Mycobacterium tuberculosis as viewed through a computer

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Mathematical models are emerging as important tools in the study of microbiology. As an illustrative example, we present results from several models each generated to study the interaction of *Mycobacterium tuberculosis* and the immune system. Different mathematical models were formulated on the basis of assumptions regarding system–component interactions, enabling us to explore specific aspects at diverse biological scales (e.g. intracellular, cell–cell interactions, and cell population dynamics). In addition, we were able to examine both temporal and spatial aspects. At each scale, there were consistent themes that emerged as determinative in infection outcome. Factors we identified include both host and microbial characteristics. The use of the models lies in generating hypotheses that can then be tested experimentally. Here, we outline the primary host and bacterial factors that we have identified as key mechanisms that contribute to the success of *M. tuberculosis* as a human pathogen. Our goal is to stimulate experimentation and foster collaborations between theoretical and experimental scientists.

Introduction

Mycobacterium tuberculosis is one of the oldest human pathogens; evidence of tubercles has been found even in Egyptian mummies. The fantastic success of this organism is highlighted by the fact that one-third of the world is infected. Given its long association with humans, one might expect that the study of this pathogen would have revealed a range of virulence factors. Without becoming trapped in a cycle of definitions regarding virulence, it is clear that standard notions do not apply for *M. tuberculosis*. This poses an interesting conundrum for microbiologists; namely, how can arguably the world's most successful pathogen lack traditional virulence factors? Is this just a matter of definitions, or has this bacteria evolved beyond our traditional concepts of such factors? To further confound things, *M. tuberculosis* has a stable genome [1] and thus mutations or phase variations probably do not contribute to its ability to evade the immune response. Here, we use a mathematical modeling approach to lend support to the idea that virulence strategies used by *M. tuberculosis* enable it to survive within macrophages and evade host immunity. We identify both microbe and host characteristics as determinative to its success.

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A role for mathematical modeling in microbiology

Most modern research in microbial pathogenesis takes place at the level of cellular and biochemical mechanisms governing host–parasite interaction; however, studies at larger scales are undoubtedly needed for a deeper understanding of infectious diseases. Components of host–pathogen systems are sufficiently numerous and their interactions sufficiently complex that intuition alone is inadequate to fully understand the dynamics of the interactions. Here, mathematical modeling becomes an important integrative experimental tool (Figure 1). Mathematical models provide a unique approach for representing and studying the integrated behavior of complex biological systems. A strength of the modeling process is that it can lend insight and clarification to existing data and theories, as well as enabling one to compare and contrast existing hypotheses. We extensively rely on collaborators and the literature to define our model structure, to decide which biological mechanisms to include, and to determine what alternative hypothesis to test. Once the structure of the model is defined, parameters that represent defined biological rates are derived from published experimental data as well as those generated from our collaborators (i.e. half-lives, infection rates and activation rates). We give weight to studies performed using human cells and *M. tuberculosis* primary and virulent laboratory strains. For full details of the modeling process, please see, for example, Wiggington *et al.* [2].

Our mathematical models represent dynamics of a general adaptive immune response and microbial factors specific to *M. tuberculosis*. Data comparison and model validation has been performed concurrently in a non-human primate (NHP) model. [3,4] When data are not available, we employ detailed statistical uncertainty and sensitivity analyses to estimate parameter values and evaluate how variations in their values contribute to infection dynamics.

We have developed several mathematical models exploring the interaction between the human immune system and *M. tuberculosis*. They have been designed to capture global dynamics regarding trafficking between the lung and its associated draining hilar lymph node [5,6] (Box 1), as well as more local dynamics occurring within a cell (Box 2), within a granuloma [7] or within lungs of infected individuals [2]. Mathematical models can be used in a variety of ways to not only reproduce bench experiments serving as validation of the model, but also to perform experiments not presently accessible in the

(a)

Growth

Bacteria

1-4 days

=

Exponential phase (linear term)

$B(t)$

$B(0)$

t [day]

$$\frac{dB_E}{dt} = \alpha B_E \implies$$

$$B_E(t) = B(0)e^{\alpha t}, \ \alpha > 0$$

(see Parameter estimation box for α)

