Reconstructing Microbial Pathogenesis

Mathematic models can simulate the complex dynamics between host and pathogen during various phases of infection

Denise Kirschner

After decades of focusing on infected patients and experimental animals, modern research in microbial pathogenesis shifted its main focus to cellular and biochemical mechanisms governing host-parasite interactions. Undoubtedly, studies at multiple scales are needed, but how can we better use knowledge from this research to improve our understanding of host-pathogen relationships?

During an infection, key elements interact in dozens, if not hundreds, of ways. This complexity imposes enormous challenges for researchers who follow standard experimental approaches in trying to understand these interactions. Thus, mathematical modeling becomes an important additional tool for them, much as it has been for studying many other complex systems in disciplines such as quantum physics, astronomy, and macroeconomics, as well as elsewhere in biology, including gene regulation, receptor-ligand interactions, microbial population dynamics, and epidemiology.

The use of "in silico" methods to analyze infectious disease mechanisms enhances understandings that come from both in vitro and in vivo research. As with in vitro research using biochemical and cell biological models, in silico analysis enables investigators to consider the host-pathogen relationship in a defined way and to study its components individually. Moreover, as with in vivo research using animal models, those relationships can be explored in all their complexity, while monitoring multiple components simultaneously. In addition, in silico analysis allows investigators to generate large volumes of data that simulate events that occur over very long time scales (on the order of months, years, or decades) and thus can extend intuitions about the behavior of key elements within biological systems. This capacity to extrapolate over extended time scales is particularly useful when analyzing latent infections.

Mathematical models of host-pathogen dynamics are built on specific assumptions regarding interactions among system components. In the same way that an experimental animal model can be so important in understanding a human biological system, a mathematical model can provide valuable insights into complex interactions and reveal key governing parameters. Unlike statistical methods that rely solely on the analysis of empirical data, mathematical models provide both qualitative and quantitative descriptions of the system under investigation. These descriptions, in turn, enable the modeler to use mathematics to manipulate specific elements and then to determine the effects of such manipulations on overall behavior of the system. In addition, once a host-pathogen system can be reliably described with a mathematical model, investigators can explore elements of the system that are problematic, or even impossible, to address experimentally.

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Modeling Principles

In applying mathematical modeling to study the interplay among specific factors during infec-

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Modeling Pathogens with Math: Interdisciplinary to the Max

"I think I was destined to like math and to be involved in it my whole life," says computational biologist Denise Kirschner. It wasn't that she planned a math career, even though she grew up doted upon by two aunts who were both math teachers and she knew the multiplication tables by heart at age four. "I was always very good at math, and I got lots of honors and awards in school," she recalls. "But I never once, ever, at any time [when I was younger] considered being a math major or that I would become a math teacher. I was always going to go to medical school."

Kirschner managed to pull together the best of her early mathematics and biology interests into her chosen career path—developing algorithms to build computer models of microbial pathogenesis. Although she nearly attended medical school, she deferred enrollment to study at the Center for Nonlinear Studies at Los Alamos National Laboratory. That supposedly brief stint turned full time, led her to do doctoral research there, and steered her clear of a career in medicine. Ironically, she now serves on the medical school faculty at the University of Michigan (UM) in Ann Arbor. "I ended up in a medical school, which is where I wanted to be since day one, except that I'm using the mathematical tools that I'm naturally good at to address biological questions," she says.

Kirschner adjusted her course to UM at several critical turning points, and she credits influential mentors for their guidance along that somewhat roundabout path. Some key advice came during a conversation with a mathematics professor who took her aside in her senior year in college and said, "'You know, Denise, we're really disappointed that you're going to medical school,'" she recalls. "He said, 'not too many people can get a Ph.D. in math, and we think that you can.'" The idea of pursuing a doctorate in math had simply not occurred to Kirschner until that moment. After all, she had never met a female mathematician with an advanced degree.

Infectious disease expert Marty Blaser, then at Vanderbilt University (VU), provided another dose of guidance while she was doing postdoctoral work in the VU math department.

Blaser knew of her work modeling HIV immunology and epidemiology. "He got me interested in Helicobacter pylori," she says, referring to the ulcer-causing microbe. "He had this vision that we could use computer modeling to understand the host-pathogen dynamics. I remember thinking, 'Wow, this is it.'" Four years later, Kirschner was attracted to the Department of Microbiology and Immunology at Michigan when it opened a search for a computational biologist studying host-pathogen interactions. Application of mathematics to microbiolical questions has had a long tradition at Michigan. Its current chair, Michael Savageau, provided critical mentoring that helped her establish a new research focus in microbial pathogenesis.

