or its time course accurately.

AIDS is fundamentally different from traditional immune deficiencies that predispose the patient to multiple resistant bacterial infections. Examples of such immune deficiencies include those experienced by transplant recipients, and severe combined immune deficiency syndrome (SCIDS), colloquially known as "boy in the bubble" disease. In AIDS, there is a complex interplay between both components of the dual immune response, with the humoral response dominating at the expense of the cellular.

All HIV models presented thus far have considered the CD4+ cell immune subset as a single entity, and their principal goal has been to model its decline, as well as its viral dynamics. However, since the beginning of the AIDS era it has become clear that the observed decline in CD4+ cells is more complex than simple depletion.[3,13-16] Moreover, all measurements of CD4+ cells, from which clinical health and drug efficacy are estimated, are taken from the peripheral blood, and it has traditionally been assumed that the observed decline represents a decline in the total population of CD4+ cells.

In the late 1980s, Mossman and Coffman presented a major study that indicated that the pool of CD4+ cells in fact contained two different types of T-helper subsets.[37] CD4+ cells, popularly known as helper T-cells, are produced in the thymus and bone marrow as immature Th0 cells. They then mature into either Th1 or Th2 helper cells, and both the functions of those helper cells, as well as their "residence" in the body, differ substantively. Furthermore, it has been shown that HIV expression is not found equally in the Th1 and Th2 subsets.[38-40]

Th1 cells are primarily responsible for cell-mediated immunity, which means that they protect against intracellular pathogens such as fungi, yeasts, viruses, and mycobacteria. Th1 cells are also believed to be involved in cancer surveillance, and as such protect primarily against lymphomas.[37] Th1 cells' production of nitric oxide (NO) gas, which destroys intracellular pathogens, is the main operational mode of the cellular arm of the immune system. The majority of Th1 cells reside in the peripheral blood, and it is their depletion that is observed in the progression to AIDS.

Th2 cells, in contrast, are responsible for extracellular immunity, and protect against bacteria and parasites.[37] They are also heavily involved in antibody production—hence the name "helper T cell" for all CD4+ cells—and they are significantly associated with autoimmunity. Th2 cells, unlike the Th1 cells, cannot produce NO gas in significant quantities, and therefore are lame against intracellular "opportunistic infections." Th2 cells reside mainly in the bone marrow and to a lesser extent in the lymph nodes, and do not appear to become depleted in the progression to AIDS. In fact, their number has been observed to increase.[41,42]

The discovery of the Th1/Th2 division sheds light on the mechanisms of AIDS-related immune dysfunction. A gradual shift

from Th1- to Th2-dominance is observed. The number of circulating T-cells in the periphery greatly decreases, and the number of T-cells in the bone marrow and lymph nodes increases.[41-43] However, the total number of T-cells remains the same. This Th1-to-Th2 shift perfectly explains some of the major conundrums of the AIDS clinical syndrome. Specifically, AIDS is unlike classical immune deficiencies in that patients experience predominantly fungal and mycobacterial infections, but very few or no "classical" bacterial immune deficiency diseases. Furthermore, elevated levels of antibodies, including autoantibodies, are characteristic of all AIDS patients—a finding consistent with a decrease in the Th1 subset coincident with an increase in the Th2 subset.

The story becomes very interesting when one considers which subset of T-cells manifests features generally considered to be indicative of HIV, such as reverse transcription and various HIV-associated proteins. Contrary to intuition, HIV is expressed primarily in Th0 and Th2 cells, and is scarcely to be found in the Th1 subset.[38-40] This is curious indeed, since it is the Th1 cells that decline, whereas the cells in which HIV prefers to reside do not decrease.

The question then becomes: What mediates the Th1-to-Th2 shift, and how can it be prevented or reversed, and the immune system restored to homeostasis?