Killing by Macrophages

Extracellular bacteria

Activated Macrophage

Dead bacteria

Activated Macrophage

→

Mass-action

For a chemical reaction   A + B → C, the law of mass action gives the rate as $dN_C/dt = \beta \ [N_A][N_B]$ where N denotes concentrations. In our model, we have # of cells (M_A) and # of bacteria (B_E) instead of concentrations.

$$\frac{dB_E}{dt} = -\beta \ B_E M_A$$

One macrophage has to physically interact with one bacteria to be able to kill it. Parameter β denotes the likelihood of killing

Infection: from extracellular to intracellular

Extracellular bacteria

Resting Macrophage

Intracellular bacteria

Infected Macrophage

→

Saturation (Michaelis-Menten)

$\frac{dB_I}{dt}$

δM_R

$\frac{\delta M_R}{2}$

s

B_E

$$\frac{dB_I}{dt} = \delta M_R \left(\frac{B_E}{B_E + s} \right)$$

Release (Michaelis-Menten type)

CTL

Extracellular bacteria

Dead Macrophage

Th1

CTL APOPTOSIS killing

Intracellular bacteria

Infected Macrophage

←

BURSTING

Dead Macrophage

Extracellular bacteria

→

(b)

$$\frac{dB_E}{dt} = \ + \alpha B_E \qquad \text{(growth)}$$

$$-\beta_1 M_R B_E - \beta_2 M_A B_E \quad \text{(killing due to macrophages)}$$

$$-\beta_3 IDC \ B_E \qquad\qquad \text{(killing due to dendritic cells)}$$

$$-\delta M_R \left(\frac{B_E}{B_E + s_1} \right) \qquad \text{(uptake by macrophages)}$$

$$+\gamma M_I \left(\frac{R_1}{R_1 + s_2} \right) \qquad \text{(release due to CTL/apoptosis)}$$

$$+\varphi M_I \left(\frac{B_I^2}{B_I^2 + (X_I)^2} \right) \quad \text{(release due to bursting)}$$

(c)

