In silico models of *M. tuberculosis* infection provide a route to new therapies

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Abstract

Tuberculosis (TB) is a global health problem responsible for ~2 million deaths per year. Current antibiotic treatments are lengthy and fraught with compliance and resistance issues. There is a crucial need for additional approaches to provide a cost-effective means of exploring the design space for potential therapies. We discuss the use of mathematical and computational models in virtual experiments and virtual clinical trials both to develop new hypotheses regarding the disease and to provide a cost-effective means of discovering new treatment strategies.

INTRODUCTION

Despite years of scientific research and effort by world health organizations, tuberculosis (TB) remains a global health problem responsible for ~2 million deaths per year. TB is primarily a pulmonary disease, although there are some extrapulmonary manifestations as well. After infection with *Mycobacterium tuberculosis* (Mtb), humans develop primary (active) TB (10%) or latent infection (90%); reactivation of latent infection can occur leading to active TB [1]. Factors that result in these different infection outcomes in humans are not well-characterized. Of great concern is that TB persists as a latent infection in ~2 billion humans worldwide, providing a reservoir of potential disease and contagion. Immunosuppressive conditions increase susceptibility to primary and reactivation TB (reviewed in [2]). Drug susceptible TB can be effectively treated with a lengthy regimen (6 months) of multiple antibiotics. This complex treatment strategy is fraught with compliance issues, and some of these drugs, notably isoniazid (INH) and rifampin (RIF), have potential toxicity [3]. Drug resistant TB is a major problem worldwide and development of both new drugs and strategies is essential to prevent further spread of these strains. Single drug therapy is not permitted in treatment of active TB in humans as drug resistance can arise, and the standard of care must be adhered to. Thus, it is difficult to evaluate effects of new TB drugs or strategies in human clinical trials. The multi-scale

nature of the disease also makes identification of new drug targets complex: interventions that show an effect at a molecular or single cell level or at a particular time scale may not have much impact on the overall course of infection. Thus there is a crucial need for novel approaches and platforms for developing, testing and optimizing new therapies for TB.

Systems Biology Approach

A systems biology approach allows us to integrate information from multiple model systems to understand, probe, and manipulate the immune response to Mtb infection. For drug discovery in TB, this includes, but is not limited to, data derived from humans, non-human primate (NHP) and other animal models of TB, and immunological and microbiological studies, together with mathematical and computational models (MCMs). In particular, MCMs provide a powerful method to integrate data generated in multiple experimental settings into a single framework for hypothesis generation and testing. Given the large numbers of possible combinations and timing of drugs to be tested, the design space of possible new strategies for TB treatment is not only enormous but also can be – due to the time and expense of working with animal models such as NHPs – inaccessible experimentally (Figure 1). Herein we briefly review MCMs of both bacterial and host dynamics during Mtb infection in lungs. We then suggest how MCMs can be used to augment existing experimental and clinical approaches by expansion and exploration of novel approaches for drug therapy for TB.

Granulomas and TB

The immune response to Mtb infection is determined by a myriad of host and bacterial factors operating at multiple length and time scales in lung and draining lymph nodes (LN). Briefly, infection occurs after inhalation of Mtb into the lungs. Resident antigen presenting cells such as macrophages and dendritic cells (DCs) take up Mtb. DCs traffic to lung draining LNs where T cells are primed. These T cells migrate to the lung and participate in the formation of a self-organizing spherical collection of cells termed a granuloma [4] (Figure 2A). Granulomas serve not only to functionally and spatially contain infection but also as a survival niche for the bacterium, highlighting the unique, complex and interdependent interaction between Mtb and host. Many data now support the idea that host-pathogen dynamics occurring within granuloma are the key to determining infection outcome [5]. In addition, new data suggest that each granuloma is distinct [6]. Thus, we focus on granulomas as the environments most likely to be impacted by new approaches to therapy.