Mathematical modeling can help understand the dynamics behind the immune system shift. The particular branch of mathematics that is useful in this case is known as bifurcation theory, and it deals with critical parameter changes in systems. Models can help illuminate which parameters regulate major changes in a system and which are incidental.

In the case of the Th1-to-Th2 shift, a crucial component is the release of NO from the cell-mediated arm of the immune system, as alluded to previously. Lowenfels has comprehensively reviewed the role of NO in the immune system.[44] Here is a brief synopsis:

NO is able to diffuse freely through cell membranes because of its gaseous nature, small size, and the fact that it needs no specialized receptors to aid with cell-to-cell communication.[45] Nitrogen oxides are regulated by the oxidative state of the immune cells. There is abundant evidence that excessive oxidation negatively affects immune function by influencing the production of cytokines from the immune cells.[46,47] Oxidative processes are counterbalanced by reduction, accomplished by sulfur-containing molecules called thiols that serve as electron donors. The primary intracellular antioxidant is glutathione, a tripeptide consisting of cysteine, glutamate, and glycine. Glutathione is found in both the reduced (GSH) and oxidized (GSSG) form. The ratio of GSH:GSSG has been shown to be important in regulating the Th1/Th2 balance.[46,48] An excess of GSSG at the expense of GSH is generally considered a marker for severe oxidative stress. Also, GSH deficiency has been shown to inhibit NO synthesis, rendering the cellular immunity defense even more helpless.[49]

If the GSH:GSSG ratio declines, Th2 cells are preferentially manufactured, as Th0 cells are instructed to mature into Th2 cells. The net effect is a shift to Th2-dominance at the expense of Th1 cells, creating a reduced T-cell count in the bloodstream, and increasing susceptibility to opportunistic infections. It is worth noting that highly active antiretroviral therapy (HAART) causes a transient increase in T-cell counts in the peripheral blood because it damages B-cells as they mature and disrupts antibody production. Unable to make contact with antibody-producing B-cells in the bone marrow, Th2 cells return to the peripheral blood.[23] Although the T-cell count increases, the cells are ineffective against opportunistic infections. The phenomenon of the so-called immune reconstitution syndrome, in which patients experience the "irony" of an increase in opportunistic infections after initiating HAART therapy, is further evidence of this effect.[50]

An Alternate Model

The role of HIV in the GSH:GSSG ratio remains unclear, and it has been proposed that the expression of phenomena believed specific to HIV is a consequence rather than the cause of the Th1-to-Th2 shift.[49] Regardless, it is crucial to understand how to control this ratio, and hence the ability of Th1 cells to persist and thus to provide protection against opportunistic infections.

Mathematics can help us to understand the dynamics of the Th1-to-Th2 shift and may be capable of illuminating how to prevent it altogether. In an alternate model for the immune decline seen in AIDS patients, the crucial components are the GSH:GSSG ratio and the Th1/Th2 balance, which are pivotal in development of AIDS. HIV itself need not even be included as a variable.

The proposed model tracks the Th0, Th1, and Th2 subsets of the T-cell pool over time. The GSH:GSSG ratio is treated as a possible bifurcation parameter. As GSSG increases, and the ratio thus declines, a greater proportion of the Th0 cells mature into Th2 cells and are diverted from the Th1 pool. A model could enable us to determine, for example, the critical ratio of GSH:GSSG below which a shift to Th2-dominance occurs. Stability analysis will assist in determining when and if an optimal balance can be reached and/or maintained.

This model has several advantages. First, it replaces viral-load measurements, which have not been shown to have good clinical predictive value, as a therapeutic endpoint. Second, determining a critical ratio—that of GSH:GSSG—rather than a critical value circumvents the problem of parameter variability among individual patients. Finally, the model explicitly considers the Th1/Th2 ratio, an important value in progression to AIDS that has been largely neglected in theoretical modeling.

The construction and interpretation of such a model will depend on patient data regarding the aforementioned parameters, and will be the subject of a future study.

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