Overcoming Math Anxiety: Malthus Meets Koch

Mathematical modeling helps us to understand host-microbe interactions, including pathogenesis

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A key challenge in modern biomedical research is to make better sense of the vast amount of data now being generated that does not consistently advance our knowledge. Here we address this challenge in the context of the host-pathogen relationship, with the key question being how we can understand this relationship and account for those aspects of it that lead to infection and immunity. Put another way, how can we take pixels and integrate them into a larger picture of what is going on?

Much of what we know about microbial pathogenesis comes from analyzing key components—either from the microbe or from the host—at a molecular level. In the case of toxin-producing pathogens, our understanding of diseases caused by such microbes often derives from rigorous biochemical analyses of toxins. Moreover, much is learned by studying how

toxins reach their cellular targets. Additionally, investigators have developed and applied genetic and imaging approaches to study when and where during infections such pathogens produce such toxins. Similarly, host responses to microbial pathogens are studied at levels ranging from molecular to organismal.

After Reductionism, Reconstructionists Seek To Describe the Big Picture

Many experimental researchers study biological systems by examining the molecular and cellular details of those systems, an approach that has been termed "reductionist." By practicing "reconstructionism," researchers take insights gained from these studies and integrate them into a more comprehensive understanding of the system in question.

Mathematical modeling provides a very useful approach toward integrating molecular and cellular data. Whatever its drawbacks, this approach can help to capture in a virtual sense the dynamics of microbial infections and host responses, enabling investigators to manipulate and analyze many features on time scales that are otherwise impossible. With such models, genes may be disrupted or neutralized, and infections then simulated within a matter of seconds. Fortunately for those microbiologists who are anxious about mathematics, plenty of math-savvy collaborators are available.

In applying this approach to microbial pathogenesis, we must first assemble what we know about a wide range of mechanisms used by specific pathogens such as toxin

- A key biomedical research challenge involves harnessing large datasets to better understand host-pathogen relationships and to account for what leads to infection and immunity.

- Through mathematical modeling, researchers "reconstruct" molecular and cellular data to capture in a virtual sense the dynamics of microbial infections and host responses, while analyzing those activities on time scales that would otherwise be impossible.

- Models are being used specifically to simulate dynamic responses to host cytokines and both macrophage and T cells to *Mycobacterium tuberculosis*, while analyzing the complex interactions that lead granulomas to form.

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activities, pilus-mediated adherence, and intracellular survival, as well as those used by the host to defend against pathogens, such as Toll-like receptor signaling, cytokine production, and macrophage function. We then use that knowledge to develop models describing host-pathogen dynamics—trying simultaneously to capture and simulate the in vivo situation but also using those models to describe or test features of particular host-pathogen interactions, much as we would do through experiments in vitro.

Various Approaches Available for Modeling Microbial-Host Interactions

What kinds of mathematical models are being applied to host-microbe interactions? In some cases, mathematical biologists produce deterministic models, studying the rates governing molecular or cellular interactions, from which new insights and hypotheses may be developed. In other cases, models that are based on an analysis of discrete events sometimes simulate "emergent" behaviors, namely those that had been neither defined nor expected.

Regardless of approach, once a system can be described mathematically, it serves as a starting point from which hypotheses may be generated and tested. The value to experimental biologists is that mathematical models are highly versatile in vitro-type tools, serving as templates for formulating and addressing a wide array of biological questions. By manipulating parameters in models, investigators may identify key mechanisms, new experiments, or design principles that are not otherwise obvious.

Reluctance in appreciating mathematical modeling perhaps reflects skepticism that the use of equations based on reaction rates can enable us to understand biological systems. It remains a challenge for some experimental microbiologists to believe that mathematical models indeed can help us to better understand complex systems, even though we rely daily on the outcomes of mathematical models. For example, they are used to forecast the weather, the economy, and the motions of the moon and planets. In these and so many other complex cases, the relative success from applying mathematical models depends on sound math, known interactions, and good data.

