Published in final edited form as:

Curr Opin Biotechnol. 2010 August ; 21(4): 524–531. doi:10.1016/j.copbio.2010.06.002.

Tuberculosis: Global Approaches to a Global Disease

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Summary of recent advances

Mycobacterium tuberculosis is a remarkably successful human pathogen. The interaction with the human host is complex and much remains unknown. Recent advances in systems biology have allowed the integration of data from humans and animal models into computational approaches. For example, mathematical models provide a platform for *in silico* manipulation of host-pathogen interactions to gain insight into this infection across temporal and biologic scales. Here, we review recent studies on global approaches toward identifying comprehensive responses of both host and bacillus during infection, and the potential for incorporation of these data into many types of useful computational systems. Systems Biology approaches provide a unique opportunity to study interventions that may improve therapy and vaccines against this major killer.

Introduction

The immunology of tuberculosis has been studied for a hundred years in humans and animal models. In the past two decades, with the advent of ever more specialized mouse models, and more sophisticated techniques that can be used on human samples, our knowledge base on the immunology of tuberculosis has increased exponentially. Nonetheless, we are still at a loss for how to fight this disease from an immunologic perspective, symbolized by the lack of an effective vaccine.

Granuloma formation in tuberculosis: the intersection of host and bacteria

M. tuberculosis infection results in formation of a structure called the granuloma, composed of varying levels of macrophages, lymphocytes, neutrophils, dendritic cells, and fibroblasts, usually organized in a somewhat spherical structure. Numerous cytokines and chemokines, both pro- and anti-inflammatory, are produced within the granuloma. There are several different types of granulomas in human tuberculosis, characterized by cellular composition, level of inflammation, necrosis, mineralization, and fibrosis, often even within one person. The granuloma serves as an immune microenvironment, where cells interact to control the infection.

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It also is a niche for mycobacterial survival, and is modulated in part by the bacillus. A balance of pro- and anti-inflammatory factors is necessary for a functioning granuloma to reduce or control bacterial proliferation and not cause excessive inflammation that drives pathology. This complex structure holds the key to control of infection and lung damage, and understanding the interaction of various cell types in granulomas is essential to elucidating mechanisms of defense.

Studies of immune response to tuberculosis in wet-lab settings and animal models

Murine models have been studied most extensively for defining immune responses (protective or pathologic) to tuberculosis (for review see [1•]). For such studies, transgenic and knockout mice have been used, which have a gain or loss of cell type or function. Antibody depletion studies to remove cell types or neutralize cytokines have also been used to identify important responses in control of tuberculosis. These studies have identified a number of important factors, including IFN- γ , TNF, CD4 and CD8 T cells, IL-12, IL-10, and inducible nitric oxide synthase, amongst others. Many of these have been identified as important in human tuberculosis, through HIV-*M. tuberculosis* co-infection studies [2], use of anti-inflammatory agents (such as anti-TNF antibody) [3], and genetic mutations [4].

Recent studies take a more global approach to defining the host response to *M. tuberculosis* infection. These are studies of individual cell types (e.g. macrophages or dendritic cells) [5-, 8•,9], data derived from blood [10,11], or data generated at the whole tissue level (i.e. lungs from mice)[12•,13•]. Transcriptomic and proteomic analyses have been performed to address the overall effects of *M. tuberculosis* infection on host responses, and vice versa. A recent study using an siRNA library to modulate gene expression in human macrophages infected with *M. tuberculosis* and a computational biology approach to interpretation of these data revealed that unique *M. tuberculosis* strains affect cells differently, possibly providing clues to the virulence of these strains [14••]. This study also implicated the avoidance of autophagy as an important survival mechanism for *M. tuberculosis*. These studies have been very informative, in terms of enhancing our understanding of the response of cells to the microbe, or overall tissue responses that occur. Integrating these global transcription and translation signatures with data from animal models and humans that address specific factors, such as loss of a certain cell type or function, are needed construct a more comprehensive picture of the immune response to *M. tuberculosis*.

Studies in the non-human primate model of tuberculosis provide an opportunity to bridge human and animal studies [15•,16•,17,18•,19]. Like humans, macaques present with a spectrum of clinical outcomes after infection with *M. tuberculosis*. The pathology in macaques is extremely similar to human pathology, including a range of granuloma types [18]. Using this model, one can obtain clinical data similar to humans, as well as immunologic data from peripheral compartments (e.g. blood). The real strength is that one can obtain serial data from airways by bronchoalveolar lavage, and from actual granulomas in the lung and lymph nodes at necropsy [16-18,20]. From these samples, a variety of immunologic assays can be performed, since there are numerous reagents available for the study of macaque immunology, and many human reagents cross-react. Immune responses at the site of infection can then be linked to responses in the blood, enabling better interpretation of peripheral data from humans. The added advantage of using this model in conjunction with human studies is that one can control strain, dose, and timing of infection in the animals, where in humans those are very difficult variables to assess, let alone control.