Mathematical and Computational Models of Mtb infection

A tractable *in silico* model of the immune response during Mtb infection can provide a new tool for developing, understanding, predicting, and optimizing drug therapy for TB treatment. Because it is important to consider molecular, cellular and tissue-level events occurring over short and long times, as well as in different biological compartments (i.e. lung, lymph node, and blood), the model developed must ultimately be both *multi-scale* and *multi-organ*. Our groups have developed multiple generations of MCMs to explore the host-pathogen dynamics of Mtb infection in the lung and draining LNs [7–10]. We have had

success building MCMs that capture stochastic events occurring at the cellular scale that manifest in an emergent fashion at the tissue scale as granulomas (Figure 2B). We use agent-based models that include macrophages (resting, infected, chronically infected, and activated), T cells (regulatory, cytotoxic, cytokine-producing), bacteria (extracellular, intracellular, non-replicating bacteria trapped in caseous/necrotic areas), and effector molecules (chemokines such as CXCR3 and CCR5-ligands: CCL2, CCL5, CXCL9/10/11; and well-studied pro- and anti-inflammatory cytokines IL-12, TNF, IFN-γ IL-10). Cells are discrete agents and effector molecules are tracked continuously and diffuse. In addition, we have developed multi-scale models that track the dynamics of molecular scale events relating to two key cytokines, TNF [11–13] and IL-10 [10]. Our current multi-scale model has been developed in both two and three dimensions. Models are validated against a variety of experimental data; NHP studies are particularly valuable as this animal model most closely replicates human infection (e.g. [14]). Thus we have the capability of studying molecular level, cellular, and tissue level events in our granuloma MCM that will provide a unique platform for studying drug therapy.

In addition to these immune-centric models, detailed models of Mtb growth and metabolism have been published over the past decade and vary from phenomenological to metabolic. Magombedze and Mulder developed an ordinary differential equation model of Mtb including different Mtb phenotypes (dormant, latent and active), *dosR* gene regulation, nitric oxide and nutrient availability to evaluate the contribution of gene regulation to latent TB development [15]. Ankomah and Levin present a similar division of bacteria into subpopulations based on their antibiotic susceptibility and advocate development of more complex pharmacodynamic data for antibiotics that are used in combination [16]. Focusing more on metabolic pathways, Beste et al. built a metabolic network model for Mtb [17], and used flux balance and flux spectral analysis to deduce intracellular diets of Mtb [18] and to identify new metabolic pathways [19]. Shi et al. subsequently used this model in combination with gene expression data to show that Mtb redirects carbon flow from biosynthetic pathways to storage compound production under stressful conditions [20]. Palsson s group independently constructed a metabolic network for Mtb to predict gene essentiality [21]. That model was subsequently used to elucidate metabolic changes during Mtb adaptation to hypoxia [22], to predict effects of enzyme inhibition [23] and the effects of INH on Mtb metabolism [24]. What is presently not considered is the role of these *bacterial* dynamics (metabolism, gene regulation, growth, etc) in the context of the *host* and *environmental* influences within a granuloma. Our group is developing a next-generation granuloma model that includes these flux-balance bacterial models running within all of the bacteria agents. This allows for a more accurate representation of bacterial dynamics and mechanisms that are possible targets for drug therapies (unpublished).

Virtual experiments

MCMs can be used to apply two useful tools for drug development, testing and assessment: virtual experiments (VEs) and virtual clinical trials (VCTs). VEs allow us to generate predictions and test hypotheses as to, for example, aspects of the immune response to Mtb that most significantly contribute to granuloma formation and function. We can perform virtual knock-out or virtual depletion experiments to test effects of different system

components, and we can simulate the effect of multiple interventions at once [8,13,25,26]. Finally, we can apply uncertainty and sensitivity analysis tools to predict specific "tipping points" in model system architecture that might be useful as therapeutic targets [27]. Granuloma outcomes in our models can be roughly classified into four categories - containment of bacteria, clearance of bacteria, non-containment of bacteria (dissemination), and containment of bacteria with excessive inflammation – these correspond well to experimentally observed situations, including the observed wide spectrum in between.

Virtual Clinical Trials

Currently, it costs 1 billion USD to test one new vaccine for TB in a clinical trial, and of course testing in humans is time-consuming and risky. VCTs can be used to simulate treatment in a collection of individuals with person-to-person variability. For example, VCTs suggested that the differential rates of reactivation TB in different populations were due to bioavailability of TNF following anti-TNF treatment [27]. In another study, variation in the number of memory T cells across a population was simulated to determine levels that offer effective protection against Mtb following vaccination [28,29]. Predictions from VCTs can be used to inform choices of drug scheduling, dosing etc in the next stage of clinical trial development for human populations.