LEGEND

$\dfrac{dB_E}{dt}$: variation in # of B_E over time

B_E: extracellular bacteria
B_I: intracellular bacteria
M_R: resting macrophages
M_A: activated macrophages
M_I: infected macrophages
IDC: immature dendritic cells
s_i: half-saturation terms (i=1,2,3)
β_i: rate of interaction (i=1,2,3)
δM_R: max uptake
γM_I: max release (apoptosis)
T_1: T helper Type 1
$R_1 = \dfrac{T_1}{M_I}$ (Th1-M_I ratio)
CTL: Cytotoxic T cells
φM_I: max release (bursting)
X_I: average # of bacteria released
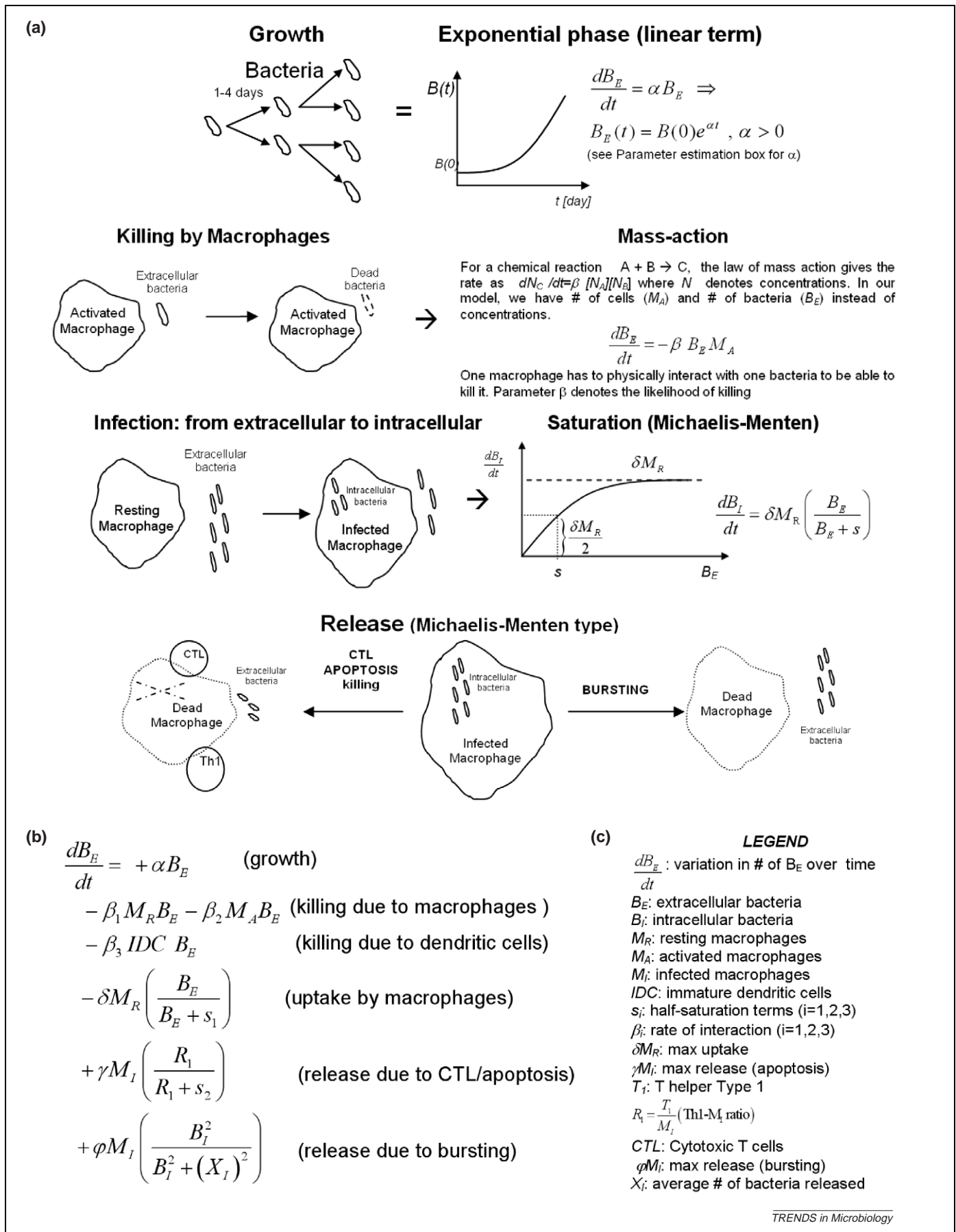
TRENDS in Microbiology

Figure 1. Extracellular bacteria dynamics: an example of the modeling process. **(a)** From biology to math: an example of how biological concepts are translated into mathematical equations. Parameter estimation of the growth rate α of *Mycobacterium tuberculosis*: *in vitro* estimates for doubling times of H37Rv laboratory strain within

Box 1. How do we track infection in *Mycobacterium tuberculosis*?

Major infection outcomes in humans are latent infection (persistent infection, ~90% of infected) and active TB (~5% of infected). Reactivation also occurs (~5–10% of latently infected) [28]. Reliable markers of the status of *M. tuberculosis* infection in humans are not presently available. A key challenge historically in the study of tuberculosis has been identifying a truly representative animal model for human latent TB. Mouse models are most often used, however latent infection (the most common outcome in humans) is not observed in the mouse and granuloma formation is less structured with a different spatial pattern than in humans. Guinea pigs and rabbits are also available models, but reagents for studying the immune response to *M. tuberculosis* are scarce in these animals. Non-human primates (NHP) more accurately reflect human disease but these animals are expensive and must be maintained under Biosafety Level 3 conditions. CFUs in tissue correlate with disease status in NHPs [29] and in mice, where a bacterial burden (in whole lung) greater than 10^8 translates to death [30].