Challenges for integration of complex data on tuberculosis

One difficulty is that studies in humans and animals are often focused on only one or two factors at a time, known as a reductionist approach, but likely what is needed is a more global or holistic approach. Although single component studies in animal models, or even in humans, are necessary and have been very enlightening, it can be difficult to put these studies in the context of what is certainly a very complex immune response. The more global studies generate vast amounts of data, but expression and function are not necessarily linked, and studies that validate the importance of factors identified in global searches are necessary to link “omic” studies with disease studies. Another challenge is that in humans, the primary compartment available for study is the blood, while in tuberculosis, immune responses that control (or exacerbate) disease are concentrated in the lungs and thoracic lymph nodes, where granulomas reside. The complexity of granulomas and the immune interactions that take place there can be particularly difficult to assess in experimental settings, as these structures to date cannot be created or maintained *in vitro*. Integration of data from studies of isolated components is necessary to gain insight into the interplay of various components of the immune response.

Systems Biology and TB

It is clear that what is needed is a complementary approach for synthesizing and expanding the individual wet-lab studies discussed above. Systems biology is an approach whereby data from several model systems (animal, human, bacterial, and computational) can be integrated and synthesized to explore a complex biological system and address relevant questions (for a review c.f. [21]). A critical step toward this goal is to create a computational model system that reflects human biology, providing an opportunity to perform experiments that are not feasible *in vivo* or *in vitro*, as well as generate testable hypotheses. With respect to tuberculosis, mathematical and computer models can be created to address biological questions, using either a temporal or spatio-temporal approach over multiple biological scales (genetic, cellular, tissue, organ). The multidisciplinary approach that we have used over the past decade integrates data from mice, non-human primates, and humans where available, together with mathematical and computational models of granulomas to provide tools for addressing questions regarding the immunology of tuberculosis. Once developed, these models can be manipulated to study how the immune response is established, regulated, evolves, and is maintained over the lifetime of the host. In addition, once a model system is in hand, it can easily be manipulated to explore interventions, specifically drug treatment and vaccines. The marriage of sophisticated computational models with an array of data from cells to organs to animal models and humans, provides a powerful platform for addressing complex questions in this infectious disease.

Mathematical and computational models of TB

Mathematical and computer models provide a unique approach to representing and studying the integrated behavior of complex biological systems. Although modeling has been used widely to understand the dynamics of epidemics and transmission [22,23], the first generation models to explicitly include a pathogen at the level of the immune system appeared more recently and contributed to our understanding of infection with HIV, *M. tuberculosis*, and LCMV [24,25•,26]. These models focused on a single biological scale, typically the cellular scale, and follow populations of immune cells over time. We have used ordinary differential equation (ODE) models, which are continuous and deterministic, to track the levels of cytokines and effector cells in the immune response to *M. tuberculosis* [27••,28•]. In contrast to ODE models, agent-based models (ABMs, also known as individual based models) are algorithm- or rule-based models that allow for a discrete and stochastic representation of cells and events [29], and these have occasionally been used in biological applications [29]. The components of ABMs are: *agents*, the *environment* where agents reside, the *rules* that govern

the dynamics of agents (movement, interactions with each other and the lattice), and *time scales* on which these rules are executed. In an ABM, cells, receptors, or any entity of interest are represented as discrete software objects (“agents”) and placed on a lattice.

We have been building and studying mathematical and computer models of tuberculosis for the last decade. Ours is the first group to create a mathematical model describing the immune response to a bacterial infection [25]. We have continued to refine our models together with our collaborators, to build comprehensive and useful models exploring granuloma formation and the dynamics within the lung draining lymph nodes [20,27,28,30-39,40•,41••-43]. For example, we have generated models of granuloma formation in a 2-dimensional setting [41•, 43]. Interestingly, a 3-D ABM gave similar results to our 2-D model, suggesting that 2-D can capture important dynamics [44].