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tions, good models use parameters that represent defined biological features, such as growth rates and nutrient uptake, rather than derived values that merely align a model with experimental data. Models also can include relevant dynamic information revealed by experiments, such as the presence or absence of specific factors or their relative temporal and spatial expressions.

A key strength of modeling is that it identifies sensitivities to parameters and initial conditions, indicating which processes and interactions are dominant in the dynamics. For example, the outcome of an infection initiated with an inoculum of 10 bacteria might be qualitatively different from one initiated with 10^4 bacteria, thus illustrating sensitivity to this particular parameter.

As with conventional experiments, in silico research relies on iterative refinements, incorporating additional details as they become available. Although a mathematical model might not fully answer questions about a system, a successful model still should enable investigators to pose questions about that system and to suggest experiments that could provide useful insights.

Testing and validation are important elements of the modeling process. A standard approach is to compare output from the model

Today, Kirschner's lab continues to study HIV and *H. pylori*, but her personal focus recently shifted to *Mycobacterium tuberculosis*. "I wanted to develop a pathogen model on my own, one that no one had worked on yet," she says. That meant spending much of her first six months as a faculty member at UM in Ann Arbor reading voraciously about tuberculosis while also sitting in on immunology and microbiology courses to learn about key subjects that were not part of her mathematics curriculum in graduate school or her subsequent research as a postdoc.

Recently, she and her colleagues submitted reports on a computer model that accurately can predict the effects of a protective host factor on the community prevalence of tuberculosis. She is also working to add a spatial component to the temporal model of tuberculosis infection that her lab developed. "We hope this will become a powerful tool that experimentalists can use to ask lots of questions," she says.

Doing interdisciplinary research can be both exciting and trying, and being one among only a handful of specialized mathematicians who focus on microbial pathogens further raises the ante. "There are very, very few of us who seriously model bugs," Kirschner says. "I can count 10 papers where people have actually tried to model the pathogenesis of bacterial infections, and we've done three of those. It's just a very young area." On one side of this interdisciplinary spectrum, some mathematicians do not regard computational biology as serious mathematics. At the other, clusters of skeptical biologists also tend to disregard her work, according to Kirschner. "I've had a few biologists tell me that they're offended by my use of the word 'experiment' to describe what I do," she says. "They're skeptical, one, because I don't think they understand what we do. I think that society has done a good job at making people math phobic. And two, because they're a little fearful about the methods, that they wouldn't be able to evaluate them. I don't want people to think that we're doing anything different than what they're doing in a wet lab. We're thinking about things in the same way. We're setting up experiments in the same way; it's just that we're doing them on the computer.

Kirschner calls her similarly interdisciplinary-minded husband, Russ Butler, a vital "soul mate," who helps her withstand such unappreciative skeptics. "He uses geography, ecology, and ornithology for his science, and I'm mixing mathematics and immunology and microbiology in my world," she explains. "Sometimes we serve as the sole person to say to one another, 'No, you're not crazy. It's ok to be mixing all these different disciplines.' Because we are moving science forward—or at least we're trying to.

"The way science has typically moved forward in the past is when different disciplines have come together," she continues. "And when that happened, it pushed both disciplines forward. I believe that's the case with computational biology from both the mathematical and the biological perspectives."