On the basis of data obtained from animal studies, we chose bacterial load as the most informative marker of TB progression in our model simulations. We recognize, however, that the status of *M. tuberculosis* infection is not reflected simply by the number of bacteria or cell types present in the lung. Our models indicate that latency is a state whereby bacterial numbers in the lung are low and relatively stable; by contrast, during active TB, bacterial burdens increase exponentially (Figure Ib). What should be emphasized is that, owing to the non-linear nature of this biological system (and the models developed to study it), there are multiple paths by which these endpoints can be reached. In other words, by playing the parameters off each other (and hence the processes they govern), the system can either attain latent infection, active TB, reactivation or even clearance. In this way, both the time it takes to reach latency and the levels of bacteria present might also vary corresponding to these changes. This has important implications for explaining differences between individual host responses during infection.
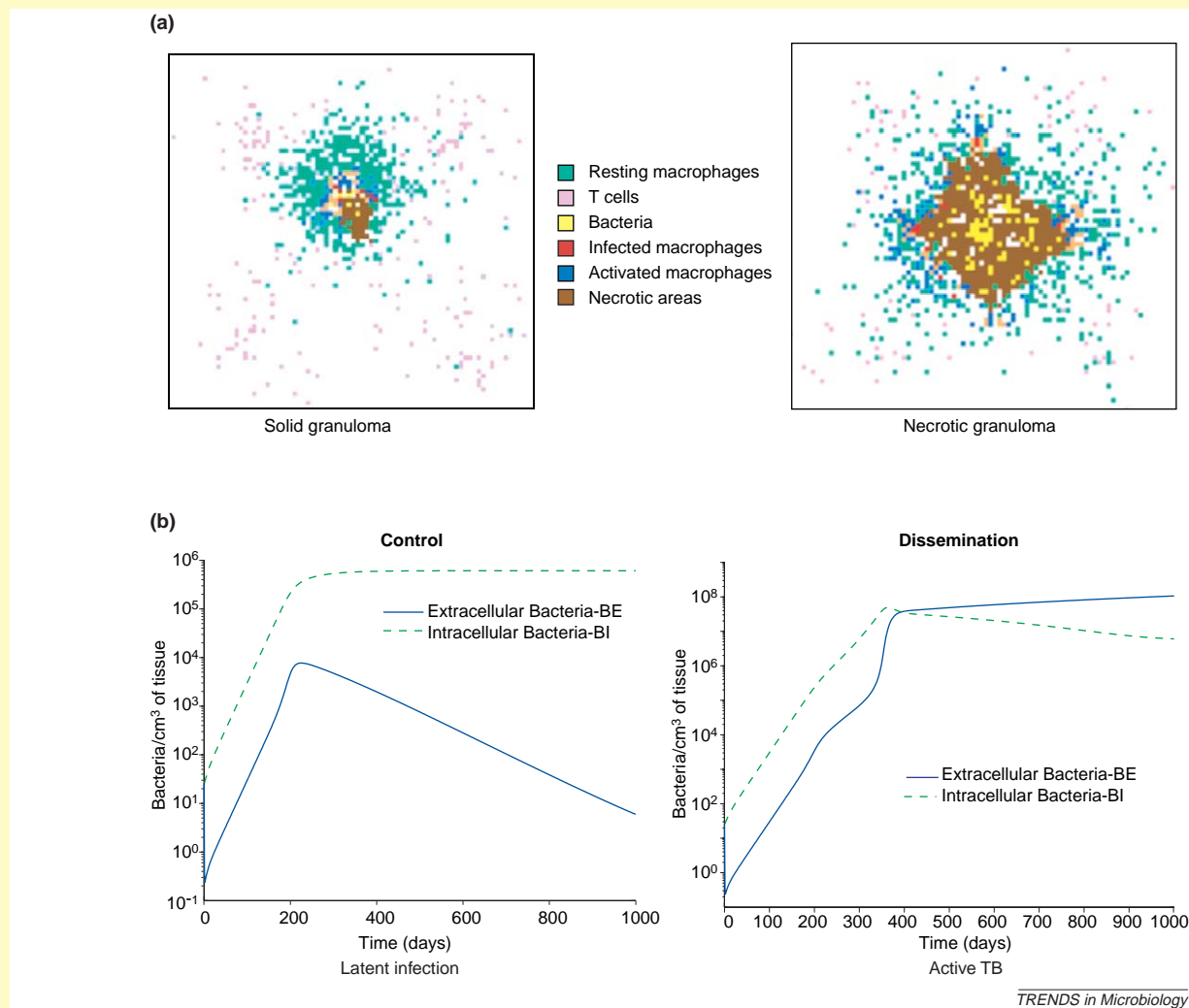
(a)