To illustrate how mathematical modeling and experimental data dynamically influence our understanding of tuberculosis, we provide as an example the conundrum of the different types of granulomas that develop during *M. tuberculosis* infection in humans and non-human primates [19•]. The mechanisms at the granuloma level that lead to different configurations of cells and differential containment of the organisms are not well understood. We have described the granuloma types in non-human primates histologically, immunologically and bacteriologically. However, there are factors that we cannot address experimentally, for example how changes in local concentrations of key cytokines and chemokines at different locations in each granuloma affect composition and control of infection. We developed an ABM that tracks dynamics of host immune cells responding to initial bacterial infection [41]. We recently revised the user interface for performing these studies so that we can easily track different aspects of the granuloma as it forms and is maintained. Our first goal is for the model to capture what is observed experimentally [19] and then to use the validated model to make predictions about, for example, the TNF gradient, a key cytokine for control of tuberculosis (Fig. 1). Here, only slight differences in certain parameter values (for example, related to TNF and macrophage activation) lead to dramatically different granuloma types, recapitulating those seen in humans. The right side panel of Figure 1 shows the corresponding TNF gradients for the respective granulomas as predicted by the model. We are now examining methods for measuring and modulating TNF at the granuloma level to determine the effects on local control of *M. tuberculosis* infection. We can perform detailed analyses of our models allowing us to predict, for example, which mechanisms define different granuloma outcomes [20]. In addition, we have studied multiple physiological compartments, specifically the role of cellular dynamics within a draining lymph node in driving DC and T cell dynamics in the lung [36, 38]. Another example of the iterative process between experimental and *in silico* modeling is the recent work by our group on the interplay between TNF and IL-10 using a multi-compartment model built on murine data from infections at different inocula doses [45]. We are currently using the *in silico* model as well as experimental approaches to address the relationship between dose of infection and priming of T cells, and exploring mechanisms in both models (mouse and computer) that affect these processes.

Recently, Schlessinger's group has explored in more detail the roles of alternatively and classically activated macrophages in lung infection dynamics [46•], with an emphasis on *M. tuberculosis* infection. Their work also considered the important predisposition of the lung to a Type 2 response. The types of specific questions discussed here can easily be addressed with modeling – and some are impossible to currently address in a wet-lab setting. Finally, a group in Africa has revisited some of our models and extended them to explore aspects of treatment during both TB and co-infection with *M. tuberculosis* and HIV-1 [47,48].

Integration of the *M. tuberculosis* bacillus into the equation

A key component in tuberculosis is obviously the microbe itself. This organism can drive and shape the immune response, and persistent infection is in part due to immune modulation and evasion strategies. The interaction of tubercle bacillus with human host has been going on for as long as humans have been around, and the evolution of pathogen and host results in a situation in which some responses can benefit both bacterium and host, at least in the short term. The outcome of infection is a balance between pro- and anti-inflammatory responses, influenced by bacterium and host, factors intrinsic to the microbe (i.e. virulence of a particular strain), and innate resistance and susceptibility of the host.

In all the models to date the populations of mycobacteria are included and subdivided into individual niches (intracellular, extracellular, replicating, and non-replicating), but we do not consider features of the bacteria that are also important to the infection dynamics. In fact, even from our simplistic representations of bacteria, this subdividing has indicated that the extracellular population levels determine whether the granuloma can control infection. The global cell based studies discussed above clearly demonstrate the modulatory roles of the bacillus on host cells. Integrating this information into mathematical models of the host response is crucial for the next phase of systems biology.

Numerous pathogenesis studies have been performed to identify mechanisms of immune evasion, persistence, and virulence of *M. tuberculosis*. In addition, transcriptional analyses of *M. tuberculosis* under several *in vitro* conditions expected to replicate conditions in the host (limited nutrients, hypoxia, drug treatment) have been published. Transcriptome analyses of the organism within cell types have been reported, as well as a few studies of gene expression from *M. tuberculosis* in mice and humans. These data present a picture of the various stresses encountered by the organism. However, in terms of systems biology, there has been very little modeling of the organism in the context of the host response.

Central to our understanding of pathogenesis is the physiological state of tubercle bacilli residing in different microenvironments provided by the range of lesions found in human TB. Measurement of very small amounts of bacterial components embedded within the vast excess of host material presents a formidable technical challenge. A realistic strategy is to develop models of mycobacterial phenotypes using data generated from *in vitro* cultures, and to map these to *in vivo* samples on the basis of a limited set of measurable parameters, to create useful computational models for assessing host-pathogen interactions, as well as intervention strategies.

One application that is useful in this context is high-throughput sequencing technologies. This provides a rich source of novel genotypic information about *M. tuberculosis*, with the potential application of linking variation in bacterial phenotype with epidemiological differences [49, 50•]. For example, although the absolute number of SNPs in *M. tuberculosis* is low in comparison to other bacteria, a high relative proportion of non-synonymous SNPs suggest significant phenotypic diversity [51••]. Chavadi *et al* [52•] explored the extensive global consequences of a single mutation in the gene encoding pyruvate kinase, which distinguishes human from bovine strains. This mutation has a very clear *in vitro* phenotype –resulting in a requirement for addition of pyruvate to culture media – although it is less clear whether it has any impact on pathogenesis *in vivo*. Mehayy *et al* [53] and Desouza *et al* [52] have studied strain variation at the proteome level, using isobaric tagging or label-free techniques.