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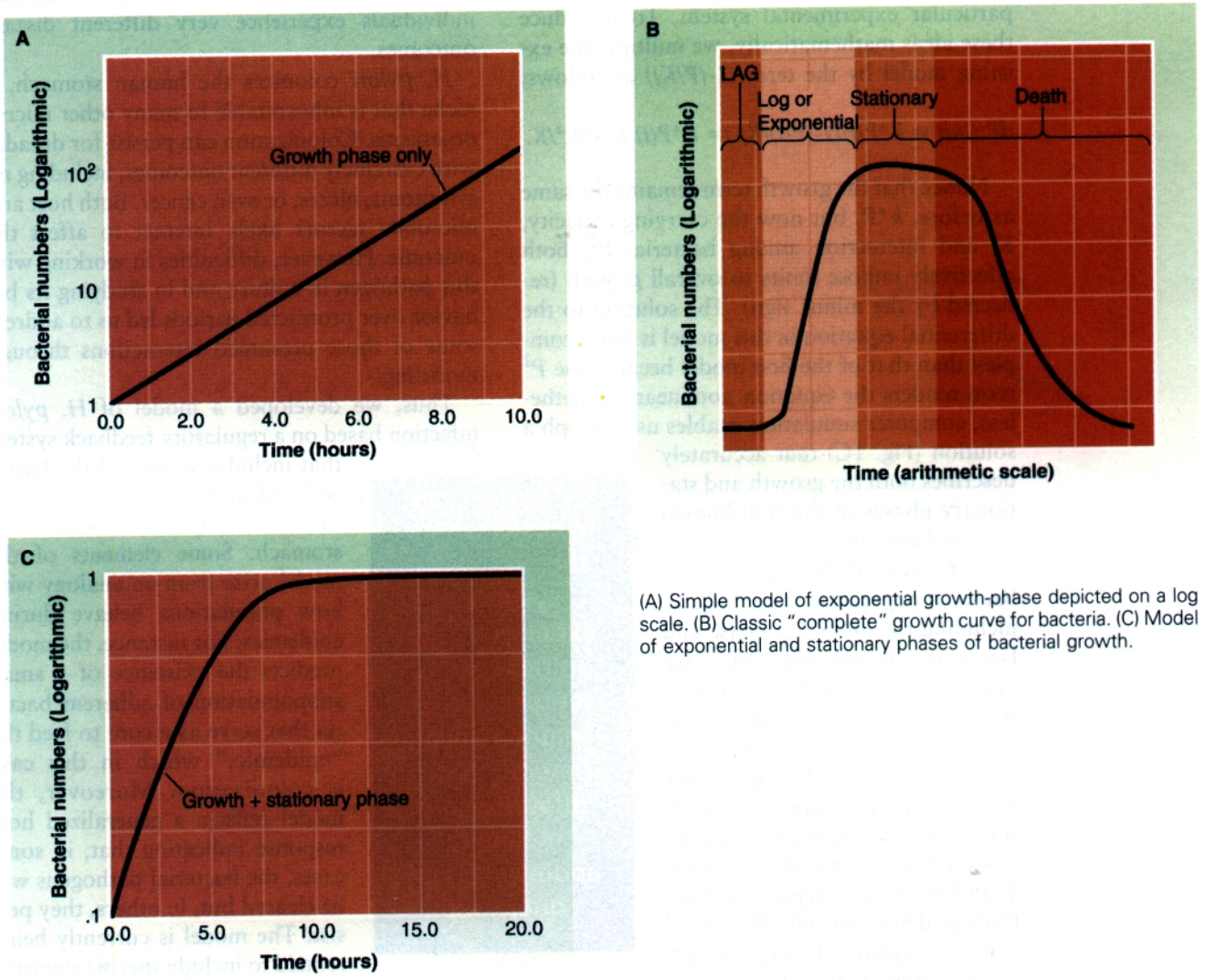
with experimental or clinical data to determine if the assumptions about the interactions of system elements used to build the model accurately capture actual dynamics. If so, the investigator can be confident in the model and the insights that it can generate.

Developing a Model To Describe Bacterial Growth Dynamics: a Simple Example

Some of the earliest mathematical modeling of population dynamics was done during the first part of the 19th century by the English economist Thomas Malthus. For instance, to describe population growth, he assumed that the population grows exponentially. In mathematical terms, this model implies that the rate of change of a population at time t, namely $P(t)$, is proportional to the size of the population: $dP(t)/dt = k*P(t)$, where k is the growth-rate constant of that change. The mathematical solution to this differential equation, where P_0 represents the initial population size, is $P(t)=P_0*e^{kt}$. When represented on a logarithmic scale, this function is linear (Fig. 1A).

Although Malthus focused on how growth in the human population and food production might affect one another, his basic proportionality assumption can readily be applied to other

FIGURE 1



(A) Simple model of exponential growth-phase depicted on a log scale. (B) Classic "complete" growth curve for bacteria. (C) Model of exponential and stationary phases of bacterial growth.

populations, such as those of bacteria. Of course for any population, the exponential growth model does not hold true over an extended time frame—in part, explaining why his doomsday prediction that the exponentially expanding human population would shortly exceed the food supply was never realized.

Over a shorter period, the same mathematics and similar graphics are useful for describing the growth pattern of a typical bacterial population (Fig. 1B). This simple model then can be expanded to deal with greater complexity of the system and to better describe its known dynamics—thus exemplifying the iterative refinement process that is integral to successful modeling.