Resting macrophages
T cells
Bacteria
Infected macrophages
Activated macrophages
Necrotic areas

Solid granuloma

Necrotic granuloma

(b)

Control

Extracellular Bacteria-BE
Intracellular Bacteria-BI

Latent infection

Dissemination

Extracellular Bacteria-BE
Intracellular Bacteria-BI

Active TB

TRENDS in Microbiology

Figure I. Model simulation results. **(a)** Simulations of a granuloma using an agent-based model. Shown are containment (left side) and dissemination (right side) outcomes for a single granuloma. Both granulomas shown are at time points 360 days post infection and are each shown in a 2 mm $\times$ 2 mm size window. **(b)** Global scale model of lung and lymph node dynamics during *Mycobacterium tuberculosis* infection. Shown are simulations of bacterial levels in the lung during latency (left side) and active TB (right side, log scale).

macrophages ranged from 28 to 96 hours. In mouse lung tissue, H37Rv was estimated to have a doubling time of 63.2 hours. We can estimate the intracellular versus extracellular growth rates from these values: rate $=$ ln2 / doubling time $\rightarrow \alpha_{BI}=$ [0.007, 0.024], $\alpha_{BE}=0.011$ (per day). **(b)** Differential equation for extracellular bacteria, B_E. Each equation of the model represents the incremental variation of a certain quantity over time (days): pg/ml (x 10^6 cells) for cytokine concentrations, cell/cm^3 of tissue for cellular variables and bacteria/cm^3 of tissue for *M. tuberculosis*. **(c)** Table of symbols.

Box 2. Models capturing intracellular dynamics between *Mycobacterium tuberculosis* and the macrophage

Antigen presentation by macrophages

Mathematical models describing the interaction of *M. tuberculosis* and the immune system can also be formulated at the intracellular level. In one such model, we can represent macrophage processes leading up to antigen presentation, including major histocompatibility complex (MHC) class II expression, antigen processing, and peptide–MHC binding [31]. Our model enables these mechanisms to be considered together rather than singly as is typically necessary in the laboratory setting. *M. tuberculosis* has been found to inhibit several of these processes but for what purpose? Are redundant mechanisms necessary for the bacterium to evade immune surveillance and survive? We suggest they might not actually be redundant but serve to ensure continuous downregulation of antigen presentation. By contrast, the inhibitory effect of a single mechanism can either be delayed or attenuated with time depending on whether the mechanism targets MHC class II expression or not. This time-dependent behavior of *M. tuberculosis* has been observed by others based on

expression profiles showing that key regulatory genes are differentially expressed. [32,33]

Iron metabolism

Clinically, excess iron has long been correlated with active TB [34], whereas increases in nitric oxide production by macrophages are closely coupled to changes in the amount of transient intracellular iron. [35] We highlight two key parameters involved in the regulatory network within macrophages; namely the enhancement of *M. tuberculosis* growth by iron and nitric oxide killing of *M. tuberculosis*. Our model simulations (J. Christian Ray and D. Kirschner, unpublished) suggest a stronger role for an indirect effect via nitric oxide, rather than a direct enhancement of *M. tuberculosis* growth by iron, as a mechanism for intracellular bacteria proliferation during TB infection. The relationship between host factors (nitric oxide and iron sequestration) and bacterial factors (metabolism) implies that a non-intuitive virulence strategy is probably at work.

laboratory setting. For example, multiple simultaneous virtual depletion experiments can be performed predicting results for gene-knockout murine models that do not currently exist. Models can be useful for testing treatment strategies or elucidating behavior that is observed, for example, using 2-photon microscopy [8]. Here, mathematical models can predict mechanisms that lead to observed phenomena.

Whose side are you on?

To study host–pathogen interactions, scientists often attack problems from either the perspective of an immunologist or a microbiologist. If one wishes to study bacterial factors and their effects on aspects of immunity while simultaneously attempting to understand how specific immune factors impinge on bacterial growth, the number of experiments that can be identified and would need to be performed becomes unwieldy. Using mathematical models we are able to approach these issues in a straightforward and systematic way, and results can be obtained in a matter of seconds. The purpose of the models then becomes to identify proposed experiment(s) that will yield decisive results. Our first goal is to reproduce typical infection progression outcomes by means of a mathematical model. Once that is achieved, we can manipulate the system to systematically ask questions about interactions and rates.