Another approach has been to use extensive transcriptomic data generated by microarray analysis for *M. tuberculosis* under a variety of conditions, including those experienced in human sputum samples [54]. This information has been collated into user-friendly databases, such as TBDB [55], and integrated into gene regulatory network models [56,57•]. Such

networks provide powerful tools for probing temporal responses of *M. tuberculosis* to changes in either the environment or bacterial growth phase [57]. Advances in sequencing technologies have facilitated high-resolution mapping of transcription factor binding sites (ChIP-on-chip and ChIP-seq) [58], including targeted regulators such as DosR [59•] and the more general nucleoid-associate protein Lsr2 [60]. Sequence-based transcriptomic approaches are also important in discovery of non-coding RNA transcripts. Small stable RNA molecules (sRNAs) are increasingly recognized as key components of post-transcriptional regulatory networks in other bacteria and a first report of sRNA in *M. tuberculosis* [61•] is likely to stimulate considerable activity in this area.

Efforts have also been put forth to collate experimental information on the proteome of *M. tuberculosis*, developing a global perspective on major protein classes including the Type VII secretion systems [62], toxin-antitoxin modules [63•] and serine-threonine protein kinases [64]. In parallel with transcriptomic approaches, the power of proteome analysis is significantly increased by temporal analysis, taking into account the kinetics of protein turnover [65,66]. *In silico* modeling approaches have been used to construct protein-protein interactomes for *M. tuberculosis*, which can be used for prediction of potential drug targets [67-69]. Modeling has also been used to analyze the expression of lipid components that are likely to be key mediators of host cell interactions during infection [70].

Finally, metabolomic analysis of *M. tuberculosis* is a critical step in identifying potential drug targets against this organism. There have been several excellent models that incorporate the metabolism of *M. tuberculosis* into genome-scale mathematical network models for analysis of potential enzymes or pathways of vulnerability that could be targeted to kill this pathogen. A flux balance analysis, which analyzes the flow of metabolites through a metabolic network, was initially used to model the mycolic acid pathway [71], which is crucial for the cell wall of *M. tuberculosis* and is the target of two standard anti-mycobacterial drugs, isoniazid and ethionamide, and more recently to identify drug targets [72•]. Targets based on critical points in the mycolic acid pathway and other pathways have been identified for experimental testing. Two re-constructions of *M. tuberculosis* were performed *in silico* to reflect the complete metabolic networks, using genomic information and flux balance analysis. This resulted in a metabolic model GSMN-TB [73] and the *in silico* *M. tuberculosis* strain iNJ661 [74], which incorporated 726 genes and 661 genes respectively, and hundreds of reactions and metabolites. The data from these networks were compared with *in vitro* and *in vivo* essentiality data and the growth of the organisms were tested on various *in silico* media. Variations from the available laboratory-generated data sets provide further experimental options for correcting gene annotations or identifying pathway interactions [74]. Finally, a complex model incorporated enzyme kinetics and a dynamic cell population growth model into the flux balance analysis metabolic model of iNJ661, providing a comprehensive systems biology framework for assessing targets and growth inhibitors for drug development [75•].

Conclusions

There is a wealth of model systems for tuberculosis, including *in vitro*, animal and computational models. What remains to be accomplished is the integration of both host and bacterium data together over both multiple time and biological scales. Mathematical and computer modeling is an ideal venue to achieve this. This will provide the best possibility for probing pathogenesis mechanisms, host-pathogen interactions at the molecular, cellular, and tissue level, and assessing therapeutics and interventions.

Acknowledgments

The authors are supported by the NIH (HL092883, (DEK, JLF); LM009027 (DEK, JLF); HL075845 (JLF), NIAID-DAIT-BAA-05-10 (JLF, DEK)) and the Bill and Melinda Gates Foundation (JLF, DY).

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Fig. 1.

(Left side) 2-D simulations of granuloma under different immune response conditions. A: Containment with small amount of intracellular bacteria and no extracellular (Be); B: Dissemination with an additional foci arising; C: clearance at the expense of large amounts of inflammation (influx of immune cells); D: dissemination with large amounts of extracellular and intracellular bacteria. Mr=resting macrophage, Mi=infected macrophage, Mci=chronically infected macrophage, Ma=activated macrophage, Tcyt=cytotoxic T cell, Tgam= gamma producing T cell, Treg=T regulatory cell. (Right side) Corresponding TNF gradients left side of figure with same letter designation. In each case A-D the corresponding TNF gradient indicates where the boundaries of the granuloma are in the left side of the figure. The bar at the bottom indicates to color code for concentrations of TNF.