In this example of bacterial population growth, for instance, we can add the concept of population-carrying capacity, called *K*. This general term may encompass various conditions affecting the growth of bacteria in culture. Growth of the bacterial population is limited by, among other things, the volume of the culture, which could moderate toxic effects, as well as the availability of nutrients in the media, which

could affect competition among bacteria. The parameter K is a measure of these elements in a particular experimental system. To introduce these ideas mathematically, we multiply the existing model by the term $(1-(P/K))$ as follows:

$$dP(t)/dt = k*P(t)(1-(P(t)/K)) = k*P(t)-k*P(t)^2/K.$$

Notice that the growth term remains the same as before, $k*P$, but now the carrying capacity, K, and interaction among bacteria, P^2, both effectively impose limits to overall growth (reflected by the minus sign). The solution to the differential equation in this model is more complex than that of the first model because the P^2 term renders the equation nonlinear. Nonetheless, computer simulation enables us to graph a solution (Fig. 1C) that accurately describes both the growth and stationary phases of the well-known bacterial growth curve.

To capture all the stages in the bacterial population growth cycle, including the initial lag phase and late-stage die-off (Fig. 1B), we again need to modify the model. While the expanded equations can accurately describe the bacterial growth curve, they do not yet constitute a useful model to understand specific conditions that control growth because the functions k and K do not represent basic biological mechanisms. The model could be improved by expressing k as a function of conditions such as nutrient concentration, temperature, and rates of nutrient uptake, and K as a function of other conditions affecting growth, including accumulation of toxic metabolites and changes in oxygen concentration.

In cases where the biological system is experimentally intractable, a representative mathematical model may offer the sole means to address certain questions

Applying Modeling Principles to Agents of Infectious Disease

We are far from fully understanding how many infectious diseases progress. This gap in understanding is particularly striking for a wide range of latent or persistent infections that are difficult to study experimentally. My lab group has focused on three such infectious agents—HIV-1, *Helicobacter pylori*, and *Mycobacterium tuberculosis*. For each of these pathogens, the major question that we are trying to address is why various members among a group of infected individuals experience very different disease outcomes.

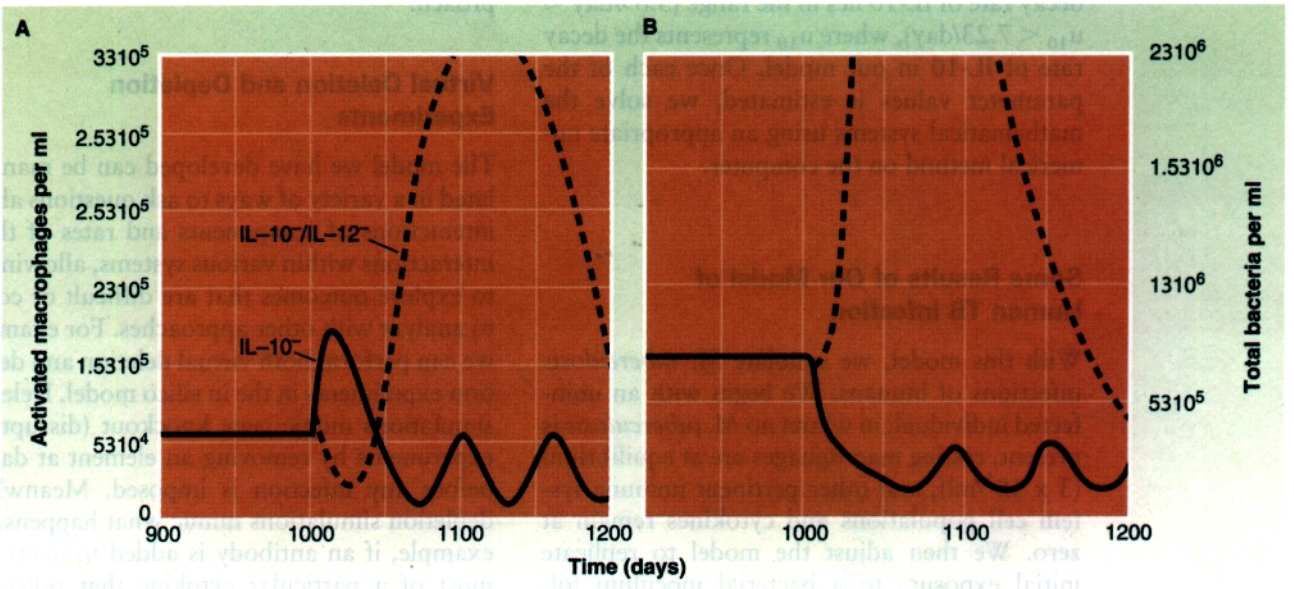
H. pylori colonizes the human stomach, a niche that is inhospitable to many other microorganisms. Colonization can persist for decades with extremely different outcomes, including no symptoms, ulcers, or even cancer. Both host and microbial factors likely interact to affect the outcome. However, difficulties in working with this pathogen in culture and in studying its behavior over protracted periods led us to address some of those presumed interactions through modeling.