From the host side

To date many factors necessary for controlling infection in *M. tuberculosis* have been identified, but no set of factors has been identified as sufficient. For example, it is well known that interferon (IFN)-γ is a key factor necessary for macrophage activation and essential for control of infection. This has been confirmed in mice (using gene KO techniques) and human studies [9–11] (based on individuals who were born without functional IFN-γ receptors). However, IFN-γ levels are high in both humans and mice with active tuberculosis (TB), even though these hosts remain unable to control infection [12,13] (see Box 1 for a description of infection outcomes). Thus, although IFN-γ is necessary, it is not sufficient for achieving latent infection. Our mathematical models also identify these same factors as necessary to *M. tuberculosis* control. But,

as outlined below, models might also identify sufficient conditions under which control can be obtained.

It is known that an effective acquired cellular immunity to *M. tuberculosis* is dependent on the ability of the host to initiate a Th1 cellular response [11]; however, this response might not be sufficient for controlling infection or preventing reactivation. From a host perspective, several factors are highlighted by our mathematical models as determinative in triggering and enhancing an efficient adaptive T-cell response: (i) cellular factors, including activation of resting macrophages and T-cell killing of infected macrophages, and (ii) environmental cues, such as trafficking and recruitment induced by different chemokine profiles and cell maturation.

Trafficking and presentation

Infection with *M. tuberculosis* has an effect on chemokine expression in the lung and on dendritic cell localization into secondary lymphoid tissues by altering expression of chemokines and chemokine receptors. [14] These signals drive dendritic cell trafficking and migration first into the lymphatic vessels and then into lymph nodes. Our mathematical simulations show how delaying this trafficking translates into altered cell-mediated immunity, leading to active TB [5].

Using our model, we identified three factors that are key: the extent of dendritic cell recruitment into inflamed tissues and subsequent migration into lymph nodes, the nature of the maturation stimulus, and the kinetics of activation. Although they represent general features of an immune response to pathogens [15], factors such as number and type of effector immune cells present (Th0 or Th precursor, Th1, Th2), or how fast cells migrate back and forth between the lymph node and lung might play distinctive roles in protection and immunoregulation, determining virulence in the context of *M. tuberculosis* infection over long time scales. Controlling overall timing of these events is crucial in elucidating *M. tuberculosis* pathogenesis and could represent a virulence strategy.

Th1-Th2 controversy

To highlight how mathematical models can be used to explore controversial hypotheses we consider the concept

that there exists a Th1-Th2 switch in the immune response to *M. tuberculosis*. Conflicting data exist in the literature regarding T-cell populations during *M. tuberculosis* infection in the lung. In the mouse and most humans, type 1 cytokines are present at high levels during infection. However, an increase in type 2 cytokines in TB patients has also been reported [16,17], but not consistently [18–20]. By contrast, our mathematical simulations [6] suggest that during latency, lymphocyte populations at the site of infection are mainly of Th0 type, with very low levels of either Th1 or Th2 cells. Thus, we propose that there is not a strict Th1-Th2 switch, but that conflicting data have probably arisen as a result of other factors. Th0 cells produce both type 1 and type 2 cytokines. The relative predominance of either Th1 or Th2 cells thus depends on several factors, including the differentiation stage of Th0 cells at the time sampling, how long after infection a sample is drawn, and the sample site (blood versus lung for example).

Recruitment

We also studied the effect of trafficking and host–pathogen interactions on a smaller scale (cell–cell interactions) using a mathematical model of granuloma formation [21] (Box 1). We are able to capture another important factor: localization of immune effector cells (macrophages and T cells) within the lung. In this setting where we capture spatial aspects of granuloma formation, the rate of cellular movement in response to signals greatly influences infection outcome. Increased recruitment of resting macrophages to the infection site is positively correlated with bacterial load, most likely by providing additional cells for productive infection. This suggests the paradoxical conclusion that inflammation might be detrimental, and further, that unless macrophages become activated, they serve to propagate infection rather than halt it. This is consistent with experimental data [22] suggesting that high levels of inflammation could be deleterious during the course of infection.