Identification of drug targets

What host factors influence the ability of a granuloma to control infection? VEs can help us understand whether subtle changes in molecular, cellular or tissue-level host parameters might identify tipping points at which a granuloma transitions from being able to contain infection to not being able to do so (Figure 1). For example, Cilfone et al. [10], using a computational model that includes both the pro-inflammatory cytokine TNF and the anti-inflammatory cytokine IL-10, demonstrated that a balance of TNF and IL-10 concentrations is essential to infection control within a single granuloma. Too little TNF leads to poor infection control; too little IL-10 leads to infection control but excessive inflammation and thus tissue damage. The appropriate ratio of TNF to IL-10 concentrations leads to infection control with minimal inflammation. Granulomas that are out of balance may improve with the addition of antibodies or exogenous cytokines to shift from poorer outcomes towards containment. Our VEs with anti-TNF drugs, which are used as treatment for inflammatory diseases such as rheumatoid arthritis and psoriatic arthritis, also show the effect of disturbing this balance. Differences in TNF/drug binding kinetics and permeability explain the differential ability of particular drugs to cause reactivation TB, and suggest characteristics of anti-TNF drugs that may lessen the probability of doing so [12,26].

Another way to shift the balance of cytokines identified above is to target physiological processes that influence IL-10 or TNF concentrations or that make up the signaling pathways downstream of those cytokines. Our work gives several examples of this. First, VEs suggest that TNF-receptor internalization kinetics play a critical role in infection control within a granuloma, regulating a balance between paracrine and autocrine TNF-induced responses [9]. Internalization that is too slow leads to clearance with excessive inflammation, while internalization that is too rapid results in dissemination. Physiological values of internalization rates lead primarily to containment. Finally, VEs of

pharmacological manipulation of the TNF-induced NFκB signaling pathway show improved infection control. Interestingly, we find that simultaneous targeting of several points along the pathway, rather than just one, produces (or leads to?) a better infection outcome [13], suggesting complex interactions are occurring between system factors that can be harnessed. Going forward, we envision that VEs will complement hands-on experiments help identify drug targets.

Optimization of antibiotic protocols

Short-course therapy for TB typically consists of a cocktail of 2–4 first-line antibiotics taken for 6–9 months. The first-line antibiotics for TB are isoniazid (INH), rifampin (RIF), pyrazinamide (PZA) and ethambutol (EMB), of which INH and RIF are arguably the most studied. Although antibiotic efficacy depends on many factors, certainly adequate exposure of the pathogen to antibiotics is key. Imaging technology has been used to demonstrate that spatial distributions of antibiotics in granulomas play a key role in treatment outcomes [30,31].

Even among approved antibiotics, the design space that includes various combinations, dosing, and timing is enormous. Recently Wallis et al. [32] counted 58 different antibiotic regimens being tested for TB treatment. Simulations of antibiotic regimens may prove useful in narrowing the treatment design space. Initial modeling studies have shed light on the success or failure of some regimens [33,34], and future work can additionally include consideration of the penetration of drugs into the granuloma [12]. For example, a computational model of the granuloma can be coupled to a pharmacokinetic model (e.g. [35]) describing blood antibiotic concentrations as a function of time.