Why should it be any more of a challenge to

imagine that complex genetic regulatory circuits, infection dynamics, or immune responses could fall within the realm of biologically astute mathematicians? Consider a much simpler system that often is cited in introductory calculus courses: a faucet delivering water into a sink with a drain releasing that water. To learn how much water is in the sink, one could experiment by adjusting the flow from the faucet, partly or fully closing the drain, and measuring the water that accumulates. But consider a more theoretical approach, based on calculating the rates of water flowing into the bowl and out the drain. A simple differential equation relates those rates to solve for the volume of water. Once developed, that equation can be used to predict how this system behaves when the rates are altered, or exactly how long it takes for the sink to fill.

In this simple example, all that is needed to solve the question is to determine the two governing rates. In studying more complex host-microbe interactions, modelers try to determine all the important rates and build equations to predict how the system behaves over time. In many cases, a model can help to ascertain whether key elements are missing and what their influence might be. No matter its complexity, the most informed model is one that starts with ample and reliable data.

Mathematical Models Extend Our Understanding of Host-Pathogen Interactions

Microbes and hosts interact in complex ways, with the outcome dependent on many factors. Infections often are not clear-cut in terms of producing symptoms or damage to a particular host. Instead, the outcome can be anywhere on a spectrum from rapid, symptom-free clearance of a pathogen to fulminant, even lethal disease. Intuitively, we recognize that many factors affect outcome, and that changing one parameter can be counterbalanced by others to produce the same outcome. For example, when a particular strain produces toxin at an increased rate, the host may adjust by making more antibody to neutralize that toxin.

Modeling host-pathogen interactions may help to identify the effects of individual factors on other variables in the system and thus to understand which factors may have a major impact on the outcome of an infection. When

theoreticians make these predictions, they enable the experimentalist to focus on the most important factors in the system.

We build mathematical models for some of the extremely complex interactions between *Mycobacterium tuberculosis* and its human host. Published data from human and animal studies and our data from studying infected animals informed the development of our mathematical systems. By continuing to incorporate new information, we are further refining these models to reflect subtleties in the system and to increase their accuracy.

One major advantage of mathematical models is that with them we can ask questions that might be impossible to address experimentally. An example is in trying to understand the factors that contribute to reactivation after latent tuberculosis. Although 90% of *M. tuberculosis* infections in humans are latent, nonhuman primates provide the only accurate animal model of latent tuberculosis (TB). In humans, such latent infections can be maintained for decades, meaning an infection may reactivate 50 years after it begins. By using experimental data to model and simulate immune mechanisms that contribute to latency, we can begin to predict which factors may prevent reactivation. Conducting equivalent experiments would be technically challenging or even impossible.

Along similar lines, mathematical models can be used to address important questions about localized immune responses and compartmentalized responses. For example, a pathogen may initially infect organs and tissues such as the skin or lung rather than a lymphoid organ. In such cases, dendritic cells ferry the pathogens to a lymph node, where they prime the adaptive immune response, triggering T cells to move to the site of infection. This process, which is split between at least two sites in the body, depends on numerous factors that direct specific actions at each site. Mathematical models can be used to describe such compartmentalized processes with far greater ease than they can be studied either in culture or fully in vivo.

At a mechanistic level, a mathematical model allows us to test specific questions about host-pathogen interactions. For instance, we can examine various effector mechanisms of T cells over the course of a chronic infection such as tuberculosis. One question might be which T cells produce a particular cytokine at each stage during an infection. We can address this question using mathematical models by varying the contribution and timing of individual cytokines produced by each T cell subset and examining the outcomes. It is also possible to study the dynamics of bacterial populations during an infection and to predict the behaviors of specific strains. In this way, both host and pathogen can be studied as an integrated system in which each element can be monitored and varied simultaneously to determine its role in infection dynamics.

Modeling Granulomas in *M. tuberculosis* Infections

Inhaling *M. tuberculosis* bacteria into the lung triggers a complex immune response involving specific cells such as macrophages, CD4+, and CD8+ T cells as well as immune effectors such as chemokines and cytokines. This spatio-temporal process yields multicellular structures called granulomas. Such granulomas serve several roles, including containing the infection; however, they also contribute to pathology. These structures are complex and diverse in type. Some granulomas may limit bacterial growth to result in a latent infection, whereas others may be incapable of containing the infection and result in active disease. Understanding what causes granulomas to form and how they function will improve our ability to diagnose and treat tuberculosis.