Thus, we developed a model of *H. pylori* infection based on a regulatory feedback system that includes several of the bacterial and host features that enable this bacterium to colonize the stomach. Some elements of the model arise from an analogy with how populations behave during epidemics. For instance, the model predicts the existence of a small subpopulation of adherent bacteria that serve as a core to feed the "epidemic," which in this case is colonization. Moreover, the model reflects a generalized host response indicating that, in some cases, the bacterial pathogens will be cleared but, in others, they persist. The model is currently being refined to include specific elements that will help to explore the mechanisms of clearance or persistence.

In Silico Model of Infection with *M. tuberculosis*

Tuberculosis (TB) is a leading cause of death worldwide, responsible for an estimated 3.1 million deaths per year. *M. tuberculosis* is not only one of our oldest microbial enemies, it remains one of the most formidable: perhaps one-third of the entire world population has latent TB. Thus, there is a great need to elucidate the mechanisms of TB disease progression and to devise ways of controlling those infections. A key issue is to understand why infected individuals experience such different clinical outcomes – latency, primary, or reactive TB.

FIGURE 2



IL-10 depletion experiments. Solid lines are IL-10 depletion results, dashed lines are double depletion (both IL-10$^-$/IL-12$^-$). both cases are shown for activated macrophages (Panel A) and bacterial load (Panel B). Simulations begin in latency, and depletion occurs on day 1000.

Janis Wigginton and I developed a model that qualitatively and quantitatively characterizes the cellular and cytokine control network operating during TB infection of humans. Our system captures host-pathogen dynamics at the infection site by monitoring bronchoalveolar lavage (BAL) fluid in the lung. During the early stages of developing the model, we identified several elements that help to regulate the host response, including CD8+ T cells that play a key role in controlling such infections. Natalya Serbina and JoAnne Flynn at the University of Pittsburgh, Pittsburgh, Pa., experimentally confirmed the importance of CD8+ T cells. The model also indicates that the role of interleukin-10 (IL-10) likely is more important than was thought.

Our model tracks mycobacteria and various host cell populations, including TH0, TH1, and TH2 cells; resting, activated, and infected macrophages; and four cytokines, interferon (IFN)-γ, interleukin-12, interleukin-10, and interleukin-4. This model then captures more than 100 interactions among these elements, enabling us to monitor them both individually and collectively, again highlighting the value of this mathematical approach. The model can be expanded by adding in other cells, cytokines, chemokines, or interactions to determine how they might influence system dynamics.

Once the mathematical expressions were developed representing the interactions among the eight cell populations and four cytokines, we needed to determine the values of the rate constants governing each of these interactions. To do so, we estimated values for most rate parameters from published experimental data, with weight given to results obtained from monitoring patients or measuring human cells; we also favored *M. tuberculosis*-specific data over results based on other mycobacterial species such as *Mycobacterium bovis* BCG. Moreover, estimates obtained from multiple studies are presented as a range of values. On those parameters for which we have a range, or those for which no experimental data are available, we performed sensitivity analyses to obtain order-of-magnitude estimates that could be used in the model.

An example of parameter estimation comes from how we established the decay rate of IL-10. Various studies estimated the range of half-life values to be 2.3–4.5 hours. The decay rate

can be estimated from half-life given by the standard formula $r = ln\ 2 / half\text{-}life$. Thus, the decay rate of IL-10 lies in the range ($3.69/day < u_{10} < 7.23/day$), where u_{10} represents the decay rate of IL-10 in our model. Once each of the parameter values is estimated, we solve the mathematical systems using an appropriate numerical method on the computer.

Some Results of Our Model of Human TB Infection

With this model, we simulate *M. tuberculosis* infections of humans. We begin with an uninfected individual, in whom no *M. tuberculosis* is present, resting macrophages are at equilibrium (3×10^5/ml), and other pertinent immune system cell populations and cytokines remain at zero. We then adjust the model to replicate initial exposure to a bacterial inoculum followed by near-immediate clearance with no immunologic memory of that response. Other outcomes for this simulation include latency, primary disease, and reactivation. Model-based simulations allow us to determine which elements of the dynamic system govern each outcome.