Immunomodulation with antibiotic therapy

Combining immune modulation strategies (immunomodulation) with antibiotics is a potential strategy for enhancing treatment of TB [3]. Immunomodulation in this context refers to the addition/subtraction of cells and/or molecules (such as cytokines) in order to enhance the immune response. It stands to reason that boosting the immune system while reducing bacterial load could lead to more rapid and effective control of infection. However, the immune response to Mtb is complex and it remains unclear which are the appropriate immune responses to modulate to increase control of infection while simultaneously reducing pathology and pathogen load. Several strategies have been tried in murine models (e.g. addition of exogenous cytokines IFN-γ, GM-CSF, TNF, IL-12; reviewed in [36,37]) and a few in humans (addition of exogenous cytokines IL-2, GM-CSF, TNF, IFN-γ) [36,38], but results are inconclusive. Likely, successful immunomodulation therapy will involve combinations of immune mediators such as the molecules TNF, IL-10, IFN-γ, IL-12 and cells (effector and regulatory T cells) as well as chemokines (CXCR3 and CCR5-ligands). Again, appropriate delivery of both antibiotic and immunomodulatory agents to granulomas and proper timing, drug combinations and dosing are all likely to be key factors in successful therapy and ones that can be explored with MCMs. VEs and VCTs applied in this setting can help address the following questions: How can immunomodulation affect the establishment of latency, reactivation, and clearance? Which immunodulators are predicted to be the most effective at improving antibiotic outcome? Is there an optimal timing or

combination of modulators and drugs that will minimize the development of active TB or reduce reactivation?

Conclusion

The development of new strategies for TB treatment is an urgent priority. We argue that virtual experiments and virtual clinical trials can complement experimental and clinical approaches toward this goal. The development of drug resistance adds additional complexity. While treatment of disease is an important goal, only vaccines will solve the TB problem. While not discussed in this article, new computational efforts also need to be extended in this direction. A systems biology approach combining multiple modalities is likely the only course to crossing the cure boundary for TB.