Fundamental challenges in using mathematics to study biological systems include choosing appropriate scale representation and mathematical tools. To model granuloma formation, we use several mathematical tools to capture this phenomenon at different biological scales, with consistent themes among each of these predictions but also clear differences that arise from each perspective and set of assumptions.

Applying the Agent-Based Model to Granulomas

Consider our results in applying the agent-based model to granuloma formation. This model contains four defined components: (i) the agents involved in the dynamics; (ii) the environment where those agents reside and interact; (iii) the rules governing the behavior of the agents; and

FIGURE 1
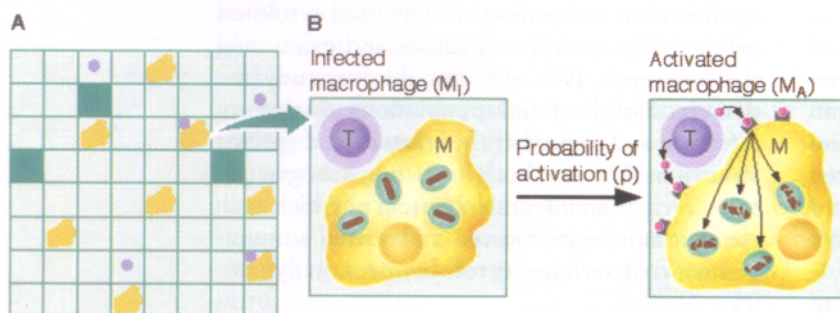
The development of the mathematical model for granuloma formation. Shown in both panels are agents (M for macrophage, T for T cell). Panel A shows a cartoon of the environment: the lattice representing the 2mm x2mm lung. In fact, there are 500 x 500 grids, but we show only a subset. The dark areas represent places on the grid that are vascular for entry of new cells. Each grid can contain at most one macrophage, but may also contain T cells as well as concentrations of bacteria and chemokines/cytokines. Panel B indicates one 'rule' for infected macrophages. If a macrophage takes up mycobacteria and becomes infected (M_I) then there is a probability (p) that infected macrophage will interact with a T cell and bind cytokine to become activated (M_A). This allows killing of its intracellular bacteria load.

(iv) estimated rates and rate constants for all agent interactions. The model tracks several agents, including chemokine concentrations and aggregate populations of both intracellular and extracellular bacteria, along with discrete macrophages in one of four states—resting, activated, infected, and chronically infected—and effector T cells.

In the simulated host environment, we assume each agent acts independently and also can interact with other agents. We also assume the environment to be a two-dimensional, 2–by-2-mm cross-section of lung tissue (Fig. 1A), which is an average cross-sectional area of granulomas. Mathematically, the structure in which we track these dynamics is known as a lattice. We assume the tissue is vascularized, enabling new cells (agents) to enter the lung onto the lattice through these sites (see darkened squares in Fig. 1A). What remains to be defined are the rules that govern the behavior of the cells in lung tissue.

In building rules, the mathematical model includes a plethora of data regarding which cell types and effector molecules are involved in *M. tuberculosis*-host interactions. Once key agents are identified, known interactions are included. For example, resting macrophages take up bacteria, thereby becoming infected. During a particular period, interactions between an infected

macrophage cell and an effector T cell produce enough of the cytokine interferon-γ (IFN-γ) to activate that macrophage, which can kill its intracellular bacteria (Fig. 1B). Although this example describes only one rule, many are included in the model. Additional experimental data, either gleaned from the literature or generated by collaborators, can thus help to ensure that key dynamics are included.

We also use experimental data to estimate parameters for many, and ideally all, of the interactions. Again considering macrophages being infected by *M. tuberculosis*, we need to estimate several rates, including the time that a macrophage must be in contact with a T cell, the concentration of IFN-γ necessary to stimulate a macrophage, the numbers of bacteria per macrophage, and the probability of activating an effector T cell that will produce IFN-γ in proper quantities for a sufficient period. When no data exist, we perform detailed uncertainty and sensitivity analyses to determine how the model output is affected by variations in parameter value choices.