Parameter values that control the rates and behavior of interactions in the model may change from individual to individual, and even within an individual over time. The virtual experiments reveal that changes in only certain parameters lead to very different disease outcomes—either latency or active disease. For instance, to simulate reactivation, one or more critical parameters needs to undergo a time-dependent change.

One key finding is that cells included in the model cannot produce as much IFN-γ as those measured in BAL fluid from patients with TB. This finding suggests that cells other than CD4+ T cells are producing IFN-γ as part of the host response to *M. tuberculosis*. A second important finding is that infected macrophages do not kill enough *M. tuberculosis* cells to suppress the infection under many situations. Taken together, these results imply that cytotoxic T cells, which produce IFN-γ, are key factors in the immune response to *M. tuberculosis*, a conclusion that Serbina and Flynn confirmed experimentally. Therefore, modeling helped lead us to recognize independently that a critical element was missing from our understanding of how the immune system responds to TB, again highlighting one of the strengths of the modeling approach.

Virtual Deletion and Depletion Experiments

The model we have developed can be manipulated in a variety of ways to ask questions about interactions of components and rates of those interactions within various systems, allowing us to explore outcomes that are difficult or costly to analyze with other approaches. For example, we can perform both virtual deletion and depletion experiments in the in silico model. Deletion simulations mimic gene knockout (disruption) experiments by removing an element at day 0, before any infection is imposed. Meanwhile, depletion simulations mimic what happens, for example, if an antibody is added to neutralize most of a particular cytokine that otherwise would be active within the system. These simulations are performed by setting a given parameter to zero after the system has achieved latency. These analyses allow us to determine what elements are needed to achieve and maintain latency.

For instance, we used the model to simulate IL-10 depletion under two different conditions (Fig. 2). When IL-10 is depleted after latency is achieved, there is an abrupt increase in macrophage activation and IFN-γ production, leading to other events that suppress bacterial numbers to one-third of those observed during latency (Fig. 2, solid lines). Once the bacterial load drops, the immune response returns to equilibrium by 100 days, with levels ranging from one-half to one-third of previous latency values. This equilibrium appears to be driven by a threefold increase in IL-4, presumably compensating for the depleted IL-10.

Our IL-10 depletion simulations do not indicate that IFN-γ production increases. Hence, we were surprised to see an increase in IFN-γ and in activated macrophages when IL-10 is depleted. Because IL-12 regulates IL-10 production, we simultaneously depleted IL-10 and IL-12 and thus abrogated IFN-γ production, and hence the level of activated macrophages (Fig. 2, dashed lines). This same effect is seen in vitro using peripheral blood mononuclear cells from TB patients.

Both the experimental and model results high-

light the complexity of immune regulation in patients with TB. Deleting IL-10 before infection may actually facilitate the clearance of subsequently added bacteria at the cost of tissue damage. Depleting IL-10 after latency throws the system out of equilibrium. Thus, the model *suggests IL-10 helps to maintain latency.*

In both humans and mice, infection with *M. tuberculosis* results in IL-10 production primarily by macrophages and, to a lesser extent, by TH2 lymphocytes. In humans, unlike in the mouse, IL-10 is also produced by TH0 and TH1 lymphocytes This difference in IL-10 production is an important example of how studying TB in mice may not accurately predict the immune response to this infection in humans. However, we can include this feature in our simulation model. Several hypotheses regarding the role of IL-10 in TB are currently being tested in the labs of our collaborators John Chan at the Albert Einstein School of Medicine, Bronx, N.Y., and JoAnne Flynn.

As more and more data accumulate on TB and other infectious diseases, we need better means to integrate this information. Mathematical modeling offers one method for doing so. Sometimes such models may appear merely to confirm what experimental scientists already surmise about a system. Properly understood, the model is only a starting point for designing crucial experiments to test hypotheses. In cases where the biological system is experimentally intractable, a representative mathematical model may offer the sole means to address certain questions. Additionally, a model may illuminate testable aspects of the system that had not occurred to the experimentalist.

Ideally, those doing modeling can collaborate closely with experimentalists who are generating data while asking pertinent biological questions. Just as genetics and computer science have spawned bioinformatics as a powerful means to explore genetics, so microbial pathogenesis and mathematics can be brought together to vastly increase our understanding of infectious diseases. A new breed of scientists needs to be trained to communicate across disciplinary boundaries if this potential is to be realized.

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