From the bacterial side

M. tuberculosis and other pathogenic mycobacteria are very slow growing. Doubling times of 24–96 hours have been reported for *M. tuberculosis* [23] (Figure 1), and this is striking when one considers that *E. coli* has a doubling time of as low as 20 minutes.

In the mathematical models that included bacterial turnover, the growth rate of *M. tuberculosis* was one of the key factors that determined whether the system achieved latent infection or active TB. In the agent-based approach [21], where interactions are tracked at their most stochastic and discrete levels, we observed a strong correlation between the intracellular growth rate of *M. tuberculosis* and granuloma size (or similarly, bacterial load). Using sensitivity analysis, we tracked this correlation during TB infection and we observed a shift from positive to negative values. In the first 12 days of infection, higher growth rates are significantly more favorable for granuloma growth (positive correlation), but between one to three months, granulomas grow larger when growth rates are slowest (negative correlation). Finally, after

three months, the correlation is again positive, where slightly higher growth rates favor granuloma growth.

From these outcomes, it is clear that growth rate is correlated with granuloma formation, although the biological basis for this result remains unclear. This counter-intuitive simulation outcome results from the non-linear dynamics that occur between T cells, macrophages and bacteria. Does the slow growth rate of *M. tuberculosis* contribute to virulence? No experimental data are available to support this finding, however our results are consistent with hypotheses drawn from earlier mathematical models suggesting that persistence of *M. tuberculosis* at low densities for extended periods in the face of immune pressure might be due to mechanisms that are associated with a very slow growth rate [24].

Extracellular and intracellular lifestyles

The status and location of bacteria during latent TB infection has long been an area of controversy. A unique feature of the mathematical model is that we are able to track at any given moment which bacteria are intracellular (within macrophages) and which are extracellular (not within macrophages). Although data on bacterial loads in murine models, for example, are usually given as cfu/gm of tissue, our mathematical models are able to distinguish total bacterial levels within these mutually exclusive compartments (Box 1).

In all but one of our cell-dynamic models (the exception being the agent-based model), levels of extracellular bacteria arose as a marker of disease progression. If bacterial levels could be contained intracellularly (within low levels of infected macrophages), then infection could be controlled. Our results suggest that all of the bacteria are harbored within a few infected macrophages during latent infection within the granuloma. New experiments by our collaborators are now in place to test this hypothesis. By contrast, when extracellular bacterial levels increase uninhibited, this reflects an immune system that is unable to control infection.

Necrosis

As with most elements of this system, there is an important balance regarding necrosis. Granulomas have a characteristic structure including a necrotic center, surrounded by macrophages, surrounded by T cells. Within the ring of macrophages these cells are lysed contributing to centralized necrosis (this structure is observed in our simulations of granulomas shown in Box 1). Our agent-based model [21] predicts that this necrotic center is a site within which extracellular bacteria reside, and unpublished data from the NHP model indicate that these granuloma are packed with bacteria in the necrotic centers (T. Reinhart, personal communication). This pattern of necrosis prevents immune cells from reaching and eliminating those trapped bacteria; however, it also prevents bacteria from spreading. Thus, in our simulations, this pattern of necrosis is a mechanism aiding stable containment of infection. Early studies on TB suggested that it might be pathology that helps limit infection spread [25,26].

From math to biology

Mathematics has long been relegated to the closet in microbiology studies. The emergence of new computational tools and other technologies provides a perfect opportunity to dust off old notions of mathematics and welcome it into the biological arena. Here, we show that it can be applied in many ways to study relevant problems in biology, using the example of *M. tuberculosis* infection. A key to successful conversations between theory and experiment is the language. If theoreticians will put their work in the proper context of the problem being studied, then experimental biologists can more easily see how mathematics can be used as an additional tool to stimulate and address important questions in microbiology. New experimental technologies [8,27] will eventually produce *in vivo* time series cell population dynamics data to support and validate mathematical model results and hypotheses. Combinations of experimental measurements and mathematical models will ultimately yield fundamental insights into biological phenomena. A new generation of students is now being trained to this end.

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