Once we complete these four steps, we can use the agent-based model to simulate a time-lapse movie of a granuloma forming de novo (see http://malthus.micro.med.umich.edu/lab/abm/movies). We then run a series of simulations, varying parameters that we identified by a sensitivity analysis as significant.

One noteworthy observation from these simulations is that two different types of granulomas emerge. The first (Fig. 2A) is smaller and more solid, showing minimal or no necrosis, whereas the other (Fig. 2B) is larger with a significant necrotic core. These two simulated granulomas show striking similarities with pictures of granulomas from a nonhuman primate model of TB (Fig. 2C and D). Both outcomes also are seen in human infection, as well as in certain animals. A key parameter difference that leads to these two outcomes was the rate and timing of effector T cells being recruited to the infection site. These findings have potential importance for vaccine design as well as for our understanding of the dynamics of granuloma formation and control of infection in vivo.

FIGURE 2
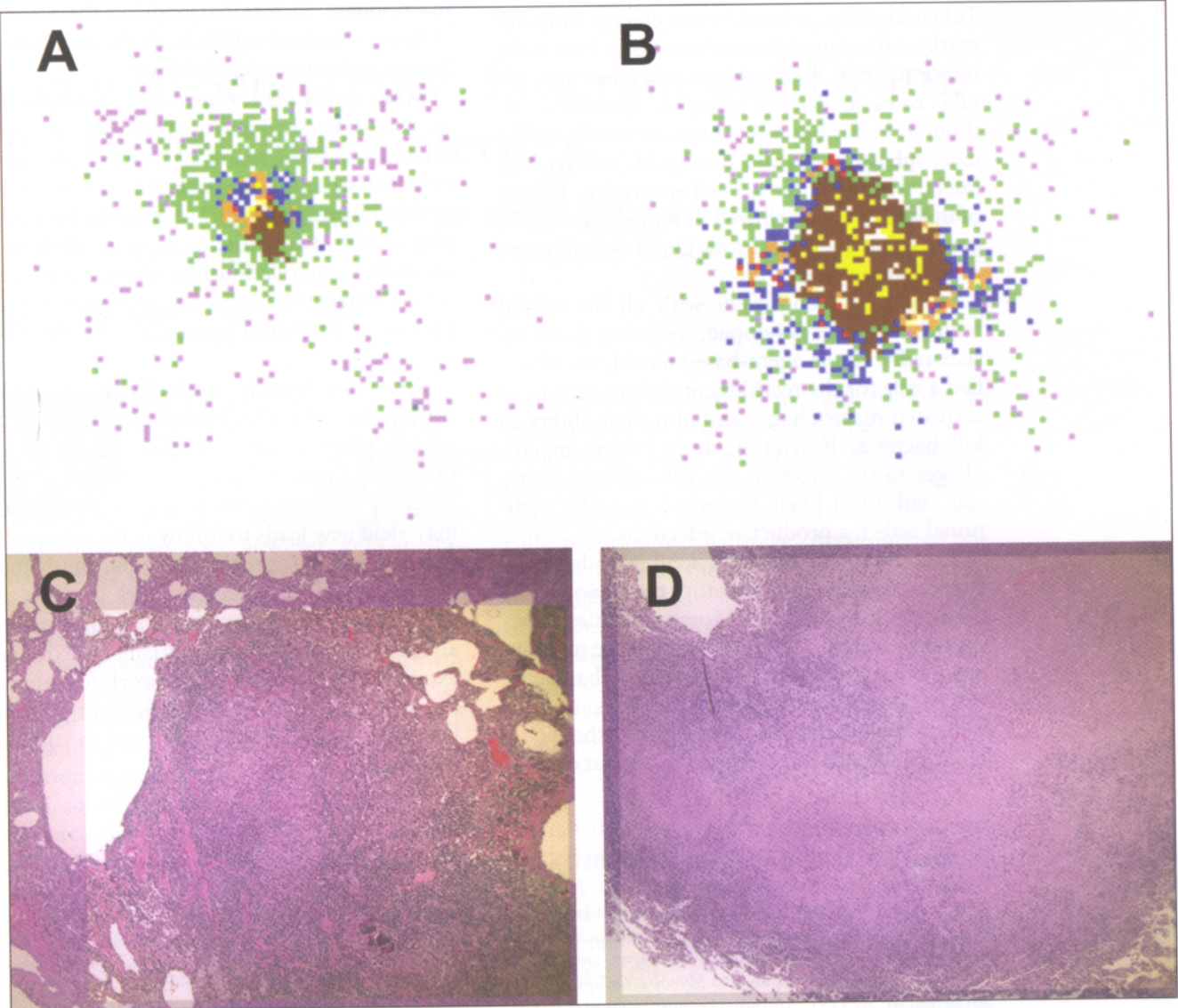
Simulations of the agent-based model of granuloma formation at day 200 postinfection with an inoculum of 10 bacteria with comparison of granulomas from the non-human primate model. Panel A shows the prediction of a solid granuloma outcome, while panel B shows the prediction of a larger, necrotic granuloma. Note that the shape of the granuloma and the arrangement of cells emerges from the computer simulation and is not specified a priori. T cells enter from a number of vascular sites on the lattice. The difference between the simulations in panels A and B was the timing and speed of arrival of effector T cells. Key for both panels: Pink-T cells, Green-resting macrophages, Tan-infected macrophages, Red-chronically infected macrophages, Blue-activated macrophages, Brown-necrotic regions and Yellow-extracellular bacteria. Intracellular bacteria are trapped within infected and chronically infected macrophages. Panels C and D show histopathologic comparison of solid (C) and caseous (D) pulmonary granulomas in the lungs of *M. tuberculosis*-infected cynomolgus macaques. Solid granulomas (C) consist of a densely populated collection of inflammatory cells that include centrally located epithelioid macrophages and histiocytes surrounded by lymphocytes. Caseous granulomas (D) are characterized by a central area of necrotic material, an outer later of epithelioid macrophages, histiocytes, and giant cells ringed by lymphocytes. Hemotoxylin and eosin stain, total magnification 10x. Both granulomas shown are 2 x 2 mm in size. (Photos donated by Dr. P. Ling Lin, in the Flynn Laboratory at the University of Pittsburgh.)