Acknowledgments

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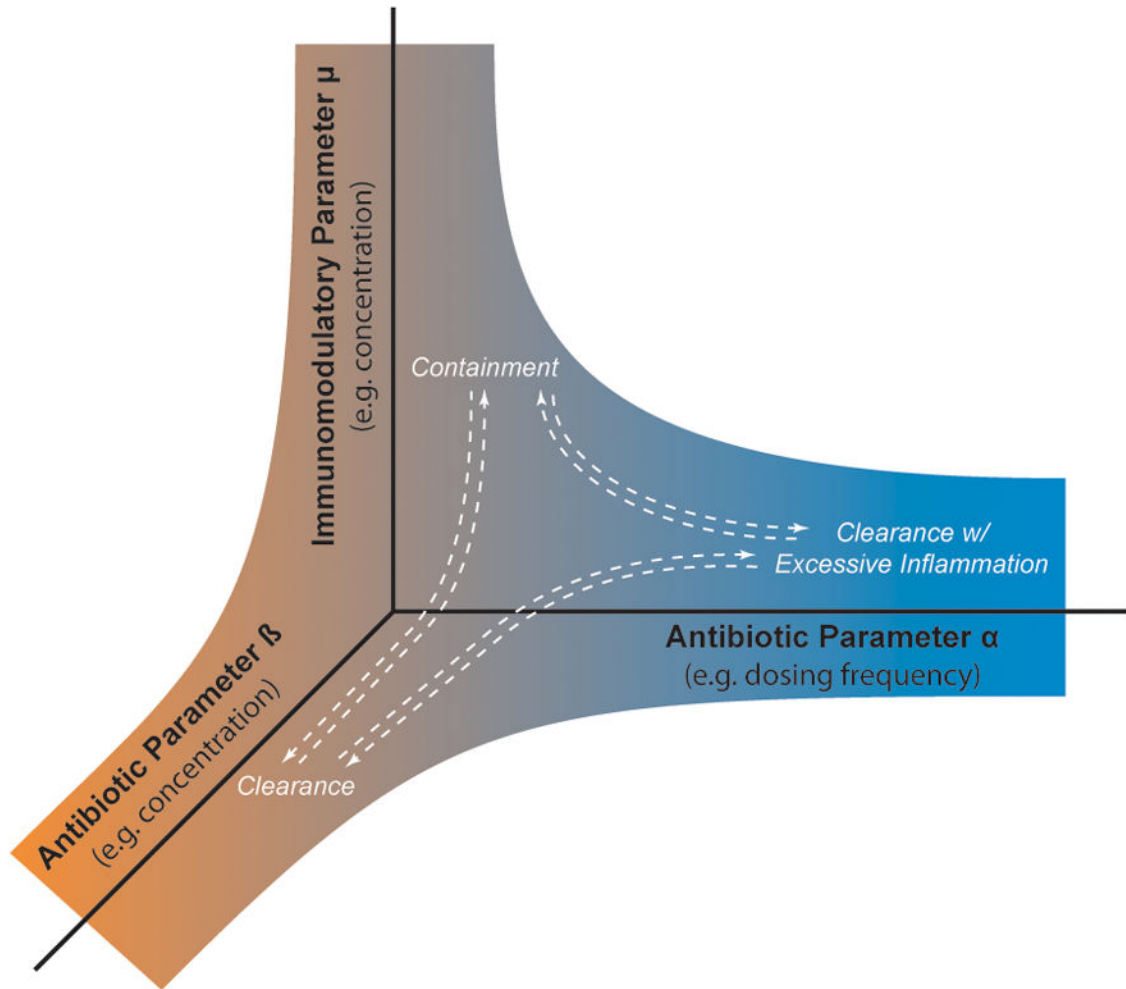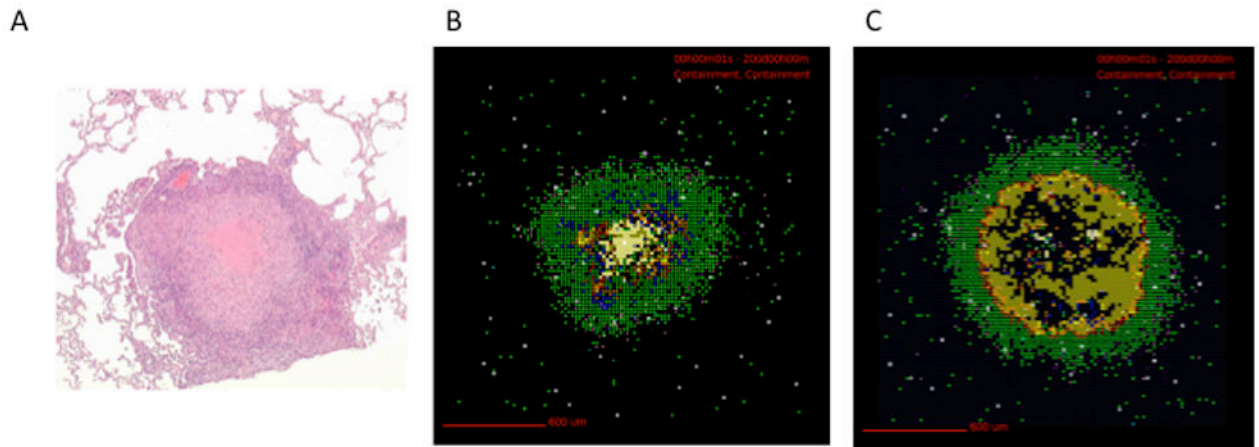
Figure 1.
Design space for treatment development. The identities, dosing schedules, and concentrations of multiple antibiotics can be varied. Similarly, the drugs, dosing schedules, and concentrations of multiple immune-modulators can be varied. Only 3 such parameters are shown. White arrows indicate movement between treatment outcomes as parameters are varied.

Figure 2.
Experimental and virtual granulomas. (A) H&E stain of a NHP lung granuloma showing macrophages and lymphocytes surrounding a necrotic core (courtesy of Flynn Lab, University of Pittsburgh). (B) VE of a granuloma at day 200 post-infection, using a model similar to Ray et al. [8]. A well-circumscribed, stable granuloma that controls bacteria (but does not clear it) emerges. See also time courses at http://malthus.micro.med.umich.edu/lab/movies). Bacterial counts for this granuloma at day 200 are: 1309 intracellular Mtb, 825 extracellular Mtb, 302 nonreplicating Mtb in necrotic areas, and 2135 total Mtb. (C) VE representing a TNF knockout. Notice the much larger region of bacteria and significantly lower levels of activated macrophages. Bacterial counts for this granuloma at day 200 are: 3494 intracellular Mtb, 46601 Extracellular Mtb, 36 non-replicating Mtb in necrotic areas, and 50195 Total Mtb. For both (B) and (C) macrophages are shown as: resting (green), activated (blue), infected (orange), chronically infected (red). T cells are pink (CD4+) and purple (CD8+). Yellow shows bacterial concentrations and white hash marks represent necrotic regions.