Models of Tuberculosis Yield General Insights and Some Surprises

Taken together, several themes emerge from our mathematical models of tuberculosis. First is the importance of macrophage activation and uptake rates in driving infection dynamics. Although the former meets expectations based on macrophage activation during M. *tuberculosis* infections, the latter proved surprising. If rates of uptake could be slowed or halted, our results predict that such infections could be contained or perhaps even cleared.

Second, and consistent with all the spatial models we have developed, including those associated with the agent-based model, we identified a role for the recruitment and movement of activated macrophages and also their ability to kill bacteria. Recruiting more resting macrophages to the infection site will increase bacterial load, most likely because it provides additional cells for productive infections.

This effect on bacterial load paradoxically suggests that this inflammatory response may be detrimental to the host. Moreover, unless macrophages become activated, they serve to propagate infection rather than halt it. By balancing inflammation and macrophage activation, T cells play a crucial role in containing these bacteria. Finally, our models predicted that chemokine dynamics, involving factors such as turnover and diffusion rates, are key to facilitating an optimal response. Factors that modulate these features in favor of the host will likely tilt the scale towards latency rather than active disease.

More generally, applying a mathematical model to a particular system provides an opportunity to organize many facts and factors into a cohesive structure. In addition, doing so may enable investigators to better visualize a complex system, while forcing them to think more carefully about relationships among specific factors. Taking such a rigorous approach thus can provide an improved appreciation of the whole system.

Even more broadly, for biologists, instead of fearing the unknown, embracing mathematical models provides an alternative means for approaching complex questions, including many that may be experimentally intractable, and thus may yield new leads to follow in the laboratory. Ideally, and to optimize benefits, mathematicians and biologists need to work together and to find a common language for asking questions about biological systems. It can be extremely exciting to build a new framework for addressing such questions, and the findings have the potential to challenge dogmas and to stimulate new ideas.

SUGGESTED READING

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